

**Slow release fluoride glass devices in the prevention of  
enamel demineralisation during fixed appliance orthodontic  
treatment.**

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## **Abstract**

Enamel demineralisation or white spot lesions (WSLs) is a risk for all patients undergoing fixed appliance orthodontic treatment (FAOT) with no consensus with regards to prevalence, risk factors, prevention and treatment. Slow-release fluoride glass devices (SRFGDs) have been shown to clinically prevent caries without relying on patient's compliance, therefore their effectiveness in preventing WSLs during FAOT was investigated.

An *in-vitro* exploratory study investigated fluoride (F) and phosphate (PO<sub>4</sub>) release from different types of powder from SRFGDs incorporated into a composite resin bonding material. Samples were stored in artificial saliva and assessed with ion chromatography for up to six months. Three types of powder showed high F release to maximise caries prevention and low PO<sub>4</sub> release to minimise degradation of powder.

A questionnaire was emailed to orthodontists' members of the British Orthodontic Society. For majority of responders the key factors to the problems related to WSLs are clinical examination, photographs, F, oral hygiene, diet and duration of FAOT.

A double-blind, randomised clinical study with orthodontic patients randomly allocated to SRFGDs or placebo devices threaded onto the orthodontic wire was conducted. Cross-polarised digital photographs of the maxillary permanent central and lateral incisors and canines were taken for 63 subjects at the start and for 40 subjects at the end of the study. One examiner assessed photographs for presence and severity of WSLs. Majority of volunteers and the smallest number of refusals lived in the most deprived areas according to the Multiple Deprivation Index. Use of SRFGDs would decrease severity by preventing 2.88 times more teeth compared to use of 225 ppm F mouth-rinse once daily and 1,450 ppmF tooth-paste twice daily. Duration of FAOT and increased gingival index at the start of FAOT increased significantly the risk of developing WSLs.

SRFGDs were effective in preventing teeth with WSLs during the course of FAOT.



**Table of Contents**

<b>1</b>	<b>Introduction .....</b>	<b>1</b>
<b>2</b>	<b>Literature Review .....</b>	<b>3</b>
2.1	Assessment of WSL(s).....	3
2.2	Rate of development of WSLs.....	7
2.3	Risk factors for developing WSLs .....	7
2.4	Prevalence and incidence of WSLs.....	9
2.5	Teeth most commonly affected by WSLs.....	17
2.6	Arrest/Repair of WSLs .....	18
2.7	The use of lasers for arresting WSLs .....	20
2.8	Treatment of WSLs following FAOT .....	20
2.9	Restoration of teeth following FAOT .....	22
<b>3</b>	<b>Prevention of WSLs .....</b>	<b>27</b>
3.1	Systematic reviews of clinical trials for prevention of WSLs .....	27
3.2	Preventing WSLs under orthodontic bands .....	32
3.3	Clinical trials in prevention of WSLs .....	32
3.4	Testing SnF <sub>2</sub> in clinical trials for prevention of WSLs .....	33
3.5	Testing chlorhexidine in clinical trials for prevention of WSLs .....	34
3.6	Testing enamel sealants in clinical trials for prevention of WSLs .....	34
3.7	Other methods tested in clinical trials for prevention of WSLs .....	35
3.8	Testing F-materials in clinical trials for prevention of WSLs .....	35
3.9	Testing F-rinse in clinical trials for prevention of WSLs .....	36
3.10	Testing F-gels and/or varnishes in clinical trials for prevention of WSLs .....	37
3.11	SFRGDs in orthodontic patients.....	37
3.12	Testing plaque and saliva in studies investigating prevention of WSLs.....	39
3.12.1	Testing plaque.....	39
3.12.1.1	Testing saliva .....	41
3.12.1.2	Testing saliva and plaque .....	41
3.13	<i>Ex-vivo</i> Studies .....	42
3.13.1	<i>Ex-vivo</i> studies investigating arrest of WSLs .....	42
3.13.2	<i>Ex-vivo</i> studies investigating prevention of WSLs.....	43
3.13.3	Other methods tested <i>ex-vivo</i> for prevention of WSLs .....	44

3.13.4	Summary of <i>Ex-vivo</i> studies.....	45
3.13.5	Difference between <i>ex-vivo</i> and <i>in-situ</i> studies .....	46
3.14	<i>In-situ</i> studies .....	46
3.15	<i>In-vitro</i> studies.....	47
3.15.1	WSLs investigated on bovine enamel .....	47
3.15.2	Investigating <i>in-vitro</i> enamel sealants in preventing WSLs .....	48
3.15.3	Investigating <i>in-vitro</i> F-releasing materials in preventing WSLs.....	48
3.15.4	Non-F based preventive regimes for prevention of WSLs tested <i>in-vitro</i> .....	49
3.15.5	Summary of <i>in-vitro</i> studies.....	49
3.16	Testing of bonding materials .....	50
<b>4</b>	<b>Aim of the study .....</b>	<b>53</b>
<b>5</b>	<b>Objectives of the study .....</b>	<b>55</b>
<b>6</b>	<b>Null Hypotheses .....</b>	<b>56</b>
<b>7</b>	<b>Materials and Methods.....</b>	<b>57</b>
7.1	Materials.....	57
7.2	SRFGDs with plastic holder .....	60
7.3	Methods of investigation .....	62
7.4	<i>In-vitro</i> exploratory study .....	62
7.4.1	Steps of the <i>in-vitro</i> study.....	62
7.5	Questionnaire to specialist orthodontist - members of the British Orthodontic Society.....	64
7.6	Socio-Economic Status (SES) of eligible participants in clinical study.....	64
7.7	Clinical study .....	64
7.7.1	Study setting .....	64
7.7.2	Study design .....	64
7.7.3	Ethics Committee Approval of the clinical study.....	64
7.7.4	Outcome variables .....	65
7.7.5	Inclusion criteria .....	66
7.7.6	Exclusion criteria.....	66
7.7.7	Sample size .....	67
7.7.8	Allocation-Randomisation process.....	71
7.7.9	Steps of the clinical study.....	71
7.7.10	Data collection and management.....	72

7.7.11	Statistical Analysis .....	79
<b>8</b>	<b>Results .....</b>	<b>80</b>
8.1	<i>In-vitro</i> exploratory study .....	80
8.1.1	Storage mediums .....	80
8.1.2	Control material.....	85
8.1.3	Assessment of powder particle size with scanning electron microscopy (SEM). .....	90
8.1.4	F and PO <sub>4</sub> release from powder from SRFGDs .....	91
8.1.5	F release from powder mixed with control material .....	94
8.1.6	PO <sub>4</sub> release from powder mixed with control material .....	98
8.1.7	SEM images of Transbond® mixed with powder from SRFGDs.....	102
8.1.8	Profilometry scan of Transbond® mixed with powder from SRFGDs.....	105
8.1.9	Bond strength of test and control (Transbond®) materials .....	109
8.2	Questionnaire to orthodontist-members of the British Orthodontic Society .....	110
8.3	Socio-economic status (SES) of eligible participants in clinical study.....	115
8.4	Clinical study .....	116
8.4.1	F release from test and placebo bead.....	116
8.4.2	Recruitment of volunteers for clinical trial.....	117
8.4.3	Randomisation .....	118
8.4.4	Demographics of volunteers .....	119
8.4.5	Participants screening failures .....	120
8.4.6	Adverse events .....	120
8.4.7	Volunteers lost to follow-up .....	120
8.4.8	Protocol deviation.....	120
8.4.9	Volunteer withdrawals .....	120
8.4.10	Duration of treatment for volunteers who completed the study .....	120
8.4.11	DMFT(S)/dmft(s) .....	120
8.4.12	Plaque Index .....	121
8.4.13	Gingival Index .....	121
8.4.14	Satisfaction questionnaire to orthodontists.....	121
8.4.15	Satisfaction questionnaire to volunteers.....	121

8.4.16	Breakage/loss of SRFGDs .....	122
8.4.17	Incidence of WSLs .....	122
8.4.18	Kappa coefficient estimation for incidence of WSLs..	128
8.4.19	Severity of WSLs.....	128
<b>9</b>	<b>Discussion.....</b>	<b>135</b>
9.1	<i>In-vitro</i> exploratory study .....	135
9.1.1	F and PO <sub>4</sub> release from powder from SRFGDs in AS..	136
9.1.2	Bond strength of test and control materials.....	136
9.1.3	Summary of <i>in-vitro</i> exploratory study.....	136
9.2	Questionnaire to orthodontist-members of the British Orthodontic Society .....	137
9.3	Socio-economic status of eligible participants in clinical study..	138
9.4	Clinical study .....	140
9.4.1	Recruitment of volunteers for clinical study .....	140
9.4.2	Randomisation .....	140
9.4.3	Demographics of eligible participants, volunteers and volunteers who failed to complete the study.....	141
9.4.4	Participants screening failures, adverse events and protocol deviation .....	141
9.4.5	Volunteers lost to follow-up and withdrawals .....	141
9.4.6	Duration of treatment for volunteers who completed the study .....	142
9.4.7	DMFT(S)/dmf(s) .....	142
9.4.8	Plaque and Gingival Index .....	142
9.4.9	Satisfaction questionnaire to orthodontists.....	142
9.4.10	Satisfaction questionnaire to volunteers.....	143
9.4.11	Breakage/loss of SRFGDs .....	143
9.4.12	Photographic technique for assessment of WSLs.....	143
9.4.13	Incidence of WSLs .....	144
9.4.14	Kappa coefficient estimation for incidence of WSLs..	146
9.4.15	Severity of WSLs.....	147
9.5	Conclusions.....	148
9.5.1	Conclusions from clinical study: .....	148
9.5.2	Conclusions from electronic questionnaire emailed to members of the British Orthodontic Society: .....	149
9.5.3	Conclusions from <i>in-vitro</i> study: .....	150
9.6	Rejection of null hypotheses .....	151

9.7	Future studies .....	151
<b>10</b>	<b>List of References .....</b>	<b>153</b>
<b>11</b>	<b>Glossary of acronyms.....</b>	<b>169</b>
<b>12</b>	<b>Appendices.....</b>	<b>171</b>
12.1	Research Ethics Committee Letter.....	172
12.2	Research and Development Approval Letter.....	176
12.3	Declaration of the end of the study.....	180
12.4	Invitation Letter for Participation in a Research Study.....	181
12.5	Parental Information Sheet About The Research.....	182
12.6	Child Information Sheet aged 6-10 years old .....	186
12.7	Young People Information Sheet aged 11-15 years old.....	188
12.8	Letter to Doctor/Dentist .....	191
12.9	Consent Form .....	192
12.10	Case Record Form (CRF) .....	193
12.11	Satisfaction Questionnaire for Orthodontists .....	216
12.12	Satisfaction Questionnaire for Participants.....	217
12.13	Randomisation codes.....	218
12.14	Questionnaire on WSLs emailed to members of British Orthodontic Society .....	219
12.15	Abstract for International Association of Dental Research (IADR) Meeting in Barcelona, Spain July 2010 .....	221
12.16	Poster presentation for International Association of Dental Research (IADR) Meeting in Barcelona, Spain July 2010.....	222

List of Tables

<b>Table 2-1: Indices used for clinical examination of WSLs.....</b>	<b>4</b>
<b>Table 2-2 Summary of studies reporting on prevalence of WSLs from 1992 onwards. ....</b>	<b>11</b>
<b>Table 2-3 Summary of studies reporting on incidence of WSLs from 1992 onwards. ....</b>	<b>13</b>
<b>Table 2-4 Teeth most commonly affected by WSLs.....</b>	<b>18</b>
<b>Table 2-5 Teeth most commonly affected by WSLs as reported in the literature published from 1992 onwards.....</b>	<b>24</b>
<b>Table 3-1 Systematic reviews of trials on prevention of WSLs and their included studies. ....</b>	<b>29</b>
<b>Table 3-2 In-vivo studies investigating prevention of WSLs by testing F- CR bonding materials.....</b>	<b>36</b>
<b>Table 3-3 Studies assessing F release from F-CR materials.....</b>	<b>52</b>
<b>Table 7-1 Composition of AS used as a storage medium.....</b>	<b>58</b>
<b>Table 7-2 Different types of powder mixed with Transbond at different ratios.....</b>	<b>63</b>
<b>Table 8-1 F standards for different types of storage mediums. ....</b>	<b>82</b>
<b>Table 8-2 PO<sub>4</sub> standards used for different types of storage mediums. ....</b>	<b>84</b>
<b>Table 8-3 F standards for Transbond®.....</b>	<b>87</b>
<b>Table 8-4 PO<sub>4</sub> standards used for Transbond®.....</b>	<b>89</b>
<b>Table 8-5 Summary of findings for F and PO<sub>4</sub> release after two weeks, four and six months. ....</b>	<b>102</b>
<b>Table 8-6 Demographic characteristics of eligible participants who declined participation n=112.....</b>	<b>119</b>
<b>Table 8-7 Demographic characteristics of study volunteers n=63. ....</b>	<b>119</b>
<b>Table 8-8 Demographic characteristics of volunteers who failed to complete study n=23.....</b>	<b>119</b>
<b>Table 8-9 Demographic characteristics of volunteers who completed study n=40. ....</b>	<b>119</b>
<b>Table 8-10 Replies from orthodontists to satisfaction questionnaire post FAOT with SRFGDs.....</b>	<b>121</b>
<b>Table 8-11 Replies from volunteers to satisfaction questionnaire post FAOT with SRFGDs. ....</b>	<b>122</b>
<b>Table 8-12 Measures of model fit for the count of teeth with WSLs after completion of the study .....</b>	<b>123</b>
<b>Table 8-13 Zero Inflated Poisson regression model on a subject level for the presence and severity of WSL. Coefficients and 95% C.I. transformed at the natural logarithm.....</b>	<b>124</b>

List of Figures

Figure 7-1 Diagram and pictures of PTFE mould. From Musanje et al., 2001. ....	57
Figure 7-2 Ion chromatography and chromatogram demonstrating the F peak in a standard 1ppm F solution sample. ....	59
Figure 7-3 Initial design of the plastic holder with the SRFGDs. ....	60
Figure 7-4 Clinical view of the holder with SRFGD placed in the lower arch. ....	60
Figure 7-5 Clinical view of the holder with SRFGD placed in the upper arch after adhesion of metallic tube on the plastic holder. ....	61
Figure 7-6 Final design of holder as provided by the manufacturer. ....	61
Figure 7-7 Clinical view of the holder with SRFGD onto the orthodontic wire. ....	61
Figure 7-8 Tooth outline with arrow denoting the WSL. ....	74
Figure 7-9 Tooth area and WSL area outline with arrow denoting the WSL. ....	74
Figure 7-10 Marks of WSL drawn freehand. ....	75
Figure 7-11 Outline of WSL drawn freehand. ....	75
Figure 7-12 Mean grey value (174) in a representative area of healthy enamel denoted by arrow. ....	76
Figure 7-13 Mean grey value (194) in a representative area within the WSL denoted by arrow. ....	76
Figure 7-14 The mean grey value of each hand placed mark was measured to a pixel level denoted by arrows. ....	77
Figure 7-15 Initial outline of WSL following placement of hand placed marks to a pixel with a corresponding mean grey value of 184. ....	78
Figure 7-16 Final outline of WSL following placement of hand placed marks to a pixel with a corresponding mean grey value of 184. ....	78
Figure 8-1 F release from different types of storage medium over n=14 days. ....	81
Figure 8-2 PO <sub>4</sub> release from different types of storage medium over n=14 days. ....	83
Figure 8-3 F release from control material (Transbond®) over 28 days. ....	86
Figure 8-4 PO <sub>4</sub> release from control material (Transbond®) over 28 days. ....	88

Figure 8-5 SEM images of powder from SRFGDs with relative solubility 3. ....	90
Figure 8-6 F release from powder from SRFGDs with different solubility and particle size, in AS after two months.....	92
Figure 8-7 PO <sub>4</sub> release from powder from SRFGDs with different solubility and particle size, in AS after two months.....	93
Figure 8-8 F release in AS after two weeks after mixing Transbond® with powder from SRFGDs.....	95
Figure 8-9 F release in AS after four months after mixing Transbond® with powder from SRFGDs.....	96
Figure 8-10 F release in AS after six months after mixing Transbond® with powder from SRFGDs.....	97
Figure 8-11 PO <sub>4</sub> release in AS after two weeks after mixing Transbond® with powder from SRFGDs.....	99
Figure 8-12 PO <sub>4</sub> release in AS after four months after mixing Transbond® with powder from SRFGDs.....	100
Figure 8-13 PO <sub>4</sub> release in AS after six months after mixing Transbond® with powder from SRFGDs.....	101
Figure 8-14 SEM images of Transbond® bonding material.....	103
Figure 8-15 SEM images of powder (relative solubility 50, particle size <38µm, ratio of bead to Transbond® 1:10) mixed with Transbond®.....	103
Figure 8-16 SEM images of powder (relative solubility 1, particle size >38µm, ratio of bead to Transbond® 1:6) mixed with Transbond®.....	104
Figure 8-17 Profilometry scan of Transbond® bonding material.....	105
Figure 8-18 Profilometry scan of powder with relative solubility 1 and particle size >38µm, mixed with Transbond®. ....	106
Figure 8-19 Profilometry scan of powder with relative solubility 1 and particle size <38µm, mixed with Transbond®. ....	106
Figure 8-20 Profilometry scan of powder with relative solubility 16 and particle size >38µm, mixed with Transbond®. ....	107
Figure 8-21 Profilometry scan of powder with relative solubility 16 and particle size <38µm, mixed with Transbond®. ....	107
Figure 8-22 Profilometry scan of powder with relative solubility 50 and particle size <38µm, mixed with Transbond®. ....	108
Figure 8-23 Flexular modulus of elasticity and stress mean±SD in n=10 samples of Transbond® bonding material. ....	109
Figure 8-24 Problems for patients with WSLs identified by dentists during FAOT. ....	110
Figure 8-25 Protocol followed to prevent WSLs during FAOT. ....	111



<b>Figure 8-26 The most important risk factor for developing WSLs identified by responders. ....</b>	<b>111</b>
<b>Figure 8-27 Second most important factors for developing WSLs identified by responders. ....</b>	<b>112</b>
<b>Figure 8-28 Third most important factor for developing WSLs identified by responders. ....</b>	<b>112</b>
<b>Figure 8-29 Agreement with risk factors for developing WSLs identified in the literature. ....</b>	<b>113</b>
<b>Figure 8-30 Methods of diagnosis of WSLs as identified by responders.....</b>	<b>113</b>
<b>Figure 8-31 Methods of assessment of severity of WSLs as identified by responders. ....</b>	<b>114</b>
<b>Figure 8-32 Methods to arrest/treat WSLs during and/or after completion of FAOT as identified by responders. ....</b>	<b>114</b>
<b>Figure 8-33 MDI of eligible participants after two years of recruitment. ....</b>	<b>115</b>
<b>Figure 8-34 Mean weekly F release from placebo and SRFGDs in de-ionised water for a month. ....</b>	<b>116</b>
<b>Figure 8-35 Flow-chart of participants in clinical study.....</b>	<b>117</b>
<b>Figure 8-36 CONSORT clinical study flow diagram.....</b>	<b>118</b>
<b>Figure 8-37 Data distribution for presence of WSLs on a subject level according to duration of FAOT. ....</b>	<b>125</b>
<b>Figure 8-38 Data distribution for presence of WSLs on a subject level according to duration of FAOT index by outcome of the study. ....</b>	<b>126</b>
<b>Figure 8-39 Data distribution for presence of WSLs on a subject level according to gingival index at the start of the study. ....</b>	<b>127</b>
<b>Figure 8-40 Data distribution for presence of WSLs on a subject level according to gingival index by outcome of the study.....</b>	<b>128</b>
<b>Figure 8-41 Number of teeth with WSLs predicted as per Multiple Deprivation Index. ....</b>	<b>130</b>
<b>Figure 8-42 Number of teeth with WSLs predicted according to GI at the start of the study. ....</b>	<b>131</b>
<b>Figure 8-43 Data distribution for presence of WSLs on a subject level according to gingival index by outcome of the study.....</b>	<b>132</b>
<b>Figure 8-44 Difference in presence of WSLs on a subject level between those who received treatment and those who received the placebo.....</b>	<b>133</b>
<b>Figure 8-45 Difference in presence of WSLs on a subject level between those who received treatment and those who received the placebo.....</b>	<b>134</b>

## **1 Introduction**

Enamel demineralisation or the so called “white spot lesion” (WSL) is the precursor stage of dental caries and a constant risk of fixed appliance orthodontic treatment (FAOT) hence of great importance to both dentists and orthodontists. The latest statistical data report that nearly 4.3 million new orthodontic courses of treatment were undertaken during the period of March 2011-2012 in England alone, an increase of 32,000 or 0.7% (Health and Social Care Information Centre, 2012). These numbers clearly demonstrate not only a great number of new cases each year but an increasing trend as well. If we were able to quantify the risk of developing caries in these patients then we might be able to quantify any costs of repair and/or any costs of prevention of such lesions for any effective method.

Introduction of brackets and bands creates plaque stagnation areas inducing changes in the oral environment, mainly increasing the intra-oral bacteria counts in both plaque and saliva (Arneberg et al., 1984), resulting in an increased caries risk compared to the general population (Lundstrom and Krasse, 1987). As a result there is extensive literature into the clinical appearance, histology, diagnosis and of course prevention and treatment/arrest of such lesions.

Comprehensive FAOT usually takes about two years to complete which maximizes opportunities for demineralisation to occur with all the aesthetic and dental health consequences. Presence of WSLs or frank caries can even delay the start of treatment until the lesions are arrested or controlled. It also appears that there is a lack of knowledge of the risk of decay among parents of children who had FAOT (Pratelli et al., 1996). These factors paired with the fact that the aim of most orthodontic treatment is to improve aesthetics leaves no doubt that an effective preventive regime is imperative to avoid new problems.

Such lesions can themselves grossly compromise dental appearance especially due to their likely location when associated with fixed appliances. Patients can therefore be left considerably worse off than if treatment had never been started. For example, if treatment has to be abandoned due to dental health problems such as development of caries, patients may have had extractions undertaken purely for orthodontic reasons and can then be left with residual spaces as well as much of the original malocclusion together with unsightly, carious lesions.

With regards to prevention of WSLs, fluoride (F) has been given much attention as it is accepted worldwide to be the main factor for the prevention of dental caries. However the main problem with use of F is patients' compliance especially during the often lengthy courses of FAOT. If the problem is not solved it is likely to result in early termination of treatment due to further development of carious lesions that may need restorative intervention.

In order to overcome the obstacle of poor compliance, the slow-release fluoride glass devices (SRFGD) have been developed in Leeds, West Yorkshire, U.K. These devices originally comprised a glass bead attached to the dental enamel hence overcoming the need for patient's compliance. The F is slowly and continuously released in the mouth for up to 18 months hence it can achieve its maximum preventive effect by being constantly present at the enamel-plaque interface (Toumba and Curzon, 1993). This study aims to investigate prevention of enamel demineralisation in patients undergoing FAOT by employing a modified SRFGD, designed for orthodontic use.

## 2 Literature Review

### 2.1 Assessment of WSL(s)

WSL(s) is one of the names used to describe demineralised dental enamel, recognised in studies as early as 1937 (Noyes, 1937). Other names used are early caries, early enamel caries, enamel demineralisation, chalky white spot, early lesions *etc.*

Clinically these lesions can be seen with the naked eye under bright white light and when dental enamel has been air-dried as a white opaque area. Water is replaced by air in the porous demineralised enamel; the lower refractive index of air compared to water results in a different appearance. The most common methods and indices that have been used to describe and/or document such lesions clinically are listed in Table 2-1.

**Table 2-1: Indices used for clinical examination of WSLs**

Index	Score			
(von der Fehr, 1961) Caries Index 1961	0= surface appears intact	1=limited greyish tinge, with or without accentuated perikymata	2=perikymata well accentuated, in some areas confluent into greyish-white spots	3=pronounced white decalcification
(Zachrisson and Zachrisson, 1971b)	0=surface appears intact	1=whitish demineralisation without cavitation of the enamel	2=whitish demineralisation with the beginning of cavitation of the enamel	3=cavitation that cannot be removed by cautious grinding of the enamel
(Curzon and Spector, 1977)	OO= absent	O1=white opaque flecks, spots, patches involving <25% of labial enamel surface	O2=white opaque flecks, spots, patches involving 25-50% of labial enamel surface	O3=white opaque flecks, spots, patches involving >50% of labial enamel surface
(Gorelick et al., 1982)	1=no white spot formation	2=slight white spot formation	3=excessive white spot formation	4=white spot formation with cavitation
(Mizrahi, 1982)	0=no enamel opacity. An opacity of <1 mm in length or diameter is considered absent	1=an opacity covering up to 1/3 of the surface area.	2=an opacity covering from 1/3 to 2/3 of the surface area	3=an opacity covering from 2/3 to the full surface area
Enamel Defect	0=no WSL	1=WSL involves less than 1/3 of	2=WSL involves more than	3= WSL involves more

Score, (Artun and Brobakken, 1986)		the vestibular enamel surface area outside the area covered by bracket and bonding material	1/3 but less than 2/3 of the vestibular enamel surface area in question	than 2/3 of the vestibular enamel surface area in question
(Geiger et al., 1988)	1=slight white spot formation	2=severe white spot formation	3= excessive white spot formation (cavitation)	
Enamel Decalcification Index, (Banks and Richmond, 1994)	0 = no decalcification	1 = mild, but clinically visible decalcification affecting <50% of the area	2=moderate to severe decalcification extending over >50% of the area	3 = decalcification covering the whole area or with obvious surface breakdown or caries

Despite the subjective nature of all these indices, they have been used in many *in-vivo* studies, even when examiner reproducibility is unreported. Their main advantages are that they are easy and cheap to use in any dental setting and in epidemiological studies requiring minimal training. Several modifications of the above can be found whereas other indices have been developed and used for the purpose of one study only (Kindelan, 1995, Melrose et al., 1996, Kindelan, 1997, Wenderoth et al., 1999, Andersson et al., 2004, Andersson et al., 2007).

More sophisticated methods have been developed for caries detection and quantification. They employ fluorescent techniques based on light scattering e.g. quantitative light fluorescence (QLF™) and DIAGNOdent with extensive literature to support them. Their main advantage is the ability to provide quantitative data but the equipment is expensive, requires training and is not easily available outside a hospital setting.

Photographs (either digital or slides with or without magnification) and computer based image analysis can document WSLs but also provide data on examiners' reproducibility. They are easily obtained with minimal training and can be easily standardised; they use inexpensive equipment but do not allow quantification of the area under examination without other technology being applied (Gorelick et al., 1982, Mitchell, 1992a). Slides performed worse than digital photos *in-vitro* and since there was poor agreement between the two techniques (86%) they should not be combined (Benson et al., 2005).

The problem of detecting changes in artificial WSLs over time and flash reflection was also examined *in-vitro* with good reproducibility when WSLs were assessed from conventional photos. Use of different software showed no evidence of systematic error but it was discussed that flash masking should be considered as well as a 20° angle to reduce flash reflection (Benson et al., 2000). In another study there was an almost linear relationship between number of days the area was exposed to a cariogenic solution and mean grey level of the WSLs assessed with digital photographs (Willmot et al., 2000).

The use of cross-polarised photography has reduced surface flash reflection allowing improved visualization of enamel defects (Robertson and Toumba, 1999, Willmot et al., 2000). Other studies do not favour use of cross-polarised photographs for early caries lesions due to lack of contrast and prints appearing "completely flat" (Hill and Geddes, 1975) or because it is difficult to focus and there is restricted flash output (Fleming et al., 1989).

Digital photographs easily allow use of image analysis software to assess surface area of WSLs. *In-vitro* comparison of QLF™ to digital photographs showed good agreement when measuring the demineralised area, therefore it is possible to combine the techniques (Benson et al., 2003a, Benson et al., 2003b). There is evidence to support use of cross-polarizing photographs in order to reduce flash reflection even when brackets are in place allowing for a 20° angle of the camera (Livas et al., 2008). Non-polarised photographs on the other hand need to be taken at a 20° angle to reduce flash reflection (Benson et al., 2000).

## 2.2 Rate of development of WSLs

A frequently quoted *ex-vivo* study showed that demineralisation around orthodontic brackets can develop within four weeks confirmed by micro-hardness (MH) testing (O'Reilly and Featherstone, 1987). Clinically WSLs were visible within five weeks (Holmen et al., 1988) and in a split-mouth design study with longer duration (6-13 weeks) 73% of surfaces developed WSL within the study period (Twetman et al., 1997). These studies show that WSLs in orthodontic patients can develop very quickly at both microscopic and clinical level, reflecting the changes in the oral micro-flora when fixed appliances are introduced in the oral environment (Lundstrom and Krasse, 1987).

## 2.3 Risk factors for developing WSLs

**Pre-existing WSLs** have been identified as a risk factor in some studies (Zimmer and Rottwinkel, 2004, Lovrov et al., 2007) but not in another study (Stecksen-Blicks et al., 2007).

The role of **patient selection and education** has been discussed in a review (Mitchell, 1992b). In an *in-vivo* prospective study in a private practice two regimes for preventing WSLs were compared in a high and a low caries risk group of patients (Zimmer and Rottwinkel, 2004). These groups were determined by assessing several factors, namely plaque index, approximal plaque index, gingival index, DMFT/dmft and initial lesions. The rigorous prevention group showed statistically significant results for both caries risk groups however participation was voluntary, introducing bias. The authors report a sensitivity of 75% and specificity of 88% for their assessment of caries risk proving their clinical validity.



Multiple regression analysis showed a weak correlation of WSL to **frequency of TB and F use** (table salt, tables, TP, gel and MW) and a highly significant relationship with the **clinical attachment** level ( $p < .01$ , multiple regression) with the incidence of WSLs of teeth increasing from 0.3% to 9.8% depending on the intensity of the prevention program (Lovrov et al., 2007).

With regards to **age**, most studies failed to find a correlation (Zachrisson and Zachrisson, 1971b, Boersma et al., 2005), however one prospective cohort study showed a lower incidence of WSLs for patients aged 19-24 years. However the cohort was not followed throughout FAOT, hence only one assessment was possible (Kukleva et al., 2002).

There is no clear answer for **gender**, with some studies finding that boys develop significantly more WSLs than girls (Zachrisson and Zachrisson, 1971b, Kindelan, 1995, Boersma et al., 2005) whereas other studies showed either the opposite (Mattousch et al., 2007) or no significant difference (Millett et al., 1999, Karadas et al., 2011).

Some clinical trials found no correlation with **length of FAOT** (Zachrisson and Zachrisson, 1971b, Boersma et al., 2005, Karadas et al., 2011) but others have reported that after 17 months (Marcusson et al., 1997) or 24 months (Geiger et al., 1988) there was an increase in WSLs.

**Poor oral hygiene** prior to FAOT appears to be a significant risk factor in many studies (Zachrisson and Zachrisson, 1971a, Zachrisson, 1972, Stratemann and Shannon, 1974, Zachrisson, 1976, Gorelick et al., 1982, O'Reilly and Featherstone, 1987, Ogaard, 1989, Boyd, 1992, Geiger et al., 1992, Gorton and Featherstone, 2003). The Plaque and Gingival Index by Loe (Loe and Silness, 1963, Silness and Loe, 1964) has been used in many studies to assess oral hygiene (Zachrisson and Zachrisson, 1971a, Wenderoth et al., 1999, Zimmer and Rottwinkel, 2004, Boersma et al., 2005, Lovrov et al., 2007). Furthermore, visible plaque at 12 weeks after bonding was significantly correlated ( $r=0.214$ ) to the presence of enamel demineralisation at the end of FAOT. However the correlation is low, so it may not be clinically significant (Ogaard et al., 2001).

**Diet** as well as **socio economic status** have not been much investigated but were not found to be correlated to the development of WSL (Boersma et al., 2005).

**Compliance with use of F rinse** has been reported to be poor in 52% and excellent in 27% of subjects when a questionnaire was given to the

parents/patients (Geiger et al., 1988). In a clinical trial of patients asked to use F rinse once every other day, 21% developed WSLs whereas the corresponding number for those who rinsed less frequently was 49%. Patients' compliance with daily use of NaF MW was reported to be full in only 13% and good in only 42% of subjects and it was significantly related to the presence of WSLs (Geiger et al., 1992) but in a more recent study, use of F showed a weak correlation to the presence of WSLs (Lovrov et al., 2007). It appears that there is an increase in WSLs with less frequent use of F rinse despite of the fact that terms like "good" and "poor" compliance do not help us quantify the problem.

A significant increase in both *lactobacilli* and *mutans streptococci* counts in saliva after insertion of fixed appliances was first reported in 1987 (Lundstrom and Krasse, 1987). Presence of WSLs has been positively related to *lactobacilli* counts in saliva but not related to *mutans streptococci* (Boersma et al., 2005). The percentage of *mutans streptococci* in plaque at the time of bonding has been reported as a good predictor for future presence of WSLs (Ogaard et al., 2001).

It appears that many potential risk factors have been investigated with conflicting results. Patients usually receive oral hygiene instructions with or without diet counselling, professional plaque removal and/or F application prior to their FAOT, therefore, the prevention package varies. Ideally identification of risk factors is possible with case-control studies where subjects are matched for confounding variables such as age or gender and the risk factor in question is identified in the groups.

#### **2.4 Prevalence and incidence of WSLs**

Over the years, many studies have estimated the prevalence and/or the incidence of this problem. In a comprehensive review of the literature figures reported to range from 2-96% of patients and 0-24% of teeth (Mitchell, 1992b). The wide range was mainly attributed to the difficulty in differentiating between WSLs and idiopathic lesions resulting in over-diagnosis. Following this review other studies investigated prevalence of WSLs as their primary outcome still with a wide range from 4.2% to 88% of teeth (Kindelan, 1997, Ogaard et al., 2001) and from 13% to 85% of subjects (Fornell et al., 2002a, Heinig and Hartmann, 2008). A summary of these studies is found in Table 2-2. Studies looking into incidence of WSLs from

1992 onwards show a wide range from 1.9 to 76.8% for teeth (Le et al., 2003, Kronenberg et al., 2009) and from 10.7 to 73% for subjects (Banks et al., 2000, van der Veen et al., 2010), as seen Table 2-3. But it is difficult to draw firm conclusions due to differences in study design, method of assessment of WSLs and heterogeneity in using clinical indices to assess WSLs.

**Table 2-2 Summary of studies reporting on prevalence of WSLs from 1992 onwards.**

Study	No of subjects	No/group of examined teeth	Detection method	Teeth most commonly affected	WSL % teeth	WSL % subjects
(Banks and Richmond, 1994)	80	1182	Clinical	Upper lateral incisors & canines, lower canines & second premolars	31	73
(Banks and Richmond, 1994)	80	1182	Clinical	Upper lateral incisors & canines, lower canines & second premolars	25	75
(Tebbett, 1995)	45	All erupted teeth	Clinical	n/a	n/a	67
(Kindelan, 1995)	52	977	Clinical	n/a	9.8	44.2
(Banks et al., 1997)	50	737 (366 experimental and 371 control teeth)	Clinical	n/a	13.5	50
(Kindelan, 1997)	42	902	Clinical	n/a	4.2	38
(Ogaard et al., 2001)	100	Upper and lower incisors, canines, premolars and first molars	Clinical	Upper lateral incisors, lower first molars and premolars	88	n/a
(Fornell et al., 2002a)	39	216	Clinical	n/a	7.4 (16/216)	13 (5/39)

<b>(Boersma et al., 2005)</b>	62	Upper and lower incisors, canines, premolars and first molars	Visual examination of QLF images on PC	n/a	30	n/a
<b>(Heinig and Hartmann, 2008)</b>	40	n/a	Clinical	n/a	9.18	85 (34/40)

(No = number, QLF=quantitative light fluorescence)

**Table 2-3 Summary of studies reporting on incidence of WSLs from 1992 onwards.**

Study	No of subjects	No/group of examined teeth (as reported in study)	Detection method	Teeth most commonly affected	Duration (months±SD)	WSL % teeth	WSL % subjects
(Mitchell, 1992a)	24	124	Photographic slides	n/a	Mean 10.5±4.2	19	n/a
(Boyd, 1992) (paste)	32/35	All incisors, canines, premolars and first molars	Clinical	First molars	Mean 26.2	14.4	n/a
(Boyd, 1992) (paste & rinse)	26/30	All incisors, canines, premolars and first molars	Clinical	First molars	Mean 24.3	10.1	n/a
(Turner, 1993)	n/a	82	Clinical	n/a	Minimum 12	25	n/a
(Boyd, 1993) (paste)	32	All erupted teeth	Clinical	n/a	Mean 26.2	14.4	n/a
(Boyd, 1993) (paste & rinse) (paste)	26	All erupted teeth	Clinical	n/a	Mean 24.3	10.1	n/a
(Boyd and Rose, 1994)	32	All erupted teeth	Clinical	n/a	Mean 26.2	14.4	n/a

<b>(Boyd and Rose, 1994) (paste &amp; rinse)</b>	26	All erupted teeth	Clinical	n/a	Mean 24.3	10.1	n/a
<b>(Trimpeneers and Dermaut, 1996)</b>	50	417	Photographic slides	Upper incisors	Mean 21	12.7	n/a
<b>(Marcusson et al., 1997)</b>	60	80	Photographic slides	n/a	Mean 22	29	n/a
<b>(Marini et al., 1999)</b>	23	Molars and upper incisors	Clinical	n/a	Mean 12	n/a	n/a
<b>(Millett et al., 1999)</b>	23	120	Photographic slides	Upper lateral incisors	Mean 15.3±3.2	72.4	n/a
<b>(Gaworski et al., 1999)</b>	16	149. Incisors, canines and premolars	Photographic slides	n/a	Range 12-14	75	n/a
<b>(Zimmer, 1999)</b>	40	All bracketed teeth	Clinical	n/a	Mean 18.32	9.8	n/a
<b>(Millett et al., 2000)</b>	45	157	Photographic slides	Upper lateral incisors and canines	Mean 21.3±6.6	26	n/a
<b>(Alexander and</b>	22	All erupted teeth	Clinical	n/a	Mean 26	3.2	n/a

<b>Ripa, 2000)</b>								
<b>(Banks et al., 2000)</b>	45	740	Clinical	Upper lateral incisors, lower second premolars	Mean 20.4±7.92	26	73	
<b>(Mattick et al., 2001)</b>	21	63	Photographic slides	n/a	Mean 25.5	n/a	n/a	
<b>(Le et al., 2003)</b>	18	47 anterior teeth	Photographic slides	Upper lateral incisors	Range 12-14	76.8 (36/47)	n/a	
<b>(Elaut and Wehrbein, 2004)</b>	45	106 upper anterior teeth	Photographic slides	Upper lateral incisors	Mean 14	54.7 (58/106)	n/a	
<b>(Ogaard et al., 2006)</b>	47	282	Clinical	Upper laterals and canines	Mean 18	7.2 (20/282)	n/a	
<b>(Vivaldi-Rodrigues et al., 2006)</b>	10	100 teeth. Upper and lower incisors, canines and premolars	Photographic slides	n/a	Mean 12	Increase of WSL index by 50.83%	n/a	
<b>(Stecksen-Blicks et al., 2007)</b>	125	2419 surfaces. Upper and lower incisors, canines and premolars.	Photographic slides	Upper lateral incisors	Minimum 6	n/a	25.7	



<b>(Kronenberg et al., 2009)</b>	20	Incisors, canines and premolars	Clinical, DIAGNOdent and QLF images on PC	n/a	Mean 26	1.9	n/a
<b>(Benham et al., 2009)</b>	14	618. All incisors and canines.	Clinical, photographic slides and DIAGNOdent	Upper lateral incisors and canines	Range 15-18	7.11 (22/309)	42.8 (6/14)
<b>(Chapman et al., 2010)</b>	332	2656. Upper incisors, canines and premolars.	Digital photographs	Upper incisors, canines and first premolars	Mean 32	36	n/a
<b>(Shungin et al., 2010)</b>	59 (30 at 12 years)	236 (120 at 12 years). Upper laterals and lower canines.	Digital photographs	n/a	Median 20.4	Sum areas	n/a
<b>(van der Veen et al., 2010)</b>	28	All bracketed surfaces	Digital photographs and QLF images on PC	n/a	Mean 18.1±5.5	20.7 (11/53)	10.7 (3/28)

(No=number, n/a=non-available, QLF=quantitative light fluorescence)

## 2.5 Teeth most commonly affected by WSLs

It has been reported that FAOT affects location of caries but does not increase its prevalence *i.e.* caries found on anterior teeth and on facial surfaces (Zachrisson, 1976). Whilst there is variation in the literature as to which teeth are most commonly affected as seen in Table 2-4 (Geiger et al., 1988, Banks and Richmond, 1994, Marcusson et al., 1997, Ogaard et al., 2001) almost all studies agree that the gingival region is the area at higher risk (Mizrahi, 1982, Mizrahi, 1983, Twetman et al., 1997).

In a review of seven studies, the prevalence of WSLs in the orthodontic population ranged from 8.5-44% for anterior teeth and 8-71% for molars (Linton, 1996). Molars therefore appear to be more vulnerable but bands are commonly placed on these teeth rather than brackets hence they have a different micro-environment whilst undergoing FAOT.

Data on the location of WSLs is shown in Table 2-4. It shows that in recent studies, the anterior teeth are commonly affected and, together with the fact that anterior aesthetics have become more important, it highlights the need to investigate more effective WSL prevention methods.

**Table 2-4 Teeth most commonly affected by WSLs.**

Study	Teeth (FDI notation)
(Meyers, 1952)	12, 22, 11, 21
(Zachrisson and Zachrisson, 1971b)	16, 26, 36, 46
(Mizrahi, 1982)	16, 26, 36, 46
(Geiger et al., 1988)	16, 26, 36, 46
(Ogaard, 1989)	16, 26, 36, 46
(Boyd, 1994)	16, 26, 36, 46
(Banks and Richmond, 1994)	12, 22, 13, 23, 33, 43
(Trimpenneers and Dermaut, 1996)	12, 11, 21, 22
(Marcusson et al., 1997)	12, 22
(Millett et al., 1999)	12, 22
(Wenderoth et al., 1999)	12, 22
(Banks et al., 2000)	12, 22
(Tobin, 2001) Thesis	12, 22
(Ogaard et al., 2001)	36, 46, 34, 35, 44, 45, 12, 22

## 2.6 Arrest/Repair of WSLs

Arrest of WSLs has mainly been attributed to salivary repair because it is supersaturated with calcium-phosphate salts identical to enamel hydroxyapatite (Gron, 1973). However, very rarely do we observe direct deposition of salts onto dental enamel, possibly because salivary phosphoproteins rich in proline have been found on the enamel pellicle, inhibiting crystal growth and spontaneous precipitation of calcium-phosphate salts (Hay et al., 1984). This is further supported by studies on the effect of acid on enamel surfaces (Garberoglio and Cozzani, 1979), which demonstrate that enamel repair after *etching* is mainly due to masking by salivary proteins than mineral deposition.

A series of *in-vitro* experiments concluded that although remineralizing solutions and/or saliva are supersaturated with respect to enamel apatite, the total amount of calcium and phosphate dissolved is so small that after precipitation of the dissolved mineral, only 1/20,000 – 1/30,000 of the volume of the mineralizing solution is occupied by mineral. There is slow

diffusion in and out of the lesion and there is rapid uptake of calcium and phosphate by the enamel apatite crystals so the aqueous phase within the pores can be presumed to be marginally supersaturated in the deeper parts of the lesion. The well mineralized surface layer on the other hand is a serious barrier therefore a subsurface area remains hypomineralized even after exposure to saliva. Nevertheless, the nucleation of new apatite crystals to substitute lost crystals especially in the deepest part of demineralized enamel lesions remains an unsolved problem (Larsen and Fejerskov, 1989).

Etched molars showed the greatest reduction in the body of lesion and tooth surface when exposed to a calcium remineralizing solution - as if *etching* provides a “pathway” to the body of the lesion for the remineralizing fluids (Flaitz and Hicks, 1994). When daily F TP was added to the remineralizing regime, weekly QLF™ measurements showed no difference ( $p > .05$ , Kruskal-Wallis) but the gold standard – transverse micro radiography (TMR) - showed statistically greater remineralization in the etched groups ( $p = .003$ , ANOVA). Irrespective of treatment, full remineralization did not occur and within weeks the process had reached a plateau.

Clinically it has been shown that complete remineralization may occur in 2.7% ( $n=10/370$ ) of teeth with WSLs two years after removal of orthodontic brackets (Mattousch et al., 2007) although it has been suggested that surface abrasion in addition to some re-deposition of minerals is the possible explanation (Artun and Thylstrup, 1986). WSLs that have developed quickly do remineralize nearly completely and within weeks *in-vivo*, in the absence of F and if the cariogenic challenge has been removed (Ogaard and Ten Bosch, 1994). If however the WSLs develop over a period of two to three years, then subsurface lesions develop that remineralize extremely slowly and, in the presence of F, the surface tends to remineralize more, forming a barrier. However, this study used optical scattering only on 14 teeth scheduled for extraction (Ogaard and Ten Bosch, 1994).

Visual examination was compared to laser fluorescence (DIAGNOdent®) when F varnish (Duraphat®) was applied weekly for four or eight weeks in children with WSLs in the upper anterior teeth. One examiner was calibrated to look into activity of the lesion (Nyvad et al., 1999), the dimensions of the WSL and the laser fluorescence readings. Results showed that after eight weekly F varnish applications there was 50% less active WSLs (Ferreira et al., 2005).

## 2.7 The use of lasers for arresting WSLs

The first report that laser irradiation makes dental enamel acid resistant was in 1965 (Sognaes and Stern, 1965). Argon laser use on dental enamel alters the surface characteristics of the crystalline structure of enamel by creating micro-spaces that stabilize ions during an acid attack rather than allowing them to be lost (Oho and Morioka, 1990, Anderson et al., 2002, Elaut and Wehrbein, 2004) SEM showed smooth enamel surfaces with small amounts of cracking, suggesting that acid resistance may be due to changes of crystallization of the enamel surface (Harazaki et al., 2001).

Lasers appear to lower the critical pH for enamel dissolution from 5.5 to 4.8 and to 4.3 in the presence of 0.1ppm F (Hicks et al., 2004) and short-term *in-situ* studies confirmed these results. Premolar pairs scheduled for extractions had a single laser exposure that showed after five weeks a 23-33% (Blankenau et al., 1999) or 44% ( $p < .05$ , ANOVA) reduction in lesion depth further enhanced to 62% ( $p < .05$ , ANOVA) by a single application of 0.5% F varnish (Hicks et al., 2004) compared to their control premolars.

The use of an argon laser on dental enamel with or without pumice/etching of premolars extracted after five weeks showed statistically significant less surface area ( $p < .01$ , ANOVA) and depth ( $p < .001$ , ANOVA) of induced WSLs when compared with control premolars (Anderson et al., 2002).

Argon laser (10sec 250mW) was compared to a halogen light (40sec) in a clinical split-mouth study for curing a CR (Transbond) but showed no significant difference ( $p > .05$ , Cochran and McNemar's test) in prevention of WSLs assessed on photographic slides in  $n=45$  subjects under FAOT for 14 months (Elaut and Wehrbein, 2004). A Nd-YAG laser combined with acidulated phosphate fluoride (APF) solution to treat WSLs showed a 51% reduction ( $p < .05$ , Mann-Whitney U-test) in the mean WSL area on photographs repeated after one year (Harazaki et al., 2001).

Even though there are positive signals from *in-vitro* work there is not enough clinical research to support use of laser for WSLs.

## 2.8 Treatment of WSLs following FAOT

A recent systematic review reported lack of reliable evidence to support effectiveness of remineralizing agents but a number of clinical trials show that routine dental home care there is improvement and also that micro-abrasion appears to be effective (Chen et al., 2013). Another systematic

review of treatment of WSLs following FAOT found seven studies with 11 evaluations, using PubMed and Research Triangle Institute/University of North Carolina Evidence Based Practice Center Criteria. Results showed that professionally applied F in high-dose and low frequency and at home use of low-dose, high frequency F is justified (Bergstrand and Twetman, 2003). An *ex-vivo* study showed that daily use of a neutral 0.2% NaF rinse may decrease the surface area but will not inhibit WSLs whereas a F (0.6% F-) MW with low pH of 1.91 managed to inhibit the lesions, hence they proposed that high concentrations of F will arrest the lesion but prevent complete repair (Ogaard et al., 1988). A clinical trial compared placebo F-rinse and TP to use of 50ppm F rinse twice daily and placebo TP (Willmot, 2004). Interestingly both groups showed a reduction ( $p > 0.05$ , 2-side t-test) by half in the lesions' size at 26 weeks raising questions about the role of F altogether since the control group appear to have no exposure to F at all.

Application of 18% hydrochloric acid and pumice abrasion (Croll and Cavanaugh, 1986) for treatment of WSLs showed a reduction in the WSL area by 83% on digital photographs. It is likely that the sample was biased, because the majority of eligible patients declined participation (42/50 or 84%) and 7/8 volunteers were females (Murphy et al., 2007).

Many clinical studies have investigated Tooth Mousse or casein phosphopeptide-amorphous calcium phosphate complexes (CCP-ACP®). One study reported significant improvement in the visual clinical scores, with almost three times ( $p < .01$ , chi-square) as many WSLs improving in the group using Tooth Mousse twice daily for three months compared to a group using once daily F rinse for six months. The examiners were not blinded to the intervention therefore it is possible that there was bias especially since a subjective method of assessment was employed (Ardu et al., 2007).

All other clinical studies found no difference when Tooth Mousse was compared to F MW and WSL were assessed with a light fluorescence method (DIAGNOdent®) (Andersson et al., 2007). Similar results when Tooth Mousse was compared to a placebo (Bailey et al., 2009) or to F TP (Brochner et al., 2011) or to TP containing calcium (Adriaens et al., 1990). All studies used light fluorescence methods to assess WSL except for one study which used ICDAS II criteria (Bailey et al., 2009). Micro-abrasion performed better in a clinical study (Fornell et al., 2002b) and in an *in-vitro* study on bovine enamel (Nazir et al., 2011).

Another paste (Enamelon®) was compared to TB in a non-randomised clinical study and after three months there was a significant decrease ( $p < .01$ , covariate analysis) in the WSL area measured with a dental probe (Kleber et al., 1999).

Other studies have found no significant differences between oral hygiene instruction given every three months compared to professional tooth cleaning ( $p = .087$ , three way ANOVA) (Aljehani et al., 2006) and bleaching to weekly F gel applications ( $p > .05$ , Mann-Whitney U test) (Knosel et al., 2007). Bleaching resulted in a more uniform look to the enamel surface and all participants appeared to be satisfied with the appearance but 30% of them reported hypersensitivity.

In 18 teeth with WSLs, a resin infiltration technique was used and photographs taken after one week showed that 11 of the 18 teeth were completely masked and only one tooth remained unchanged. Colour differences between sound enamel and WSLs showed a significant decrease ( $p < .05$ , Wilcoxon signed rank test) (Kim et al., 2011).

F has been identified as playing a significant role in the treatment of WSLs however recent studies use it as control in order to investigate new products and/or methods. A reduction in the area of WSLs is a common finding without a significant difference between test and control groups. Understandably it may be disappointing for a new product/method not to be effective but equally these results strengthen the role of F when tested in the control group. The only exception that showed significant results was the use of Enamelon® TP and Nd-YAG laser combined with an APF solution. However, there was no control group hence their effectiveness should be interpreted with caution. When subjective clinical indices are combined with a lack of blinding then bias is likely to be introduced into the study, decreasing validity.

## **2.9 Restoration of teeth following FAOT**

Data on restorative care are limited even though there is much less subjectivity in diagnosing cavities as opposed to diagnosing WSLs and the majority of indices have a corresponding score for cavities. This lack of data may be because clinicians will stop treatment before a cavity develops or even before they fear that a cavity might develop. In the latest literature review on prevalence of WSL there is no report of any index on restorative care (Mitchell, 1992b). In recent studies restorative care is rarely reported

but results range from zero to 4.8% (Mitchell, 1992a) as seen in Table 2-5. Possibly the only study where restorative care was clearly reported as a primary outcome two years after completion of FAOT 5% (n=19/370) of teeth with WSLs had restorative work (Mattousch et al., 2007).



**Table 2-5 Teeth most commonly affected by WSLs as reported in the literature published from 1992 onwards.**

<b>Study</b>	<b>No/group of examined teeth (as reported in study)</b>	<b>WSL (%) teeth</b>	<b>Teeth (%) in need of restorative care post-FAOT</b>
<b>(Mitchell, 1992a)</b>	124	19	4.8
<b>(Boyd, 1992) (paste)</b>	All incisors, canines, premolars and first molars	14.4	0.9
<b>(Boyd, 1992) (paste &amp; rinse)</b>	All incisors, canines, premolars and first molars	10.1	0.8
<b>(Turner, 1993)</b>	82	25	0
<b>(Boyd, 1993)</b>	All erupted teeth	14.4	2.3
<b>(Boyd, 1993)</b>	All erupted teeth	10.1	1.0
<b>(Boyd and Rose, 1994)</b>	All erupted teeth	14.4	0.9
<b>(Banks and Richmond, 1994)</b>	1182	31	n/a
<b>(Tebbett, 1995)</b>	All erupted teeth	n/a	n/a
<b>(Kindelan, 1995)</b>	977	9.8	n=0
<b>(Trimpenneers and Dermaut, 1996)</b>	417	12.7	n/a
<b>(Banks et al., 1997)</b>	737 (366 experimental and 371 control teeth)	13.5	n/a

<b>(Kindelan, 1997)</b>	902	4.2	n=0
<b>(Marcusson et al., 1997)</b>	80	29	n/a
<b>(Marini et al., 1999)</b>	Molars and upper incisors	1 tooth	1 tooth
<b>(Millett et al., 1999)</b>	120	72.4	1 tooth
<b>(Gaworski et al., 1999)</b>	149. Incisors, canines and premolars	75	n/a
<b>(Zimmer, 1999)</b>	All bracketed teeth	9.8	n/a
<b>(Millett et al., 2000)</b>	157	26	1.3
<b>(Alexander and Ripa, 2000)</b>	All erupted teeth	3.2	0.2
<b>(Banks et al., 2000)</b>	740	26	n/a
<b>(Mattick et al., 2001)</b>	63	n/a	n/a
<b>(Ogaard et al., 2001)</b>	Upper and lower incisors, canines, premolars and first molars	88	n/a
<b>(Fornell et al., 2002a)</b>	216	7.4	n/a
<b>(Le et al., 2003)</b>	47 anterior teeth	76.8	n/a
<b>(Elaut and Wehrbein, 2004),</b>	212 upper anterior teeth	54.7	n/a

<b>(Boersma et al., 2005),</b>	Upper and lower incisors, canines, premolars and first molars	30	n/a
<b>(Ogaard et al., 2006)</b>	282	7.2	n/a
<b>(Stecksen-Blicks et al., 2007)</b>	2419 surfaces. Upper and lower incisors, canines and premolars	n/a	in graphs
<b>(Heinig and Hartmann, 2008)</b>	n/a	9.18	1.76
<b>(Kronenberg et al., 2009)</b>	Incisors, canines and premolars	1.9	n/a
<b>(Benham et al., 2009)</b>	618. All incisors and canines.	7.11	n/a
<b>(Chapman et al., 2010)</b>	2656. Upper incisors, canines and premolars.	36	n/a
<b>(Shungin et al., 2010)</b>	236 (120 at 12 years). Upper laterals and lower canines.	Sum areas	n/a
<b>(van der Veen et al., 2010)</b>	All bracketed surfaces	20.7	n/a

### 3 Prevention of WSLs

#### 3.1 Systematic reviews of clinical trials for prevention of WSLs

The long-standing problem of WSLs in orthodontic patients has attracted many systematic reviews. Following the first review in 2001 (Bader et al., 2001) the first Cochrane Systematic review published (Benson et al., 2004) had only one study in common in both reviews (Hirschfield, 1978). Another systematic review in the same year (Derks et al., 2004) had one study in common with the Cochrane review and none with the 2001 review. A review published in 2005 (Chadwick et al., 2005) had one study in common with the 2004 Cochrane review, two studies in common with the 2004 review by Derks and two other studies in common with the 2001 review. No single study was included in all four reviews as seen in Table 3-1.

The latest updated systematic review published by the Cochrane Collaboration in 2013 identified three studies and n=458 participants all published from 2005 onwards hence they would not have been included in any of the previous systematic reviews (Benson et al., 2013). The study with low risk of bias showed moderate evidence that F varnish (Fluor Protector® 0.1%F) applied every six weeks resulted in an almost 70% reduction in incidence of WSLs and number needed to treat of 5.5 (Stecksen-Blicks et al., 2007). The study with high risk of bias due to large number of volunteers drop-outs showed no statistically significant difference on number of WSLs between use of a SRFGDs or a daily F mouth-rinse (225ppmF) (Luther et al., 2005). The third study had an unclear risk of bias and reported a statistically significant mean increase in the WSL index used when two mouth-rinses were used daily. The amine fluoride/stannous fluoride mouth-rinse (140ppmF, pH 4.5) group performed better compare to the sodium fluoride mouth-rinse group (250ppmF) (Ogaard et al., 2006). It appears that there is no agreement even between systematic reviews as to which is an effective method of preventing WSLs in orthodontic patients. There are several possible reasons why this might be as each systematic review is discussed in detail.

There are different levels to test effectiveness of any given method. Firstly, statistically there should be a significant difference in favour of the test group. Secondly, and probably more importantly, the question whether this difference in numbers mirrors an equally important significant clinical difference. Thirdly, cost-effectiveness of the method should ideally be

investigated as it is an important factor that could potentially prohibit use of the method. With the exception of a few studies that will be discussed later the clinical significance of reported differences and cost-effectiveness are rarely documented.

**Table 3-1 Systematic reviews of trials on prevention of WSLs and their included studies.**

(Bader et al., 2001)	(Benson et al., 2004)	(Derks et al., 2004)	(Chadwick et al., 2005)	(Benson et al., 2013)
(Hirschfield, 1978)	(Hirschfield, 1978)	(Banks et al., 1997)	(Hirschfield, 1978)	(Luther et al., 2005)
(Ullsfoss et al., 1994)	(Ogaard et al., 1986)	(Alexander and Ripa, 2000)	(Alexander and Ripa, 2000)	(Ogaard et al., 2006)
(Buyukyilmaz et al., 1994)	(Banks et al., 2000)	(Lundstrom and Krasse, 1987)	(Boyd, 1992)	(Stecksen-Blicks et al., 2007)
(Boyd, 1993)	(Czochrowska et al., 1998)	(Twetman et al., 1995)	(Boyd, 1993)	
(Holmen et al., 1987a)	(Gillgrass et al., 2001)	(D'Agostino et al., 1988)	(D'Agostino et al., 1988)	
(Ogaard et al., 1996)	(Gorton and Featherstone, 2003)	(Mitchell, 1992a)	(Denes and Gabris, 1991)	
(Lundstrom et al., 1980)	(Marcusson et al., 1997)	(Marcusson et al., 1997)		
	(Ogaard et al., 2001)	(Millett et al., 1999)		
	(Pascotto et al., 2004)	(Turner, 1993)		
	(Twetman et al., 1997)	(Trimpeneers and Dermaut, 1996, Turner, 1993)		
	(Dyer and Shannon, 1982)	(Wenderoth et al., 1999)		
	(Sonis and Snell, 1989)	(Fornell et al., 2002a)		

The 2001 review was based on guidelines from the Research Triangle Institute – University of North Carolina Evidence-Based Practice Center (Bader et al., 2001). Methods of prevention and the arrest of progression of non-cavitated dental lesions were investigated. The target group was caries active or high caries risk individuals but not necessarily patients undergoing FAOT. They identified 22 studies and seven of them, using 11 methods were evaluated separately as they all investigated prevention in orthodontic patients. The primary question was “...*efficiency of methods to reduce incidence of caries in teeth with orthodontic bands...*” In general the evidence for efficacy was characterized as insufficient due to small sample sizes and small number of studies per method. They computed a quality score based on twelve elements such as blinding, sample size, study type *etc*; each element had a given weight in the calculation of the quality score. Based on this assessment the mean quality score of the identified studies was 57/100 (range 25-80). A statistically significant difference was found when any of the following methods were employed: F in any of the following formats: titanium tetra-F solution (TiF<sub>4</sub>); APF and NaF rinse; NaF varnish; SnF<sub>2</sub> gel; also plaque removal by prophylaxis or a combination of NaF and a CHX rinse were proven to significantly reduce mean lesion depth. However the authors judged the evidence for efficacy to be insufficient for any given method.

The Cochrane Systematic review published three years later, (Benson et al., 2004) concluded that daily use of 0.05% NaF (225ppmF) can reduce the severity of WSLs whereas use of GIC for bonding can reduce both the severity and prevalence. They identified 15 trials which fulfilled most of their criteria. The methods tested in these trials were F (varnish or rinse as NaF, SnF<sub>2</sub>, acid phosphate F); CHX varnish; F elastomeric ligatures and GIC and/or RM-GIC bonding material.

A systematic review based on PubMed and Medline databases only, examined studies published from 1970 onwards on methods used to prevent caries during FAOT. The prevented fraction (PF) and standard error (SE) were used to assess efficacy of methods. Preventive fraction is an index less sensitive to the experimental circumstances *e.g.* age of patients, duration of the study and has the following formula:  $PF = \frac{\text{Incidence (control)} - \text{Incidence (experiment)}}{\text{Incidence (control)}}$ . Their aim was to perform a meta-analysis but it was not possible due to lack of data homogeneity and insufficient data to calculate 95% Confidence Intervals therefore a systematic review was undertaken. They identified 15 studies with 16 interventions, grouped into four groups. The F group showed no statistically significant difference but

showed positive results for use of 1,500/5,000ppm F TP and 5,000ppm F gel. The group of CHX showed again positive results and included either 40% varnish or 1% preparation placed in mouth-trays either alone or combined with 1% thymol. The enamel sealants group showed no positive results and the bonding agent group showed an overall PF of 20% for use of GIC with a SE of 9% in seven studies but the PF was not deemed statistically significant even though they do not explain how they reached that conclusion. Surprisingly no studies with use of F mouth rinse were included and the results are quite different to the Cochrane Systematic Review published in the same year (Derks et al., 2004).

A systematic review based on guidelines published by the Centre for Reviews and Dissemination at the University of York in U.K. (Chadwick et al., 2005) identified seven studies with six trials investigating rinses (APF, amine F, NaF), TP (1,500 ppmF) or gel (amine F, SnF<sub>2</sub>, NaF). They concluded that topical F, in addition to use of F TP, reduced the incidence of WSLs both in fluoridated and non-fluoridated areas. No specific recommendations were made as no method was superior to any other although high-potency preparations might offer benefits. Their question was prevention of incidence of WSLs and the outcome variable would be severity of WSL, DMFT or DMFS. They also used the preventive fraction however it was not possible to accurately calculate it due to lack of data. Even though they contacted the authors of the included studies, a measure of variance and the variance of the differences were not available to be able to calculate PF. They also highlighted the importance of developing guidelines on reporting results of clinical trials in order to provide material for future use in a systematic review and/or meta-analysis.

The latest literature review into the prevention of WSL highlighted the importance of patient education and oral hygiene practices whereas methods of F administration (water fluoridation, use of TP, MWs, gels, varnishes, within orthodontic bonding agents or in elastomeric modules and ligature ties) have been reported to be effective. They also report on CCP-ACP® (casein phosphopeptide-amorphous calcium phosphate in the form of sugar-free chewing gums (Recaldent®), mints (Recaldent Mints®), topical gel (Tooth Mousse) which has also shown promising results. They report that there is a dose-related increase in enamel remineralisation within already demineralised lesions however their ability if any to prevent WSL has not been proven yet (Sudjalim et al., 2006). This is the latest review on



the subject however it was not a systematic review so the findings have not been filtered through strict inclusion/exclusion criteria.

Systematic reviews have very strict and rigid inclusion and exclusion criteria for the studies they review. This may result in very few studies that would meet the criteria and a rather disappointing conclusion where “...no studies were found to meet all the inclusion criteria...” Secondly, the outcome variables may also differ between reviews investigating different things. One review may have as a primary variable incidence of WSLs whereas another one may have reduction of surface area of WSLs, however both reviews are investigating methods to prevent WSLs but in different ways. These variables may not be directly comparable and if not enough data is provided in the results e.g. confidence intervals then it is not possible to perform any statistical comparisons or use other indices like the preventive fraction discussed earlier. It appears that even though the systematic reviews have covered the same topic and have assessed the same or similar literature, their findings differ because their questions/outcome variables were slightly different. Each systematic review should be assessed on its own merit and their findings evaluated accordingly. Even though there was no agreement on a specific method, F appears in all systematic reviews as an efficient method in preventing WSLs during FAOT. Other methods identified were plaque removal, CHX rinse combined with NaF rinse and use of GIC for bonding.

### **3.2 Preventing WSLs under orthodontic bands**

The mechanics of the FAOT need the edge of the orthodontic wire to pass through a metal tube which can be either welded onto bands or attached directly *i.e.* bonded, onto the tooth. A Cochrane systematic review (Millett et al., 2011) (Millett et al., 2011) identified only one study with better results for prevention of WSLs when bands were used with GIC compared to tubes bonded directly with CR (Nazir et al., 2011).

### **3.3 Clinical trials in prevention of WSLs**

One of the first clinical split-mouth studies was published in 1952 but FAOT was very different then with bands used on all teeth whilst F was not routinely used. Nevertheless, a single application of a chloroform based

varnish (Copalite) prevented WSLs assessed visually after six months (Meyers, 1952).

### **3.4 Testing SnF<sub>2</sub> in clinical trials for prevention of WSLs**

Studies published in the 1970's and 1980's investigating the potential of SnF<sub>2</sub> had no statistical analysis and subjects were not randomised. Results were favourable for daily use of a 0.4% SnF<sub>2</sub> gel throughout FAOT which lasted from 18 to 24 months (Stratemann and Shannon, 1974). The same protocol was followed by n=78 subjects with banded teeth showing again favourable results (Shannon and West, 1979).

Combining 0.4% SnF<sub>2</sub> solution with APF solution (0.31% F) also showed favourable results for banded teeth (Magness et al., 1979). Even one year after band removal results showed that only two subjects and three surfaces in the MFP group developed WSLs but there were no lesions in the SnF<sub>2</sub> group (Dyer and Shannon, 1982).

In the 1990's studies employed statistical analysis but randomisation was used randomly. There was no difference between the groups testing 0.05% NaF (225ppmF) rinse once daily alone or in combination with 0.4% SnF<sub>2</sub> gel applied twice daily ( $p = .06$ , ANOVA) but both of them were significantly better than the control group using 1,100ppm F TP twice daily ( $p < .05$ , ANOVA). Conclusion was that probably F TP alone is not adequate to effectively prevent WSL (Boyd, 1993). In a follow up paper both Plaque ( $p < .01$ , ANOVA) and Gingival Index ( $p < .001$ , ANOVA) were also significantly better for the SnF<sub>2</sub> gel group (Boyd, 1994). Elastomeric chains with and without SnF<sub>2</sub> were replaced every four to six weeks during FAOT (mean 1.7 years  $\pm$  6 months) in n=94 subjects, following a sample size calculation of n=40 subjects per group. One examiner used the Enamel Decalcification Index (Banks and Richmond, 1994) and results showed significantly less WSLs ( $p < .001$ , Chi-square test) in the F chain group at subject level (63% Vs 73%) and tooth level (16% Vs 26%). There was no randomization of the subjects hence there is bias but there were minimum drop outs (6/94) and good follow up of the subjects; a cost analysis would also provide more information on the overall effectiveness of the method (Banks et al., 2000).

Even though there is a number of papers published supporting use of 0.4% SnF<sub>2</sub> as gel, solution or elastomeric chains tested in clinical trial with duration ranging from 14 months (Magness et al., 1979) to 24 months (Boyd,

1993). Factors which could introduce bias to the studies hence interpretation of the results should be treated with caution is the lack of randomisation, the lack of statistical analysis in the studies published in the 70's and 80's and in cases use of indices made and used by the authors for the purposes of one study only with no report on examiner's reproducibility.

### **3.5 Testing chlorhexidine in clinical trials for prevention of WSLs**

Many clinical studies have tested CHX applied either as a varnish, gel or solution during course of FAOT. Results yielded no statistically significant difference in caries/WSL assessed by Koch index (Koch et al., 1979, Lundstrom and Krasse, 1987) or using Gorelick index (Ogaard et al., 1997, Ogaard et al., 2001) or reporting D3/4MFS (Jenatschke et al., 2001). There was statistically significant but not clinically significant reduction of salivary *mutans streptococci* counts (Ogaard et al., 1997) when 1% CHX and 1% thymol varnish (Cervitec®) was additionally applied to 0.7% F varnish (Fluor protector®). Significantly less DMFS was reported in favour of Cervitec® varnish application after one year. However the split-mouth design of the study indicates that there may have been an overlap effect between the test and placebo varnish (Madlena et al., 2000).

### **3.6 Testing enamel sealants in clinical trials for prevention of WSLs**

The idea that sealing the enamel would protect against WSLs showed when tested *in-vitro* that under the microscope there were small isolated areas representing "breaks" in the sealant (Frazier et al., 1996).

Compared to frequent applications of an enamel sealant every three months (Fornell et al., 2002b), it seems that the single application provides some protection against demineralisation (Heinig and Hartmann, 2008). The risk of enamel breaks that could induce WSLs over the length of FAOT warrants further investigation to decide on the cost-effectiveness of this method. To this end when a primer was compared to an enamel sealant in order to save chair time the results favoured time consuming application of enamel sealant (Ghiz et al., 2009).

### **3.7 Other methods tested in clinical trials for prevention of WSLs**

A number of different methods to prevent WSLs have been tested clinically. For some the results were statistically significantly different to control groups favouring use of xylitol (Rekola, 1986), electric tooth-brushes (Boyd and Rose, 1994), F elastomeric modules (Mattick et al., 2001), combined use of Cervitec-Fluor Protector varnish (Kronenberg et al., 2009) and lingual brackets (van der Veen et al., 2010).

Other studies found non-statistically significant differences testing argon laser for CR light curing (Anderson et al., 2002) and F slow release intraoral devices (Marini et al., 1999). Identification of subjects at high caries risk appeared to be an explanatory variable for presence of WSLs in two studies from the same group of authors with non-random allocation of subjects into groups (Zimmer, 1999, Zimmer and Rottwinkel, 2004). Similar findings were reported in a retrospective study assessing DMFS and not specifically WSLs (Karadas et al., 2011).

Interesting point to note is the increased risk of bias in some of these studies mainly due to lack of randomisation (Rekola, 1986, Marini et al., 1999, Zimmer, 1999, Zimmer and Rottwinkel, 2004). Non-validated indices were developed and used for the purpose of a single study (Boyd and Rose, 1994), subjective assessment of caries risk (Zimmer, 1999, Zimmer and Rottwinkel, 2004) and split mouth design that could possibly favour one intervention also increase risk of bias (van der Veen et al., 2010, Mattick et al., 2001). When objective methods based on light fluorescence were used there was no good agreement with the clinical examination therefore results need to be carefully interpreted. For example QLF™ showed poor agreement with clinical examination and DIAGNOdent did not diagnose any WSLs that were diagnosed clinically (Kronenberg et al., 2009).

The plethora of methods tested highlights the fact that the problem of preventing WSLs has still not been addressed effectively and needs further investigation.

### **3.8 Testing F-materials in clinical trials for prevention of WSLs**

Majority of split-mouth studies comparing various F-releasing bonding materials failed to detect a significant difference (Mitchell, 1992a, Turner, 1993, Millett et al., 1999, Gaworski et al., 1999, Paschos et al., 2009).

Studies seen in Table 3-2, favour use of F-CR compared to CR (Sonis and Snell, 1989, Trimpeneers and Dermaut, 1996) or diacrylate (Unite) compared to GIC (Marcusson et al., 1997) or CR compared to a compomer (Millett et al., 2000). One study found difference on a subject level with twice as many patients developing WSLs when a chemically cured CR (Lee®) was used (14/23 subjects) compared to a light cured CR with F (Orthon®) (5/19 subjects) (Trimpeneers and Dermaut, 1996).

**Table 3-2 In-vivo studies investigating prevention of WSLs by testing F- CR bonding materials.**

Study	No of subjects	No of teeth	Design	Duration (months)	Assessment	Result – WSLs	Result – Bond strength
(Sonis and Snell, 1989)	22	412	split-mouth	25	Visual exam	CR 12.6%	No SSD
			F-CR Vs CR		Curzon index (0-3)	F-CR 0%*	
(Trimpeneers and Dermaut, 1996)	50	836	split-mouth	9-33	Visual exam	No SSD	n/a
			F-CR Vs CR				

There seems to be no consensus whether different F releasing bonding materials do actually prevent WSLs compared to CR bonding materials but there will always be similar studies published as new materials enter the market. When bonding materials are tested prevention and/or arrest of WSLs would always be a secondary variable. The primary aim of these materials is to show adequate bond strength to serve their purpose; that is to bond brackets onto human dental enamel.

### 3.9 Testing F-rinse in clinical trials for prevention of WSLs

Almost all studies agree that use of F rinse reduces severity/prevalence of WSLs depending on the level of compliance. However, few studies report differences between products (Hirschfield, 1978, Geiger et al., 1992, Boyd, 1992). When studies follow their participants for the duration of the FAOT, it

mirrors clinical reality. As discussed before, randomisation plays a crucial role in clinical studies to avoid bias and this is a point where some studies show real weakness (Geiger et al., 1992, Boyd, 1992).

### **3.10 Testing F-gels and/or varnishes in clinical trials for prevention of WSLs**

Application of Fluor Protector varnish (0.1% F) every six weeks compared to a placebo varnish was tested in a double-blind randomised controlled clinical trial in two hospitals over a period of at least six months (6-30 months). Two examiners used an index (Gorelick et al., 1982) to examine digital photographs. Statistically significant higher incidence (25.7%-7.4%) ( $p < .001$ , Wilcoxon test) and progression (2.6-0.8) of WSL ( $p < .001$ , Wilcoxon test) was reported for the placebo group. The absolute relative risk (ARR) was 18% and number to treat (NNT=1/ARR) was 5.5 *i.e.* five subjects to be treated to obtain one patient free of WSL which indicates a not cost-effective method (Stecksen-Blicks et al., 2007). This is one of the few studies investigating a method in depth to provide a comprehensive answer with regards to effectiveness.

### **3.11 SRFGDs in orthodontic patients**

In order to achieve the topical action of F *i.e.* present as a free ion in the plaque-enamel interface so as to effectively prevent caries (Fejerskov et al., 1981), devices that release F slowly but continuously in the mouth were developed both in the U.S.A. and in Leeds, U.K. This method was targeted at people in high risk caries groups where compliance with TB and use of F products was a problem.

The copolymer membrane F releasing device that has been developed in the U.S.A. contains an inner-core of hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) copolymer in a 50/50 mixture that contains a precise amount of sodium F. The core is surrounded by a HEMA/MMA 30/70 mixture copolymer membrane which controls the rate of F release. This can vary between 0.02 and 1.0 mg F/day. The duration of F release has been estimated to last from 30-180 days (Mirth et al., 1982, Toumba and Curzon, 1993).

A study published in 1999 (Marini et al., 1999) tested the efficacy of a copolymer F releasing device using customized holders and releasing 0.04mg/day of F has been tested in n=76 patients undergoing FAOT for

12 months, with some of them living in a fluoridated area (0.12 ppmF) and with no intervention for the control group. No information was provided regarding any use of F and/or oral hygiene practices during the study. No carious and/or early enamel lesions developed in the devices group whereas in the control group with no devices, 2/23 subjects developed caries, one requiring restorative care and another developed a WSL. There was no significant difference between the two groups for Plaque Index, Gingival Index or bleeding on probing. The presence or absence of WSLs was assessed by visual clinical examination with no use of any index and no report of reproducibility of the method. One out of 53 devices was detached with no adverse reactions reported. They also reported that the F in the whole saliva (determined using an ion-specific F electrode) increased from 0.05µg/ml to 0.46µg/ml. However these numbers are not reflected on the corresponding graph and also the available data available on the graph go as far as 200 days (6.6 months) *i.e.* short of the 12 months duration of the study (Marini et al., 1999).

The SRFGD was developed in Leeds, West Yorkshire, U.K. and its' last form it is a glass bead attached to a plastic bracket. It contains 13.3% F in the form of sodium fluoride (NaF). There are four different relative solubility (1, 3, 16 and 50) types depending on the rate of F release. A randomized double blind controlled clinical trial in n=174 children showed that the SRFGD can elevate salivary F levels to 0.11ppm compared to a placebo group whose levels were 0.03ppm after two years (Toumba and Curzon, 2005). The same study reported a statistically significant reduction in caries increment in the test group (n=31 children who retained the SRFGD for two years) associated with 67% fewer carious teeth, 76% fewer carious surfaces and 55% fewer occlusal carious surfaces in primary and permanent dentition. This was the only study included in a Cochrane systematic review (Bonner et al., 2006) however the evidence was considered to be clinically weak because statistical analysis excluded 52% of participants who had lost the SRFGD during the trial and since the salivary F levels should be 0.02ppm F to prevent dental decay (Duckworth and Morgan, 1991).

The SRFGDs has also been tested in patients undergoing FAOT in a pilot study (Tobin, 2001) that showed positive results for the reduction in severity and incidence of WSLs. The interim report provides data for 21/70 subjects that were randomly allocated to SRFGD or no device, all living in non-fluoridated area and using 1,100-1,450 ppmF tooth paste twice daily and 225 ppmF mouth rinse once daily. WSLs were examined by one

examiner using cross-polarizing photographs at the start and end of the study. Presence/absence of lesions and white spot area in mm<sup>2</sup> were assessed using computer image analysis software for the upper anterior teeth. An error study was performed to test reproducibility of the image analysis method and 20% of the photos were randomly selected and re-examined by the same examiner with results showing borderline acceptability. For the incidence of WSLs there was an overall increase of 15% (19% in the control group and 8% in the test group) with no statistical testing. Lateral incisors were more frequently affected (58%). The control group demonstrated a statistically significant increase in the surface area compared to the test group ( $p < .001$ , paired t-test). However, the difference in the mean change of the surface area between the two groups was not statistically significant ( $p = .007$ , Student's t-test). The interim report also showed that the design of the holder for the device had to be changed since there were 19 breakages of the device from the metallic eyelet in eight patients (Tobin, 2001).

Intraoral devices that slowly and continuously release F have been developed and tested for a number of years with two studies investigating orthodontic patients. One study appears to have a heterogeneous sample with some subjects exposed to systemic F in drinking water. Results appear to favour the device based on clinical examination. The pilot work cannot provide any definitive conclusions but the interim report suggests a positive indication in favour of the SRFGD but also highlighted the need for a change in the design of the device.

### **3.12 Testing plaque and saliva in studies investigating prevention of WSLs**

Since plaque and saliva have an important role in the caries process - saliva being the medium to transport minerals in the oral environment and the presence of plaque being a prerequisite for caries development-it was only logical that studies would investigate possible ways to influence these vehicles so as to prevent the development of WSLs.

#### **3.12.1 Testing plaque**

Plaque presence was analysed with image analysis software and was evident around brackets and near the gum level in  $n=52$  subjects, with 37%



of them having plaque present in over 50% of the dentition (Kuklowska et al., 2011).

The potential of GIC materials to release F and its' proximity to the areas where WSLs develop resulted in a number of studies investigating the effect on plaque.

In a split-mouth clinical study a GIC (Aqua-Cem, DeTrey®) and a CR (Concise, 3M®) bonding material were compared in n=12 children who lived in a low F area. The children were asked to use daily 225ppmF rinse. 48h old plaque samples were obtained at three, eight, 28 days and six months. Results showed significantly lower ( $p < .01$ , student's paired t-test) plaque levels of *mutans streptococci* in the GIC group on all sampling occasions, hence it could act as long term F releasing reservoir (Hallgren et al., 1993).

One study investigated WSLs alongside *mutans streptococci* counts for six months (Twetman et al., 1995). WSLs were found in 6% (n=11) of teeth and there was no difference between test and control group (Twetman et al., 1995). Significant differences were found only after one week ( $p < .01$ , ANOVA) and one month ( $p < .05$ , ANOVA) for *mutans streptococci*.

Only one study found a significant difference in the long term favouring use of GIC after six months (Hallgren et al., 1993), other studies found statistically significant differences mainly during the first month (Twetman et al., 1995, Wright et al., 1996, Pellegrini et al., 2009, Jose et al., 2013).

A chair-side method called rapid adenosine-triphosphate (ATP) - driven bioluminescence assay was used to compare plaque bacteria adjacent to self-ligating and elastomeric-ligating brackets at one and five weeks after bonding in a split-mouth clinical study in n=14 patients. Less plaque bacteria were found by the self-ligating brackets and the difference was statistically significant ( $p < .05$ , paired t-test). The method showed excellent correlation coefficients (r) to findings assessed with the gold standard by obtaining plaque samples diluted in phosphate buffered saline and plated on enriched blood agar (r was 0.895 for total oral bacteria and 0.843 for total oral *streptococci*), therefore could be used for future studies (Pellegrini et al., 2009). Unfortunately however, no other study has been found using the same chair-side method to be able to follow up their findings.

The most recent clinical study showed significantly lower *mutans streptococci* counts in plaque in 60 patients who used either a probiotic TP or curd for 30 days (Jose et al., 2013).

### 3.12.1.1 Testing saliva

One study investigated salivary changes and reported only a minor improvement in prevention of DMFS. Results cannot justify routine clinical use of the tested varnish containing 40% CHX which was compared to a placebo varnish and was applied every eight weeks in n=33 subjects during their FAOT (median duration 21 months) (Jenatschke et al., 2001).

### 3.12.1.2 Testing saliva and plaque

The effect on both saliva and plaque would provide a more complete picture of intraoral changes and the potential to prevent WSLs. The only significant decrease for plaque *mutans streptococci* in a group exposed once to F varnish (Fluor Protector 0.7% F-) and Cervitec varnish (1% CHX & 1% thymol) during six months of FAOT ( $p < .01$ , t-test) occurred 12 weeks after bonding (Ogaard et al., 1997).

When a single varnish (Cervitec - 1%CHX & 1% thymol) was compared to a placebo varnish, both applied every three months for one year in alternate quadrants in n=24 subjects living in a 0.1ppmF area. Results showed that the Cervitec group had significantly less DMFS ( $p < .05$ , Student's paired t-test) whereas in plaque only *mutans streptococci* counts were significantly less (Madlena et al., 2000). This cross-over design may not be the ideal design for such a protocol because there does not seem to be a wash-out period between treatments and the duration of the effect of treatment is unknown. As a result, there may be an overlap of the effect of one treatment over the other.

Comparing two bonding materials, a GIC (Fuji Ortho) or CR (Concise) following one application of 0.4% SnF<sub>2</sub> showed no effect in plaque 30 days after bonding but a significant reduction of *mutans streptococci* in saliva ( $p = .638$ , paired t-test). Results showed that the antimicrobial activity of GIC occurred only in the initial phase and had no long-term activity (Mota et al., 2008).

With the exception of the self-ligating brackets assessed with a new chair-side method (Pellegrini et al., 2009) all other methods tested showed

no significant long term decrease in bacterial counts in plaque or saliva. More promising results were found when Cervitec was used for six months (Hallgren et al., 1993) or a year (Madlena et al., 2000) with significant decreases in *mutans streptococci* occurring but only in plaque.

### 3.13 *Ex-vivo* Studies

#### 3.13.1 *Ex-vivo* studies investigating arrest of WSLs

A plaque retaining band was introduced in the 1980's to promote caries formation within four weeks (Arneberg et al., 1984). The first study using this band showed 80% reduction in mineral loss when 0.2% NaF rinse was used in such lesions examined with MR and micro-densitometry (Ogaard et al., 1986). The same band was placed in four premolars for up to one month with one premolar extracted every week; SEM showed that functional wear and TB arrested the lesions by disturbance and removal of bacterial deposits rather than incorporation of F (Holmen et al., 1987b). A later study confirmed the formation of WSLs within four weeks under SEM examination (Melrose et al., 1996).

The same model was used to compare daily F rinse to combined use of F and CHX rinse in n=14 premolars for one month. MR showed significantly better results for lesion depth, mineral loss, plaque and saliva counts for lactobacillus and streptococcus mutans for the combination group. A possible explanation for their findings is that CHX acts on streptococcus mutans and has a long lasting effect on plaque acid formation whereas F alone cannot repair mineral loss at a very low pH (Ullsfoss et al., 1994).

The daily use of 0.2% NaF (900ppmF) rinse in five subjects was compared to a control group with no intervention, all having banded premolars scheduled for extraction after four weeks. MR showed a reduction by 80% for mineral loss ( $p<.05$ , t-test) and by a factor of 3 for lesion depth, highlighting the ability of F to quickly remineralize WSLs in poorly accessible areas. However, the small sample size doesn't provide us with conclusive findings (Ogaard et al., 1986).

Studies used QLF™ and TMR on artificial lesions created on enamel specimens with a low ratio of mineral loss to lesion depth, by leaving these specimens for two 24h periods in a partially saturated acetic acid solution. These lesions further demineralized when brushed twice daily with 1,100ppm F TP for a month, whereas high ratio lesions showed

remineralization. This model showed that there seems to be a dose response to F however this needs to be ideally tested on natural WSLs (Lippert et al., 2011).

Formation of WSLs by using an orthodontic band provided a study setting that was considered close enough to a real life scenario, enabling assessment of the efficacy of various protocols to remineralize *i.e.* arrest/repair/treat WSLs already present *in-vivo*. These studies show not only that TB may have a more important role but also that there seems to be a dose response in remineralizing such lesions. F appears to have a key role in the process - either as a MW or TP.

### **3.13.2 *Ex-vivo* studies investigating prevention of WSLs**

During the 1970's, some studies followed groups of children throughout their FAOT whereas other studies examined WSLs on premolars scheduled for extraction and as a result had a shorter duration which ranged from one to six or seven weeks (Landry and Shannon, 1973) with the majority lasting for four weeks (Ogaard et al., 1986, Holmen et al., 1987a, O'Reilly and Featherstone, 1987, Buyukyilmaz et al., 1994, Ullsfoss et al., 1994, Melrose et al., 1996, Chung et al., 1998, Czochrowska et al., 1998, Gorton and Featherstone, 2003, Pascotto et al., 2004, de Moura et al., 2006, Gontijo et al., 2007). A few studies lasted longer; from six to 13 weeks (Twetman et al., 1997), eight weeks (Underwood et al., 1989), three months (Farhadian et al., 2008) or even six months (Chatzistavrou et al., 2010).

A frequently quoted study reported that mineral loss assessed by MH and localized in an area 50 to 75µm beyond the periphery of the bracket could develop *in-vivo* within four weeks, even if clinically the teeth appear to be sound. Prevention was more effective when 0.05% NaF MW was used daily in combination with 1,100ppm F TP (O'Reilly and Featherstone, 1987).

The caries model with bands cemented onto premolars scheduled for extraction but leaving 2-3mm of space for plaque accumulation was used in 20 subjects over a nine week period (Arneberg et al., 1984). QLF™ showed that brushing with a 5,000ppm F TP with no rinsing prevented significantly more WSLs compared to a control group using 1,450ppm F TP ( $p < .005$ , unpaired t-test) (Al-Mulla et al., 2010).

Studies with a single application of F varnish show a significant difference ( $p < .05$ , Mann-Whitney U test) in lesion depth, mineral loss (Buyukyilmaz et

al., 1994) and higher calcium and F content ( $p < .05$ , Wilcoxon test) in extracted premolars examined with MR and X-Ray spectrometry even in subjects living in a fluoridated area (.7ppm F) (Gontijo et al., 2007). A number of such studies favoured GIC materials by taking advantage of the initial “burst” effect of F release therefore results should be interpreted with caution for their long term effectiveness.

Many studies have found that various GIC products performed significantly better compared to different CR products for both lesion depth ( $p = .024$ , Wilcoxon signed rank test) (Czochrowska et al., 1998) and mineral loss (Czochrowska et al., 1998, Gorton and Featherstone, 2003, Pascotto et al., 2004). WSLs were tested with both MR and MH. Similar results ( $p = .016$ , Sign test) were found even when WSLs were assessed on photographs by one calibrated examiner using a study made index (Chung et al., 1998).

In the studies with longer duration, the results fail to favour the GIC and showed no statistically significant differences from control groups who had CR. Two studies investigated bonded premolars scheduled for extraction. The first split-mouth study showed no difference ( $p > .05$ , Wilcoxon test) between GIC (Aqua-Cem, De Tray®) and CR (Concise®) when premolars were examined with stereomicroscope after 6-13 weeks (Twetman et al., 1997). The other split-mouth study testing CR (Transbond XT, 3M Unitek®) and GIC (Fuji I GC®), showed after six months higher F- concentrations ( $p < .05$ , 2-way ANOVA) on premolars bonded with GIC both in the outer and deeper enamel surface originating from the cement particles and not ionic uptake from the oral environment (Chatzistavrou et al., 2010). One study with premolars banded with either GIC or zinc phosphate cement and extracted after three months failed to show a statistically significant difference ( $p < .05$ , Student’s t-test) in the F and  $Ca^{++}$  concentration (Akkaya et al., 1996). It appears that the initial “burst” effect of F release from GIC materials reaches a plateau in these longer duration studies but at least short term GIC used to bond brackets on premolars offers protection against WSL. Question remains whether this protection is available long term especially since it is not common practice for orthodontists to bond with GIC.

### **3.13.3 Other methods tested *ex-vivo* for prevention of WSLs**

Novel protocols preventing WSLs that have been investigated over the years include an experimental F-exchanging agent that was compared to CR over 2 months under PLM. Forty bonded premolars scheduled for extraction in

ten subjects were tested. Results showed a 93% reduction ( $p < .05$ , ANOVA and Student-Newman-Keuls test) of occurrence only for dark zones in the test group (Underwood et al., 1989). No follow up study was found on this experimental agent.

A triclosan TP with 1,100ppmF was investigated in a split-mouth study where premolars were bonded with plaque accumulating brackets. There was no effect ( $p > .05$ , Tukey test) in the RM-GIC group but it performed significantly better ( $p < .05$ , Tukey test) in the CR group for both depth and area of demineralisation (de Moura et al., 2006). No other study was found investigating the same TP.

Significantly harder human enamel was found at 20 $\mu$ m depth when premolars were exposed to F elastomers continuously for one month (Wilson and Love, 1995). Another study tested a single five minute exposure to either CPP-ACP or NaF gel provided statistically significant protection against demineralisation ( $p < .001$ , Kruskal Wallis rank test) compared to a control group after two months in  $n=21$  subjects with no difference between test groups. No subjects had any exposure to any other F source for 11 weeks in total (Uysal et al., 2010a). In a follow up study they compared CR with a new CR containing CPP-ACP in  $n=14$  patients. Premolars were extracted after 30 days and MH showed statistically significant and favourable results for the new material at 10 $\mu$ m distance from the buccal enamel surface ( $p < .001$ , Tukey post hoc). However, the subjects lived in a fluoridated area and didn't use any extra F for seven weeks (Uysal et al., 2010b).

These studies show that F was the main method investigated in various ways either alone or incorporated into GIC or combined with other methods. It is important to note the overall exposure to F in these studies, as some volunteers lived in fluoridated areas but didn't use any F products during the study period. These are situations that do not mimic a real life scenario where it would be difficult if not unethical to ask volunteers not to use any F products for the duration of the FAOT.

#### **3.13.4 Summary of *Ex-vivo* studies**

There is a plethora of *ex-vivo* studies examining premolars scheduled for extraction after being exposed to real clinical conditions. The finding that WSLs can develop within four weeks even if they are not visible clinically dictates the minimum test period for any such protocol (O'Reilly and

Featherstone, 1987). Introduction of a plaque retaining band may help development of WSLs but it is questionable whether it reflects reality (Arneberg et al., 1984). Nevertheless this model has provided valuable information especially when testing different bonding materials. However, the main critical disadvantage is that the duration of these studies is just a fraction of the duration of the FAOT and this factor needs to be taken into consideration as short term studies would favour materials which initially have an increased F release such as GIC.

### **3.13.5 Difference between *ex-vivo* and *in-situ* studies**

In *ex-vivo* studies the experiment is done on a tooth in the mouth but the measurements are done in the lab. This is a significant difference from *in-situ* studies where the experiment and the measurement are done outside of the mouth. Common practice is to attach dental tissue onto a removable appliance hosted in the mouth to mimic clinical conditions.

### **3.14 *In-situ* studies**

A few *in-situ* models have been used to test various protocols either to arrest WSLs.

In terms of arresting WSLs a cross-over study in n=15 subjects who used twice daily F TP and once daily F rinse, had a pair of enamel slabs with pre-formed WSLs placed bilaterally on an arch wire in the lower arch. The control slab had an orthodontic bracket attached. After 52 days TMR results showed a statistically significant ( $p=.006$ , one-way ANOVA) increase in remineralisation for the non-bracketed sample (Benson et al., 1999). Time was not correlated to any parameters of the lesions. However, this doesn't agree with an earlier study which found an approximately linear relationship for bands left *in-situ* (O'Reilly and Featherstone, 1987). The main difference however is that in the recent study the question is not prevention or arrest of development of a WSL but regression of a lesion already developed and located underneath a bracket.

The same model was used in n=12 individuals undergoing FAOT to test elastic ligatures with or without F, replaced for an average of 15 times during the two experimental periods (6 weeks each) with participants using 1,055ppmF TP. TMR images were quantified by computerized image analysis but results showed no significant difference ( $p=.0376$ , one way

ANOVA) between the two interventions or the control group (Doherty et al., 2002). In contrast with their earlier study (Benson et al., 1999) there was a trend towards more mineral loss during the study period. This was explained as possibly due to the higher mineral loss at baseline (Doherty et al., 2002).

A recent *in-situ* cross-over study compared GIC to F varnish on remineralisation of artificial WSLs on human enamel slabs with orthodontic brackets bonded on posterior teeth in six volunteers. Results from PLM showed a significant difference ( $p < .05$ , unknown statistical test) favouring GIC possibly due to the initial “burst” effect of F release during the 30 days study period (Trairatvorakul et al., 2010).

In terms of preventing WSLs, bovine enamel blocks have also been used as test specimens. Brackets were ligated with elastomeric rings or stainless steel wire, placed palatally onto removable appliances dipped eight times daily into a 20% sucrose solution. The four volunteers lived in a fluoridated area (0.6-0.8 ppm F) and used 1,000ppm F TP for two weeks, three times daily. Results of this pilot study showed no difference between the groups in their microbiological profile ( $p > .05$ , Wilcoxon paired test) and percentage mineral volume ( $p > .05$ , ANOVA) assessed by cross sectional MH (Gameiro et al., 2009).

It is important to note that some studies (Benson et al., 1999, Gameiro et al., 2009, Trairatvorakul et al., 2010) have investigated remineralisation of already established artificial WSLs hence they investigate arrest/repair of WSLs whereas one study investigated prevention of WSLs using bovine enamel slabs (Gameiro et al., 2009).

### **3.15 *In-vitro* studies**

#### **3.15.1 WSLs investigated on bovine enamel**

Bearing in mind the differences between bovine and human dental enamel not only in dental morphology but also in chemical composition, crystal structure and physical properties *i.e.* refractive indices (Yassen et al., 2011), results cannot be directly translated into clinical practice. Only one study used bonded bovine incisors and light microscopy and image analysis showed 38% less mean lesion depth ( $p < .01$ , Student's t-test) for Duraflor® F varnish after been exposed to a cariogenic solution for 35 days (Demito et al., 2004).



### 3.15.2 Investigating *in-vitro* enamel sealants in preventing WSLs

Many *in-vitro* studies favour use of enamel sealants compared to other materials especially in studies with longer duration. The surface area of WSLs appears to be directly related to the time premolars are left in demineralizing solution and sealant materials appear to offer protection ( $p < .05$ , Chi-square test) against demineralisation (Hughes et al., 1979). These results were confirmed by an identical study from the same group of authors (Younis et al., 1979). A highly filled enamel sealant (Pro Seal, Reliance, Orthodontic products®) showed statistically significantly less demineralisation ( $p < .05$ , Newman-Keuls test), compared to etched enamel, F varnish application and an unfilled enamel sealant. The enamel specimens were tested with micro-hardness (MH), after 14 days of pH-cycling and TB (Hu and Featherstone, 2005). The same material and protocol was tested with QLF™ and CFLM on premolars showing significantly ( $p < .05$ , Kruskal-Wallis test) less lesion depth (Behnan et al., 2010). “Breaks” have been found in sealed slabs when left in a cariogenic solution for 95 hours (Frazier et al., 1996). Other *in-vitro* studies failed to find positive results ( $p = .621$ , Chi-square test) when enamel sealant was applied on the bracket periphery of extracted premolars left for 10 days in a demineralizing solution (Farrow et al., 2007) or when enamel sealants were compared to a control CR ( $p < .05$ ) (Tecco et al., 2008).

### 3.15.3 Investigating *in-vitro* F-releasing materials in preventing WSLs

The plethora of bonding materials and their proximity to the area where WSLs develop have prompted many studies investigating their preventative efficiency. Traditional CR materials have been tested (Basdra et al., 1996, Vorhies et al., 1998, Todd et al., 1999) alongside GIC (Glasspoole et al., 2001) and RM-GIC (Schmit et al., 2002, Paschos et al., 2009) which already have a good record of studies showing F release hence great potential to prevent dental caries. Application of F varnish also enhanced remineralization mainly for CR (Kindelan, 1996). Recent studies have also tested CCP-ACP® materials *i.e.* Tooth Mousse (Sudjalim et al., 2007, Uysal et al., 2010a). F rinses (225 and 50ppm F) were compared to a placebo solution as a daily 5min dipping solution for a period of up to 30 days in extracted molars left in demineralising solution for two weeks. Clinical photographs were compared with quantitative microradiography and results showed that the 50ppm F performed significantly better (Linton, 1996).

However all these studies investigated remineralisation of artificially induced WSLs hence they do not test prevention but arrest or treatment of WSLs.

The only study investigating prevention of WSLs showed that bonding materials exposed to F varnish (Vanish 3M®) or resin-sealer (Pro-seal, Reliance, Orthodontic products®) significantly resisted demineralization ( $p < .05$ , Kruskal-Wallis test) assessed with QLF™ and CLSM after 15 days of pH cycling (Behnan et al., 2010).

It appears that bonding materials, especially CR, are enhanced by F application in preventing WSLs. It is difficult to distinguish between the effects of a single F application or of the bonding material especially in *in-vitro* studies with short duration as there is not enough time for the effect of the F to be “washed” out. Such studies though provide evidence for testing materials *in-vivo* in clinical trials.

#### **3.15.4 Non-F based preventive regimes for prevention of WSLs tested *in-vitro***

Laser application for 5 seconds resulted in a lower severity index for WSLs (Geiger index) on molars bonded with CR compared to visible light curing. In the same study there was no difference ( $p = .055$ , ANOVA) in surface area and lesion depth for WSLs assessed with PLM (Noel et al., 2003).

The role of different ligation methods was explored on premolars exposed for 5 weeks to a cariogenic biofilm model *i.e.* a cylinder with a two-organism (*Streptococcus mutans* and *Lactobacillus acidophilus*) continuous flow culture. Even though TMR showed no significant differences ( $p > .05$ , ANOVA) orthodontic brackets ligated with elastomeric rings tended to encourage more demineralisation compared to non-ligated or self-ligating counterparts (Amaechi et al., 2006). This highly cariogenic challenge failed to find a difference between groups in this short period of time and once again, the study could be criticised for not reflecting a more realistic clinical time period.

#### **3.15.5 Summary of *in-vitro* studies**

The *in-vitro* studies initially used destructive methods such as TMR where only a slice of a tooth is examined but more recently, light scattering methods such as QLF™ and DIAGNOdent have more frequently been used.

However, another problem has now occurred with CR itself generating fluorescence.

Recent studies included pH cycling with/without TB in the test protocol, trying to mimic oral conditions. Results seem to favour use of enamel sealants; however the risk of breaks within the sealant is always highlighted. F releasing materials also seem to perform better compared to CR bonding materials whose performance is enhanced by application of F varnishes/gel. One study has investigated the role of a solution with a low F concentration with promising results.

None of these short term studies assessed the cost effectiveness of their test method in the long term and again, use of clinical subjective indices without blinding introduces bias to the study; it is not uncommon to find significant differences when subjective clinical indices are used and the opposite results when more objective – but maybe destructive methods - are employed.

### **3.16 Testing of bonding materials**

Many studies have tested the efficacy of different materials in preventing and/or arresting WSLs. The primary purpose of these materials though is to have adequate bond strength to allow bonding of brackets onto human dental enamel throughout the course of FAOT. For this reason new materials should be tested for both outcomes if planned to serve a dual purpose. Comparing argon laser with light curing of a CR bonding material showed no significant differences in WSLs changes throughout FAOT, which lasted on average 14 months, but the bond failure rate was significantly higher in the light curing group (5.7%) compared to the control group (2.4%) (Elaut and Wehrbein, 2004). Comparison of two enamel sealants showed no difference in bond failure but the chemically cured sealant showed increased favourable changes WSLs by 13% (Banks and Richmond, 1994). The only study investigating banded first permanent molars showed better results for GIC (Ketac-Cem, ESPE®) for band failure whereas there was no significant difference in WSLs changes compared to CR (Band-Lok, Reliance®) (Gillgrass et al., 2001). When different materials were compared no significant differences were found in WSLs changes in any study. Bond failures though were significantly higher for cyanoacrylate (Smart Bond) compared to a CR (Light Bond®) (Le et al., 2003) and for a RM-GIC (Fuji II Ortho LC) compared to a CR (Light Bond Reliance®) (Gaworski et al.,

1999). No significant difference in bond failure was reported between a CR (Rely-a-Bond, Reliance®) and its' modified version with incorporated F (Banks et al., 1997).

Provided there is adequate bond strength and no difference in bond failure, prevention of WSLs was significantly better in only one study (Banks and Richmond, 1994) when two enamel sealants were tested. Another study reported no difference in bond failures and prevention of WSLs when two versions of the same CR were compared (Banks et al., 1997). Even though all these split-mouth studies followed subjects throughout the course of FAOT no comparisons can be made due to different methodologies.

Systematic reviews identified use of GIC bonding materials which offer the advantage of releasing F- (Derks et al., 2004, Benson et al., 2004). Still one of the main problems with GIC bonding materials is poor retention of orthodontic brackets (Cook and Youngson, 1988, Cook and Youngson, 1989, Klockowski et al., 1989, Cook et al., 1996, Ortendahl and Thilander, 1998). Studies show fairly consistently that CR materials have better bond strength both *in-vitro* and *in-vivo* (Pickett et al., 2001, Penido et al., 2009) compared to GIC (Ortendahl and Thilander, 1998) and/or RM-GIC (Cook et al., 1996, Reddy et al., 2003).

To overcome these problems F-CR materials have been investigated not only for their bond strength but for their F release as well. The small quantity of measurable F- in a CR, with the Transbond releasing  $0.00007 \mu\text{g}/\text{cm}^2/\text{day}$  (Cacciafesta et al., 2007), could be due to the presence of small amounts of F- containing glass in its dispersed inorganic phase. It may also be due to a constant F- reading being noted in the storage medium *e.g.* distilled water or due to TISAB in the test solution that frees F- bound to hydrogen and is recorded by the F- specific electrode ( $0.1 \mu\text{g}/\text{cm}^2/\text{six months}$ ) (McNeill et al., 2001).

Results for F release from F-CR materials *in-vitro* can be seen in Table 3-3. All studies used the F electrode and concluded that no clinical effect is to be expected due to small amount of F release, ranging from 0.42ppm (Bishara et al., 1991) after 40 days to  $212\mu\text{g}$  after 20 weeks (Chadwick and Gordon, 1995).

**Table 3-3 Studies assessing F release from F-CR materials.**

Study	Method (N=number)	Materials	Duration (weeks)	Storage medium	F release from F-CR
(Fox, 1990)	N=10 specimens 10x10x1mm	F-CR Vs GIC Vs CR	20	De-ionised water	Cumulative F F-CR=68µg
(Chan et al., 1990)	N=40 human molars	F-CR Vs chemically cured CR	6	Water	No figures given
(Bishara et al., 1991)	N=40 human teeth	F-CR Vs chemically cured CR	6	De-ionised	Day 43=0.42ppm
(Ghani et al., 1994)	N=48 premolars	Two F-CRs	1	De- mineralizing solution	No figures given
(van Rensburg and Wiltshire, 1994)	N=40 discs	Two F-CRs	28	Distilled water at 37°C	No figures given
(Chadwick and Gordon, 1995)	N=5/group 10x10x1mm specimens	RMGIC Vs F-CR	20	De-ionised water	212µg
(Basdra et al., 1996)	N= 5 discs 5.3x0.8mm	F-enamel sealants Vs CR	12	Distilled water	Plateau within 14 days (.019-.023 mg/L)
(Trimpeneers and Dermaut, 1996)	N=5 discs 13x1.2mm	Four F-CR Vs GIC	72	Double- distilled water &.1mol/L NaCl	No figures given

#### 4 Aim of the study

With regards to prevention of WSLs even though there is a plethora of studies available, the systematic reviews that have been published have conflicting results and a meta-analysis was not possible due to lack of data. Nevertheless the latest four systematic reviews all identified F as playing an important role in preventing WSLs but the method of F application has not been agreed. It is interesting to note that even though the systematic reviews were published from 2001 to 2005 they identified different studies that report significant findings. The 2001 review identified F, plaque removal by prophylaxis or combined use of NaF and CHX rinse (Bader et al., 2001). The Cochrane review identified use of daily F MW (225ppm) or use of GIC as a bonding material (Benson et al., 2004). The 2004 review identified use of 1,500/5,000ppm F TP, 5,000ppm F gel or use of CHX (Derks et al., 2004). Another systematic review published in 2005 concluded that topical F in addition to use of fluoridated TP, reduced the incidence of WSLs commenting that high-potency preparations might offer benefits (Chadwick et al., 2005). The latest Cochrane systematic review identified moderate evidence favour application of F varnish every six weeks during FAOT (Benson et al., 2013)

The long standing problem of WSLs during FAOT has been investigated for many years by *in-vitro*, *in-situ*, *ex-vivo* and *in-vivo* in clinical trials during the course of orthodontic treatment, testing various protocols and applying different assessment methods. Initially there was no consensus with regard to the scale of the problem with a wide range of prevalence/incidence reported (Mitchell, 1992b). It appears that the standard preventive methods of using F TP and F MW are not adequate to address the problem with the issue of compliance having been frequently highlighted. The scale of the need for restorative care appears to be underreported although it is a critical outcome following FAOT and an important variable in terms of cost effectiveness of any given method to prevent WSLs.

However, although the methodology is still an issue, there is little doubt that F plays a role in preventing WSLs. Bearing in mind that the FAOT may take two years or more to be completed, any given method should not only be effective in the long-term but ideally would need to be cost-effective as well *i.e.* avoid extra visits or use of costly materials and/or use of auxiliary staff that would increase the cost of treatment and/or prolong appointment time.

Compliance of the patient is also paramount for any method to be successful especially when the patient is a child and/or a young teenager. Bonding materials, especially F-CR, could be the answer to these problems since they combine anti-cariogenic properties and adequate bond strength. Two clinical split-mouth studies followed participants throughout their FAOT for a mean period of 21-25 months and support their use (Sonis and Snell, 1989, Trimpeneers and Dermaut, 1996).

For these reasons the aim of this study is to test the FSRGD not only because it is a method which has been shown to prevent caries in high caries risk children (Toumba and Curzon, 2005) but because it is based on long-term and continuous intra-oral release of F, which is the key factor in preventing caries and highly relevant to FAOT. It should also prove to be a cost-effective method because it is applied once either the introduction of the glass bead at the time of bonding and/or incorporation in the CR bonding material. Most importantly it does not rely on patient's compliance and there is pilot data to support a clinical trial (Tobin, 2001).

## **5 Objectives of the study**

The objectives were to compare SRFGDs and placebo devices in patients scheduled to have FAOT in a randomised, double-blind, controlled clinical trial. Buccal surfaces of the upper six anterior teeth (upper central, lateral incisors and canines) were examined with quantitative light fluorescence (QLF™) and with cross-polarising digital photographs analysed with image analysis software - Adobe Photoshop® (Adobe Systems Inc., California, USA). Photographs were taken on the day when fixed appliances were placed and following removal of fixed appliances and bonding material.

An exploratory study will investigate the F and PO<sub>4</sub> release of a composite resin material enriched with SRFGDs in the form of powder. The potential of caries prevention effect from F release of a bonding material needs to be investigated against the ability to display sufficient bond strength for orthodontic brackets to adhere to tooth enamel.



## **6 Null Hypotheses**

There is no difference in the incidence of WSLs in patients having SRFGDs compared to a placebo device, during their course of FAOT on the upper anterior teeth.

There is no difference in the severity of WSLs as assessed by the number of teeth with WSL(s) on each participant having SRFGDs compared to a placebo device, during their course of FAOT on the upper anterior teeth.

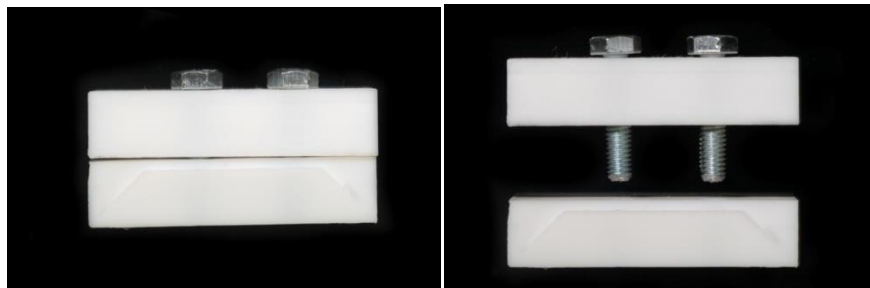
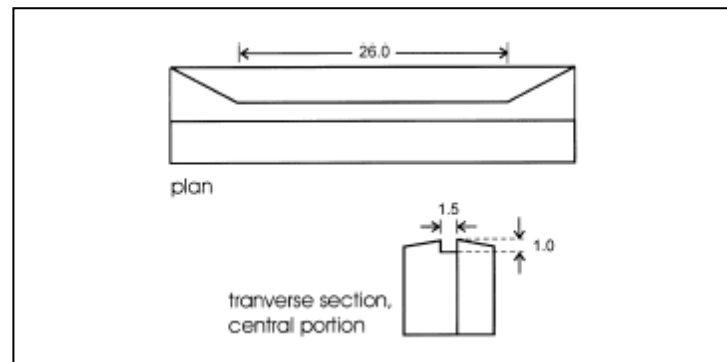
## 7 Materials and Methods

### 7.1 Materials

The following materials were used:

Test materials were prepared using moulds as described by (Musanje et al., 2001) in order to allow fabrication of specimens that comply with British Standard BS EN ISO 9917-1:2007. Bar specimens (26 X 1.5 X 1.0 mm<sup>3</sup>) of each material were fabricated in the mould made from poly-tetra-fluoro-ethene (PTFE) as seen in the following figure (Figure 7-1).

**Figure 7-1 Diagram and pictures of PTFE mould. From Musanje et al., 2001.**



Composite resin for bonding of orthodontic brackets (Transbond, 3M®)

Grinding of SRFGD glass (containing sodium 21.2%, phosphorus 20.7%, aluminium 6.8%, fluorine 19.5% and oxygen 31.9%) to powder of various particle sizes with steel dish (Gyro Mill) and fractionated using sieve stacks (30µm).

Composition of AS used as a storage medium (Leung, 1991) is seen in the following table (Table 7-1).

**Table 7-1 Composition of AS used as a storage medium.**

	/g dm <sup>-3</sup>	Moldm <sup>-3</sup>
Stock Solution A		
NaH <sub>2</sub> PO <sub>4</sub>	28.0	0.2333
KCl	86.8	1.164
NaCl	7.21	0.123
NH <sub>4</sub> Cl	11.0	0.205
Trisodium citrate 2H <sub>2</sub> O	1.1	3.74 X 10 <sup>-3</sup>
Lactic acid	3.5	0.039
Stock Solution B		
Urea	10.0	0.167
Uric acid	0.75	4.46 X 10 <sup>-3</sup>
NaOH	0.2	5.00 X 10 <sup>-3</sup>
Stock Solution C		
KSCN	12.0	0.123

A Fuji S3 Pro Fine Pic Macro Lense with a Sigma ring flash and cross polarising filter. To operate the camera F11 was used at 1:25 of the second and 1:4 ring flash speed.

Digital photographs were uploaded to a computer and analysed using the Adobe Photoshop® Software (Adobe Systems Inc California, USA).

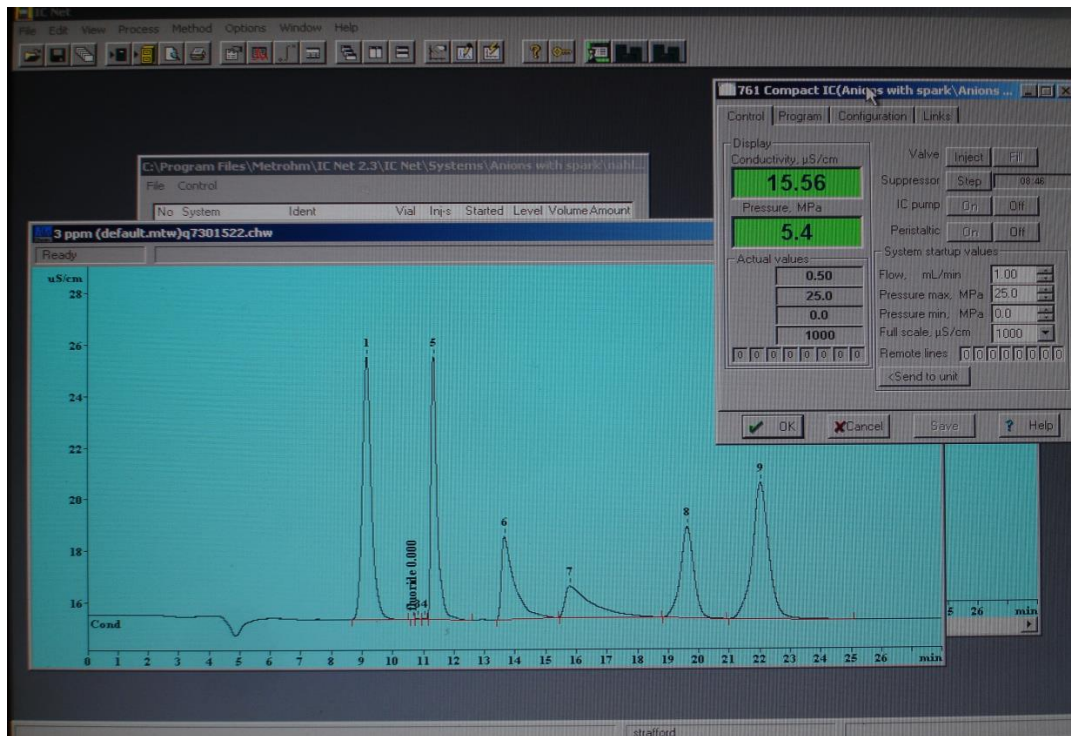
SRFGDs and a prototype plastic holder manufactured by Ultradent®<sup>1</sup>.

Ion Chromatography (761 Compact, Metrohm RP) seen in Figure 7-2 was used to assess ion release. For the suppressed analysis the Hamilton PRP x 110S 7µm 250X4.1mm column with the Metrohm RP Guard column was selected for analysing the F and PO<sub>4</sub> anion with a carbonate eluent (NaHCO<sub>3</sub> 1.7 mmol/l and Na<sub>2</sub>CO<sub>3</sub> 1.8 mmol/l) at 0.5ml/min flow rate. The y axis presents conductivity (uS/cm) and the x axis retention time (min). Note the F peak obtained at 9.3 min.

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<sup>1</sup>Ultradent Products Inc., Utah, USA.

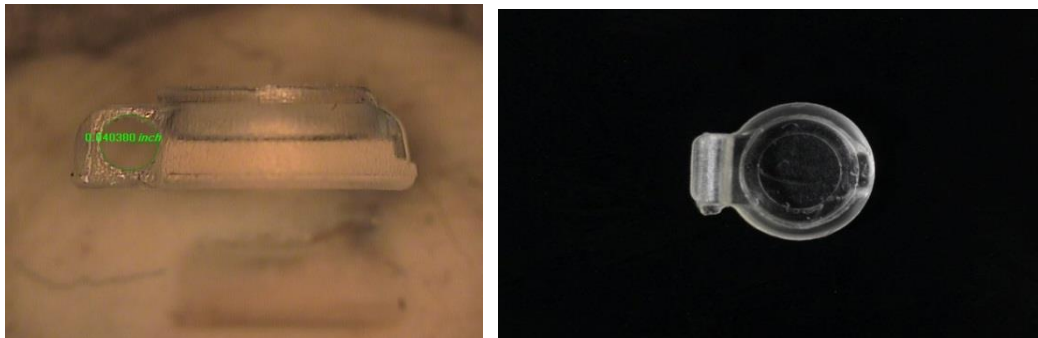
Figure 7-2 Ion chromatography and chromatogram demonstrating the F peak in a standard 1ppm F solution sample.



## 7.2 SRFGDs with plastic holder

The initial design provided by the manufacturer had a hole on one side for the orthodontic wire to be threaded as seen in Figure 7-3. When tested in the mouth the holder was lying above the occlusal plane thus interfering with the occlusion. The holder was tested in the lower arch as seen in but it was still interfering with the occlusion as seen in Figure 7-4. An adjustment was made and a metallic tube was attached on the posterior side of the plastic holder. Following this modification, the device was at the same level as the orthodontic brackets as seen in Figure 7-5. This meant it no longer interfered with the occlusion and was also unable to rotate around the orthodontic wire hence improving necessary retention and stability in the mouth in order to minimise the risk of breakage and/or loss of the device. The new design was discussed with the manufacturer and was adopted to provide a plastic holder with a hole on the posterior side as seen in to improve retention and avoid occlusal interference as seen in Figure 7-6 and Figure 7-7.

**Figure 7-3 Initial design of the plastic holder with the SRFGDs.**



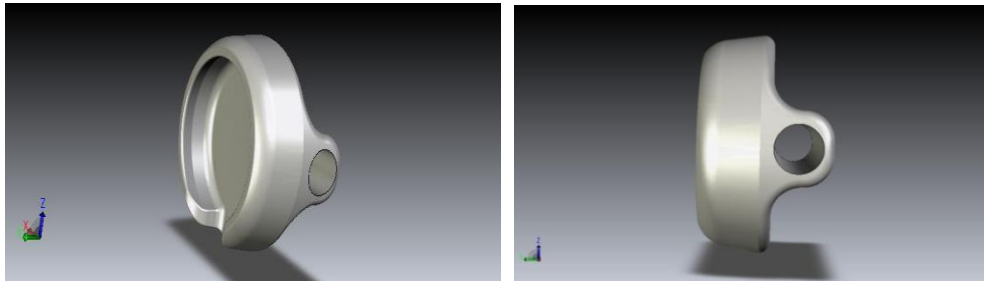
**Figure 7-4 Clinical view of the holder with SRFGD placed in the lower arch.**



**Figure 7-5 Clinical view of the holder with SRFGD placed in the upper arch after adhesion of metallic tube on the plastic holder.**



**Figure 7-6 Final design of holder as provided by the manufacturer.**



**Figure 7-7 Clinical view of the holder with SRFGD onto the orthodontic wire.**



### 7.3 Methods of investigation

This section will cover the following:

- *In-vitro* exploratory study
- Questionnaire to orthodontist-members of the British Orthodontic Society (BOS)
- Socio-economic status (SES) of eligible participants in clinical study
- Clinical study

### 7.4 *In-vitro* exploratory study

#### 7.4.1 Steps of the *in-vitro* study

Five different liquid storage mediums were assessed for their  $F^-$  and  $PO_4$  release using IC.

SRFGDs were ground into powder and sieve stacks were used in order to obtain powder with a known particle size.

Since solubility is affected by surface area, SRFGD with a given weight was compared to the same weight of powder with different particle sizes in order to obtain similar  $F^-$  and  $PO_4$  release to the SRFGD used as a control. Ion chromatography was used to measure concentration of  $F^-$  and  $(PO_4)^{3-}$ .

Morphology of powder with particle size that exhibited similar solubility to SFRGD was assessed using SEM.

In order to explore  $F^-$  and  $PO_4$  release different types of powder were mixed with the control material (Transbond®) at different ratios, giving 40 different combinations as seen in Table 7-2. All 40 samples were prepared using PTFE mould and stored in AS.

Transbond® was hand mixed with powder using spatula on a glass pad. The test material was placed in PTFE mould and covered with glass before been light cured.

Table 7-2 Different types of powder mixed with Transbond at different ratios.

	Solubility 1		Solubility 3		Solubility 16		Solubility 50	
	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m
Ratio 1:6	*<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m
Ratio 1:7	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m
Ratio 1:8	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m
Ratio 1:9	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m
Ratio 1:10	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m	<38 $\mu$ m	>38 $\mu$ m

\*particle size of <38 $\mu$ m or >38 $\mu$ m

Universal Testing Machine (UTM) (Lloyd LR10K) was used to test flexural modulus of elasticity and flexular stress of samples prepared in a PTFE mould to comply with British Standards EN ISO 9917-1:2007. These two measurements will allow assessment of the debonding force at a cross-head speed of .1mm/min and load cell of 20N. Depending on the surface area of the sample the debonding strength of the test material would be estimated.

For the purpose of the *in-vitro* study, AS was used as a storage medium in order to create an environment for the specimens close to intra-oral conditions (Leung and Darvell, 1997). The composition of AS used in this study is seen in Table 7-1 and was originally developed by (Darvell, 1978) based on human saliva analysis and improved by adding potassium (Leung, 1991). It has been extensively used in studies looking into mechanical behaviour of dental materials including glass-ionomer (Musanje et al., 2001, Musanie and Darvell, 2003, Musanie and Darvell, 2004).

Determination of phosphate shows degradation of the glass bead as it is the core ion of the glass. Test specimens and control specimens of the material were assessed to compare concentration for these ions. Ion Chromatography is considered the method of choice for analytical determination of free ions (Fritz, 2004). Two studies in dental research have used ion chromatography for the determination of F<sup>-</sup> in distilled or de-ionised water from dental materials *in-vitro* (McCabe et al., 2002, Itota et al., 2004).



## **7.5 Questionnaire to specialist orthodontist - members of the British Orthodontic Society.**

A questionnaire was emailed to all specialist orthodontist members of the British Orthodontic Society ([www.bos.org.uk](http://www.bos.org.uk)) to screen current clinical practice. The questionnaire can be found in the appendix (Appendix No 14).

## **7.6 Socio-Economic Status (SES) of eligible participants in clinical study**

The address/postcode of the eligible participants was used to identify their Multiple Deprivation Index (MDI) (McCabe et al., 2002) in order to identify significant differences between volunteers and those who declined participation.

## **7.7 Clinical study**

### **7.7.1 Study setting**

The study took place in the Orthodontic Department at the Leeds Dental Institute, in Leeds, West Yorkshire, U.K. and the author (CT) was responsible as the principal investigator for identification, recruitment, obtaining consent and follow-up of the participants according to the study protocol.

### **7.7.2 Study design**

The study design was a prospective, randomised, controlled and double-blind clinical trial using test and placebo devices with a follow-up period of at least one year. The treatment /intervention was placement of SRFGDs.

### **7.7.3 Ethics Committee Approval of the clinical study**

Approval from Leeds Central Research Ethics Committee, Leeds, UK was obtained for this study on 29/04/2008, Reference Number 08/H1313/6. An amendment of the protocol was also reviewed and approved by the same Research Ethics Committee on 10/6/2008. The amendment was an additional examination of teeth for signs of WLSs with a method called

“quantitative light-induced fluorescence” or “QLF™”, which is based on the auto-fluorescence of teeth. Approval from Research and Development Office at the Leeds General Infirmary, Leeds, West Yorkshire, U.K. was also obtained on 15/05/2008, Reference Number DT08/8473. The relevant documentation can be found in the appendix (Appendix No 1,2). Following ethical approval all clinicians in the orthodontic department at the Leeds Dental Institute were informed about the study via email; the protocol of the study was circulated and comments and questions were welcomed. Each clinician was contacted personally by email to establish whether patients routinely have any F application prior to FAOT; to identify at which stage the orthodontic brackets were removed in relation to their retention phase of treatment and by whom and when the bonding material was removed. This information was important because it provided a better insight into how clinicians work; it assessed the F exposure and confirmed the homogeneity of the study sample. In total, 23 members of staff were contacted; 12 consultants, seven senior specialist registrars and four specialist registrars.

#### **7.7.4 Outcome variables**

The primary outcome was: the incidence of WSLs on the buccal surfaces of the six upper anterior permanent teeth. Presence/absence of early enamel lesions was defined by visual examination of digital photographs and clinical examination. The type of variable: nominal.

The secondary outcome was: severity of WSLs on the buccal surfaces of six upper anterior permanent teeth. The surface area of the WSLs was drawn and expressed as a percentage of the total buccal tooth area using a computer software package to analyse digital photographs. The type of variable: metric continuous.

Satisfaction questionnaires were given to the participants and the orthodontists responsible for the FAOT of the participants at the last appointment for their FAOT. This documentation can also be found in the appendix (Appendix No 11,12). Type of variable: nominal.

Loss and/or breakage of the device were documented in the CRF form by the responsible clinician orthodontist. Type of variable: nominal.

In order to account for any confounding factors as identified in the literature the following data were collected: age, gender, address/postcode, DMFS/T, dmfs/t, duration of orthodontic treatment, Plaque Index (PI) and Gingival Index (GI).

### **7.7.5 Inclusion criteria**

The eligible participants had to meet the following inclusion criteria:

- Volunteers could be included up to 18 years of age at the start of the study.
- Volunteers should have no relevant medical history and should not be under any regular medication that was known to affect the oral cavity and oral flora status.
- Volunteers should have given consent to have FAOT.
- Volunteers should not be pregnant.
- The following permanent teeth had to be present in the upper dental arch: incisors and/or canines.
- Valid (informed) consent obtained from parent(s)/legal guardian(s) using a consent form approved by the Ethics Committee. All participants were below 18 years of age at the start of the study and were encouraged to sign, should they wish to do so, a consent form approved by the Ethics Committee.
- Exposed to water F levels of <.1 ppm F, considered to be the limit below which there is no protective effect against dental caries (ten Cate, 2001), in order to minimise any systemic exposure to F in the drinking water. This was established by contacting the local water provider - Yorkshire Water (<http://www.yorkshirewater.com>) and the British Fluoridation Society (<http://www.bfsweb.org/index.htm>).
- Willing to refrain from using any additional F products during the period of the study, other than standard adult F toothpaste (1,100-1,450ppm F) and F mouth-rinse (225 ppmF) but maintaining normal dietary habits.

### **7.7.6 Exclusion criteria**

Volunteers were excluded if any of the following applied:

- Volunteers who were older than 18 years of age at the start of the study.
- Volunteers with a relevant medical history and/or under regular medication known to affect the oral cavity and oral flora status.

- Volunteers who had not given consent to have FAOT.
- Pregnant female volunteers.
- The following permanent teeth were not present in the upper dental arch: incisors and/or canines.
- Exposed to water F levels of  $>.1$  ppmF, considered to be the limit above which there is a protective effect against dental caries (ten Cate, 2001), hence it would be impossible to differentiate whether protection (if any) against development of WSLs would have resulted from the devices or exposure to F in the drinking water.
- A signed, valid (informed) consent form was not obtained from the parent(s)/legal guardian(s) and/or participants.

### 7.7.7 Sample size

The level of significance for the study was set to .05 ( $\alpha=0.05$ ) and the power of the study to 90% ( $1-\beta=.90$ ). The number of participants was established by analysing data from previous, related studies (Artun and Brobakken, 1986, Ogaard, 1989) and a pilot study (Tobin, 2001) and using this data to support a formal sample size calculation formulated with the assistance and guidance of Mr Andrew Blance, Lecturer in Statistics and Mrs Theresa Munyombwe Lecturer in Biostatistics, Centre of Epidemiology and Biostatistics, Faculty of Medicine, University of Leeds, Leeds, West Yorkshire, U.K. These statisticians also provided guidance and assistance for the statistical analysis of this research project.

The sample size calculation was based on the primary outcome *i.e.* incidence of WSLs by assessing previous studies with related research questions. The first stage of the sample size calculation treated observations as independent and in the second stage accounted for clustering. The following steps were undertaken for the sample size calculation:

The clinically relevant significant difference was a 10% reduction in the number of teeth with WSLs.

We assessed available data for the six (6) teeth to be examined in this study and/or incidence of WSLs in teeth overall.

The sample size calculation was based on the difference in proportions in treatment and control groups assuming our observations were independent. The reference table 3.1 page 40 and equation 3.7 page 21, Chapter 3 were used from “Sample size tables for clinical studies” (Machin et al., 1997). The power was set at 90%;  $1-\beta=.90$  and  $\alpha$  (2-sided)=.05.

Since 6 teeth per subject were examined therefore our observations were not independent, we needed to account for the effect of clustering using the design effect *i.e.* to establish what increase in the sample size was required to account for the natural association between the teeth within the same individual. Data on a single tooth per individual would provide us with information only for this particular tooth. As there is no single tooth most commonly affected by WSLs during FAOT this approach allows us to investigate more teeth in fewer individuals but still taking into account the effect of clustering and increasing the sample size as appropriate (Thompson et al., 2012).

Design effect= $1+(m-1) \times ICC$ , where  $m$  is the size of our cluster ( $m=6$ ) and ICC (intra class correlation coefficient) is an estimate of the variation attributable to the cluster, as a proportion of the total variation, set to be quite small at .04.

$$Deff=1 + (m - 1) \times ICC = 1 + (6-1) \times ICC = 1 + 5 \times 0.04 = 1 + 0.2 = 1.2$$

The final calculation step was as follows:

Sample size=Sample size from tables $\times 1.2$ (design effect)/6(size of cluster).

The older study published in 1986 (Artun and Brobakken, 1986) was used as it had adequate follow up (1-1.8 years) and an adequate number of participants (180 subjects).

The percentage of teeth with WSLs in the corresponding groups was 5% and 16.9%.

Based on the findings from this study the sample size was estimated following the previously stated steps:

Stage one:

$$m = [Z_{1-\alpha} \sqrt{\{2\pi(1-\pi)\}} + Z_{1-\beta} \sqrt{\{\pi_1(1-\pi_1) + (\pi_2(1-\pi_2))\}}]^2 / \delta^2$$

m = sample size

$$Z_{1-\alpha} = 1.96$$

$\pi_1 = 0.05$  = success under treatment

$\pi_2 = 0.169$  = success under placebo

$$\pi = \pi_1 + \pi_2 / 2 = 0.05 + 0.169 / 2 = 0.1095$$

$$Z_{1-\beta} = 1.2816$$

$\delta = 0.169 - 0.05 = 0.119$  = anticipated increased proportion of successes

$$m = [1.96 \sqrt{\{(2 \times 0.1095)(1 - 0.1095)\}} + 1.2816 \sqrt{\{0.05(1 - 0.05) + 0.169(1 - 0.169)\}}]^2 / 0.119^2$$

$$m = [1.96 \sqrt{\{(2 \times 0.1095 \times 0.8905)\}} + 1.2816 \sqrt{\{(0.05 \times 0.95) + (0.169 \times 0.831)\}}]^2 / 0.014161$$

$$m = [1.96 \sqrt{0.1950195} + 1.2816 \sqrt{(0.0475 + 0.140439)}]^2 / 0.014161$$

$$m = [1.96 \times 0.4416101 + 1.2816 \sqrt{0.187939}]^2 / 0.014161$$

$$m = [0.8655557 + 1.2816 \times 0.4335193]^2 / 0.014161$$

$$m = [0.8655557 + 0.5555983]^2 / 0.014161$$

$$m = [1.421154]^2 / 0.014161 = 2.0196786 / 0.014161 = 142.62259$$

$$n = (2 \times SD^2 \times \text{magic number} / \text{difference in means}^2) + 1$$

Magic number is 10.5 for alpha =.05 and power 90%; 1- beta=.90

Based on the above equation and use of tables the sample size was

n=26 subjects/group

Stage two:

A sample size calculation was also undertaken for the secondary outcome based on the pilot work (Tobin, 2001) for the severity of WSLs by comparing means and using tables from the above mentioned reference and equations to compare means of independent groups. Based on the findings from this study the sample size was:

Sample size =

Sample size from tables x 1.2 (design effect) / 6 (size of cluster)

Sample size = 143 x 1.2 / 6 = 29 subjects/group

The risk of patients failing to complete their orthodontic treatment has been reported in the dental literature. Two hospital based studies which together investigated over 700 patients, reported rates of 17.6–19.5% over a four year period (Roberts et al., 1994, Trenouth, 2003). Based on these figures the number of participants was increased from 29 per group.

$$29 + 18\% = 34.22$$

$$29 + 20\% = 34.80$$

### **7.7.8 Allocation-Randomisation process**

Randomisation of participants into two groups with equal allocation was carried out by a statistician using S-Plus programme and 5984 random seed to provide a random number generated list. The code could be accessed by two supervisors only. The statistician provided us with a random list of numbers produced by a random number generator with equal allocation of participants based on their gender to placebo/treatment. A printed list was also available in clinic stating whether the volunteer was allocated to group A or B according to their gender and in numerical order e.g. first female allocated to group A. The principal investigator, the clinician orthodontists, the participants and the members of staff on clinic were blinded whether group A corresponded to treatment or placebo. The codes were kept in a sealed envelope in the office of two supervisors and in the master file and were revealed at the end of the study. Glass beads were kept in two separate plastic boxes named A or B by the responsible supervisors.

### **7.7.9 Steps of the clinical study**

Potential participants were identified through the computerised appointment booking system.

Information sheet for parents and potential participants were posted together with an invitation letter for participation in the study separately to their appointment letter. The documentation can be found in the appendix (Appendix No 4,5,6,7).

Potential participants, who expressed an interest in participating in the research study, when they attended for their scheduled orthodontic appointment, had a dental clinical examination to record their dental status and to assess if they fulfilled the inclusion criteria. On the day of the dental examination, the consent forms and the parent's and patient's information sheet were available for any questions to be answered and the potential participant together with their parent(s)/legal guardian(s) were given time until their next scheduled appointment for orthodontic treatment to decide on their participation in the study. A letter was to be posted to the volunteer's General Dental Practitioner. The documentation can be found in the appendix (Appendix No 8).

For each participant, cross-polarising digital photographs were taken on the day of placement of the fixed appliances and SRFGDs and following



completion of FAOT and on the day of removal of fixed appliances and bonding material. The SRFGDs rested in a plastic holder threaded onto the orthodontic wire and placed by the clinician orthodontist anterior to the last banded molar and posterior to the adjacent tooth (e.g. between 16 – 15 and 26 - 25, FDI notation).

The Case Record Form (CRF) of the participants was filled in to document any adverse event and to collect all relevant documentation for participants. All Case Record Forms together with the Master File of the research study with all relevant documents were kept on clinic in a location known to staff members of the Orthodontic Department. The documentation can be found in the appendix (Appendix No10).

#### **7.7.10 Data collection and management**

The following data were collected from the patient's dental notes in order to account for possible confounding factors for development of WSLs: age, gender, address/postcode, DMFS/T, dmfs/t, duration of FAOT, PI and GI.

Satisfaction questionnaires for participants and orthodontists were provided at the end of the study. It was piloted amongst members of staff before finalized and it can be found in the appendix (Appendix No11,12).

Loss and/or breakage of the device were documented in the CRF form by the responsible clinician orthodontist.

Three digital photographs were taken; one from the maxillary right canine and lateral incisor, one from the maxillary central incisors and one from the maxillary left lateral incisor and canine. The cross-polarising technique was used (Robertson and Toumba, 1999) with the same equipment and under the same conditions by photographers at the photography department at the Leeds Dental Institute, Leeds, UK. The photographers and the principal investigator were calibrated against each other by examining a photograph of artificial WSL on a tooth, using the same equipment on a premolar tooth with an artificial WSL on the buccal surface used as a prototype.

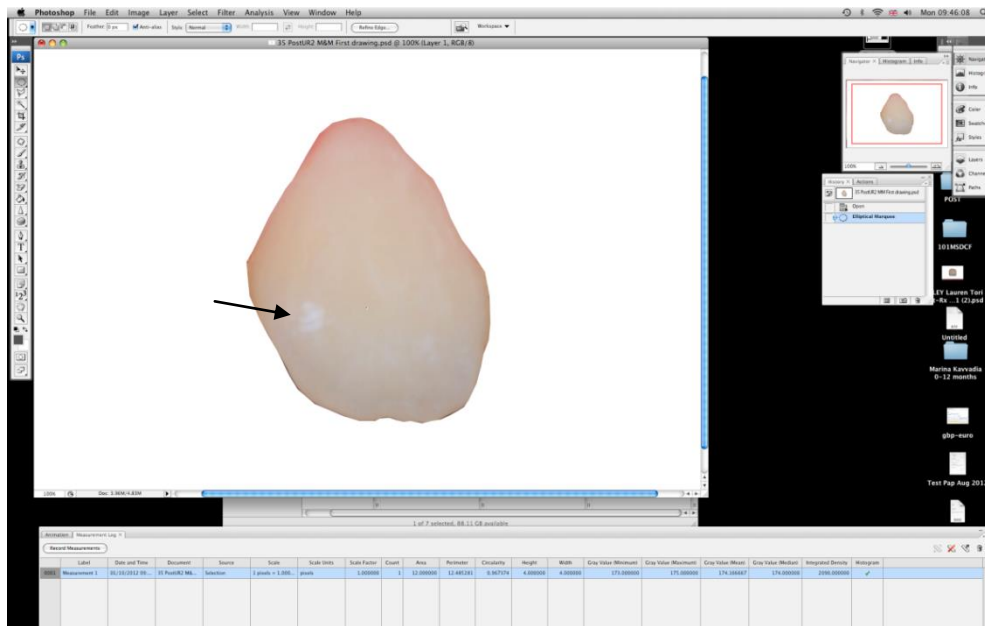
Presence/absence of WSLs was determined on the digital photographs loaded onto a computer. One examiner performed all assessments to improve the reproducibility of the procedure. Cohen Kappa scores were obtained by randomly re-examining 10% of the sample.

Presence/absence of WSLs was determined clinically using a hand-held QLF™. One examiner performed all assessments to improve reproducibility

of the procedure. Cohen Kappa scores were obtained by randomly re-examining 10% of the sample.

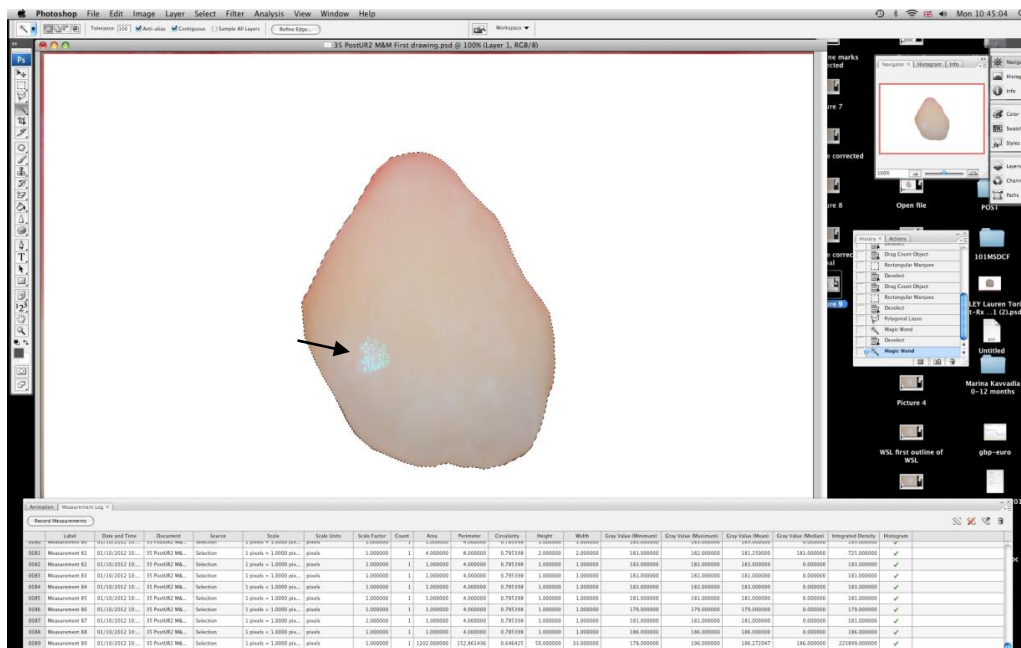
Severity of WSLs was determined by measuring the surface area of the lesion using Adobe Photoshop® Software (Adobe Systems Inc California, USA). The digital images were saved as JPEGs (Joint Photographic Experts Group) files. The computer monitor screen resolution was set at 1920 x 1200 and colour resolution at 32-bit true colour. The outline of the buccal surface of the teeth was drawn freehand using the magnetic lasso tool and was cropped of gingival tissue and surrounding teeth and stored as a new JPEG image as seen in Figure 7-8. A unique code number was given prior to analysis (Kanthathas et al., 2005a).

Figure 7-8 Tooth outline with arrow denoting the WSL.



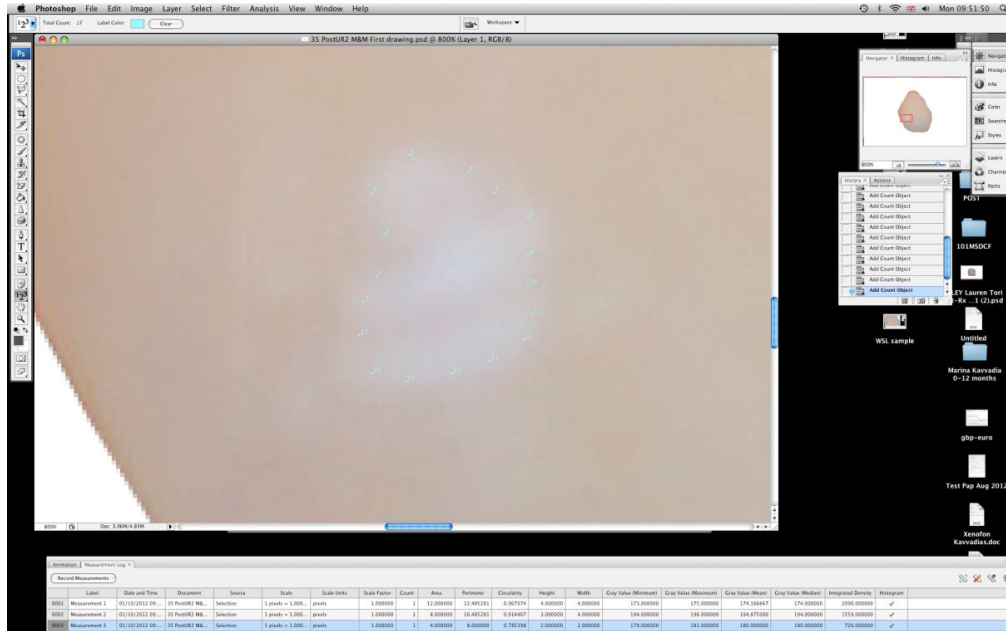
The number of pixels within the WSL and the buccal surface were recorded as seen in Figure 7-9. WSL area was defined as a percentage of the total labial surface *i.e.*  $WSL\% = (\text{Area of the lesion} / \text{Area of the tooth}) \times 100$  following the method used in a previous study (Kanthathas et al., 2005a). One examiner performed all the assessments in order to improve the reproducibility of the procedure. Cohen Kappa scores were obtained by re-examining 10% of the sample.

Figure 7-9 Tooth area and WSL area outline with arrow denoting the WSL.

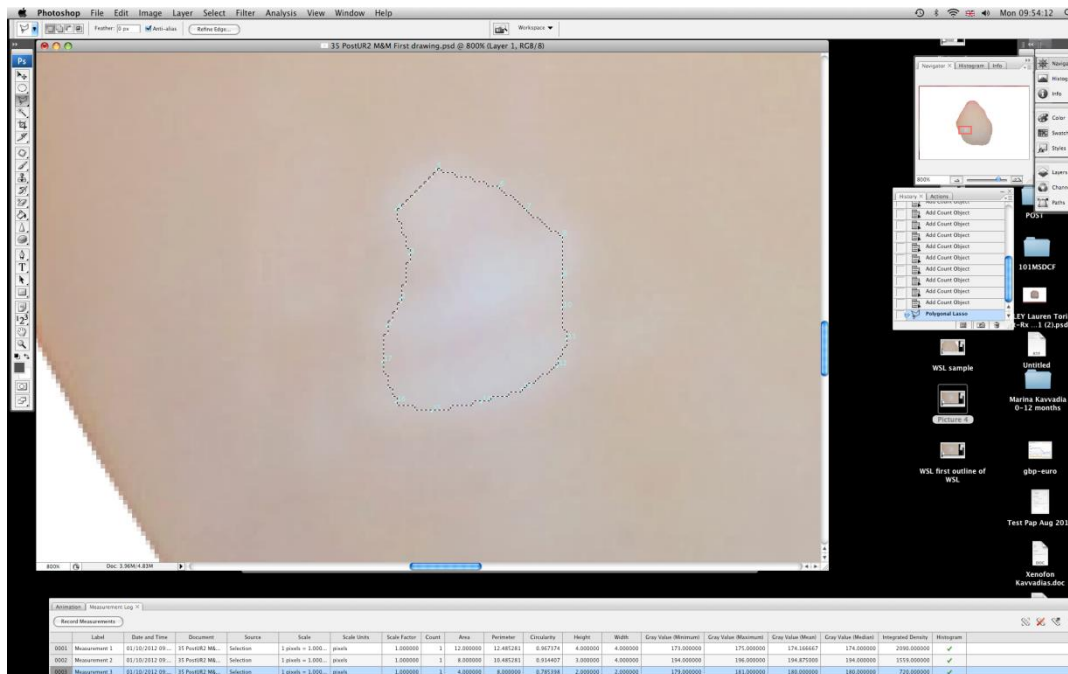


WSL area was defined using the count tool to place marks freehand on the borders of the lesion as seen in Figure 7-10 and Figure 7-11.

**Figure 7-10 Marks of WSL drawn freehand.**



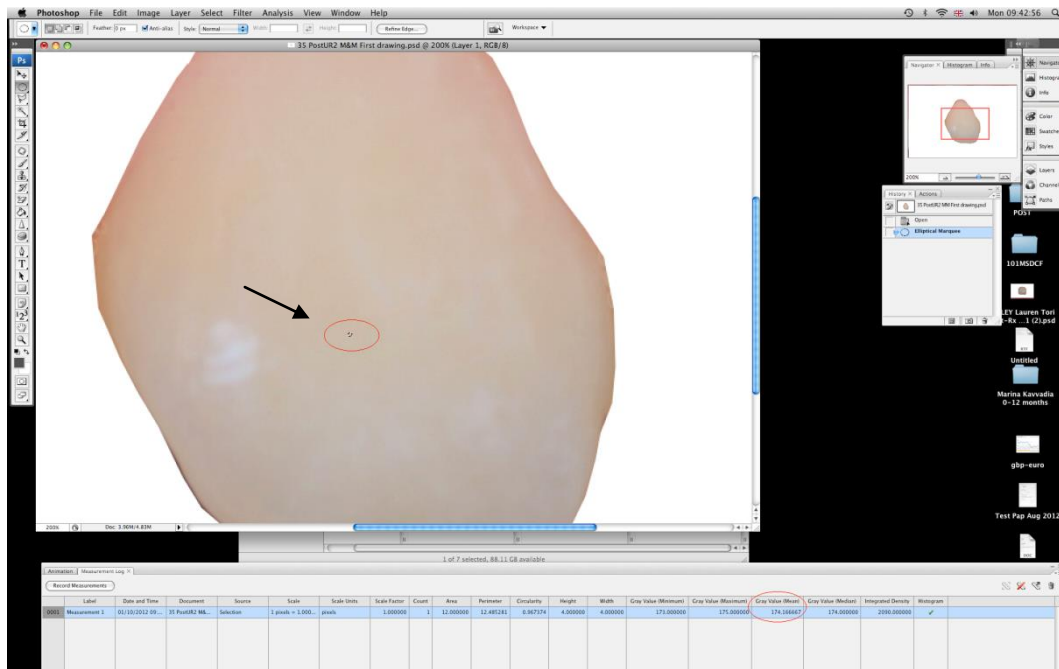
**Figure 7-11 Outline of WSL drawn freehand.**



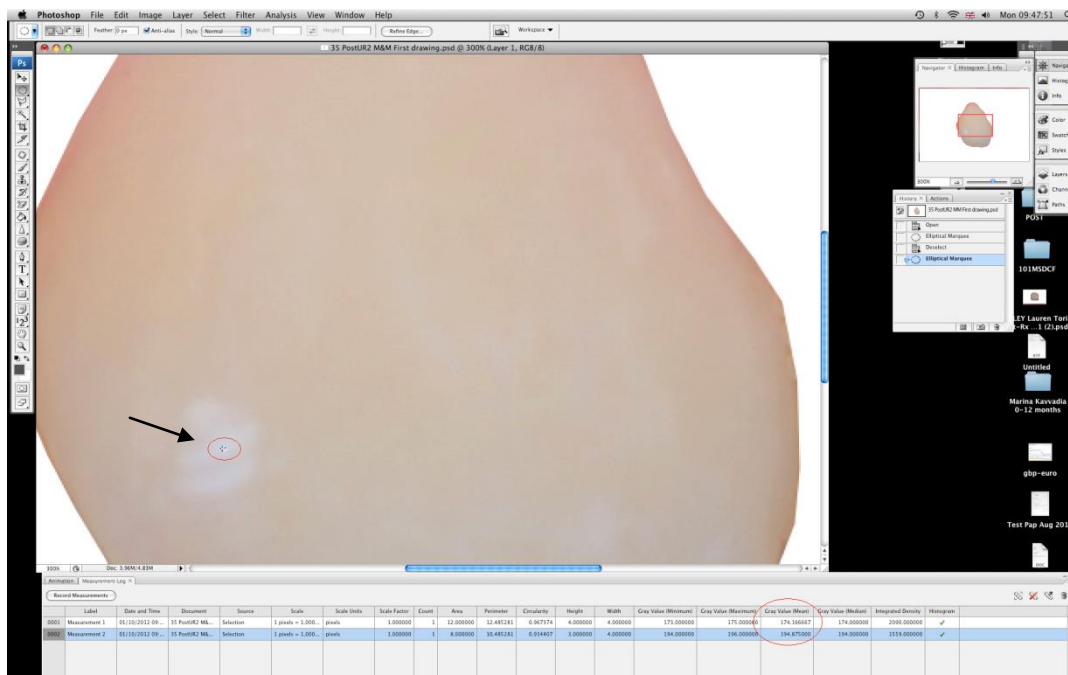
In order to reduce the subjective nature of freehand placement of marks to define WSLs, the following steps were taken; the mean grey value in healthy

enamel as seen in Figure 7-12 and in a representative area within the WSL were measured using the elliptical marquee tool as seen in Figure 7-13.

**Figure 7-12 Mean grey value (174) in a representative area of healthy enamel denoted by arrow.**



**Figure 7-13 Mean grey value (194) in a representative area within the WSL denoted by arrow.**



The mean grey value ranged from 0 representing black to 255 representing white. The mean of the difference in the grey value between healthy enamel and WSL was added to the grey value of the healthy enamel. This value was used as a reference point to outline the periphery of the WSLs *i.e.*

$$\text{WSL} = [(\text{Lesion} - \text{Healthy}) / 2] + \text{Healthy}$$

For example:  $\text{WSL} = [(194 - 174) / 2] + 174$

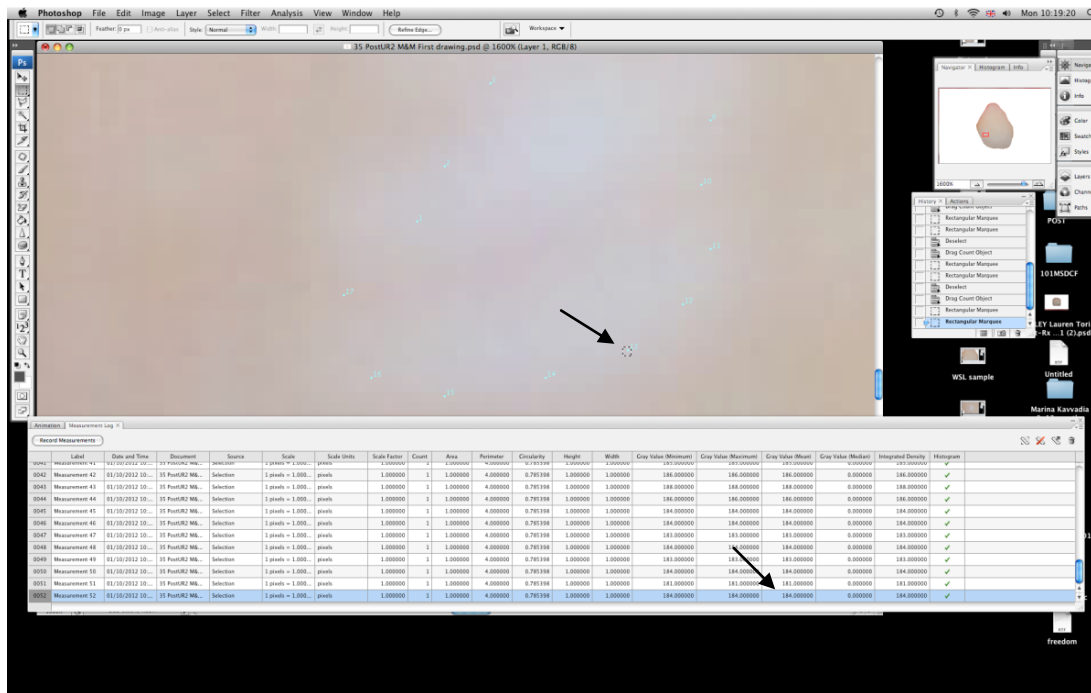
$$\text{WSL} = (20 / 2) + 174$$

$$\text{WSL} = 10 + 174 = 184$$

$$\text{WSL} = 184$$

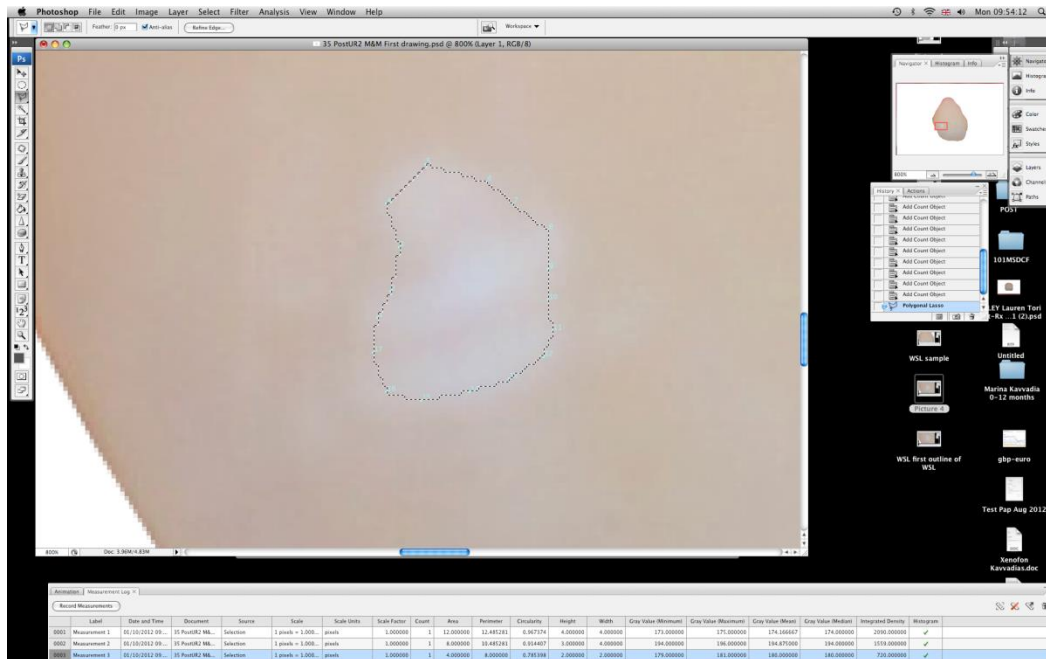
The mean grey value of the free hand placed marks was measured using the rectangular marquee tool to a pixel level as seen in Figure 7-14 and was compared with the reference value.

**Figure 7-14** The mean grey value of each hand placed mark was measured to a pixel level denoted by arrows.

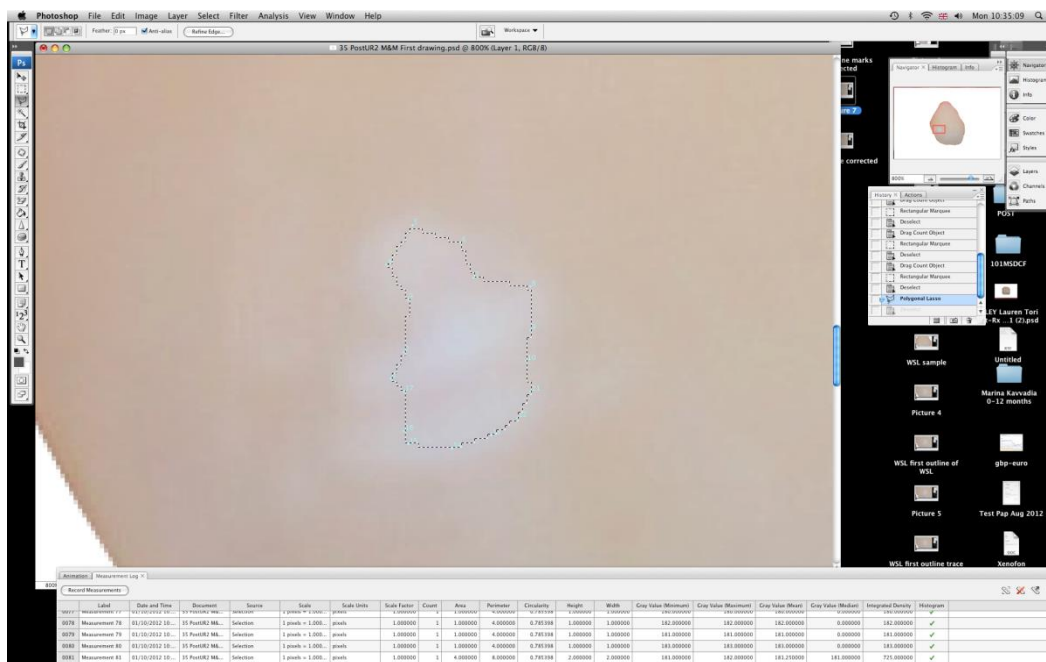


Using the count tool the freehand placed marks were moved accordingly and re-measured to a pixel level until their mean grey value met the reference value on the borders of the WSLs to provide the final outline of the WSL as seen in Figure 7-15 and Figure 7-16.

**Figure 7-15 Initial outline of WSL following placement of hand placed marks to a pixel with a corresponding mean grey value of 184.**



**Figure 7-16 Final outline of WSL following placement of hand placed marks to a pixel with a corresponding mean grey value of 184.**



The surface area within the corrected outline of the WSL was measured as number of pixels and expressed as a percentage of the total tooth surface area. To minimise human error the marks placed freehand to define the WSLs were defined by their mean grey value. The mean grey value of the difference between healthy enamel and WSLs was used as the cut-off point

to mark the WSLs. It appears from the photos that a significant area of WSL is “excluded” in the final outline but this is a systematic and reproducible method of defining WSLs.

### **7.7.11 Statistical Analysis**

For descriptive statistics and functional data statistical analysis the IBM SPSS Statistics 22® software package was used<sup>2</sup>. Zero inflated Poisson regression was applied to model the effect of all variables on number of teeth with WSL. All statistically significant variables at  $p_1=0,20$  were entered in the initial model and the results were compared to those of the simple Poisson regression model with the use of the vuong test and model fit was assessed as significantly better as 44 individuals showed no WSL (zero counts) at the end of the study. Model fit was assessed with the use of Akaike's information criterion and Bayesian information criterion. Variables were considered as statistically significant at  $p<.05$ . Analysis was carried out using STATA® v.13.0<sup>3</sup>

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<sup>2</sup>IBM SPSS Statistics 22, IBM Corporation, New York, USA.

<sup>3</sup>Stata v13, StataCorp LP, Texas, USA.



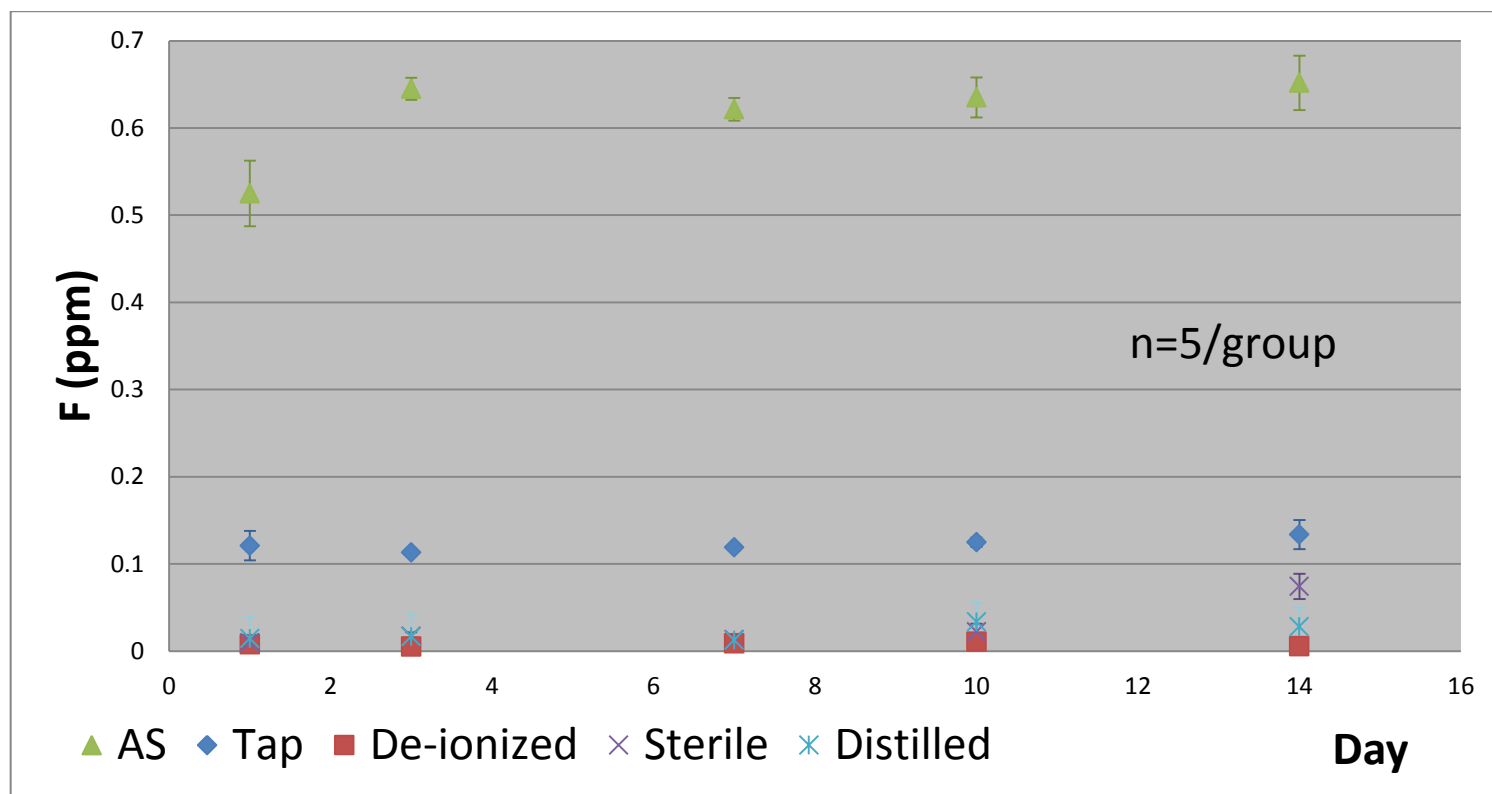
## **8 Results**

### **8.1 *In-vitro* exploratory study**

#### **8.1.1 Storage mediums**

Firstly various storage mediums were assessed for their F and PO<sub>4</sub> release over two weeks. These were AS and water either distilled, deionised, tap or sterile; the results can be seen in Figure 8-1 and Figure 8-2 and the standards used in Table 8-1 and Table 8-2.

Figure 8-1 F release from different types of storage medium over n=14 days.

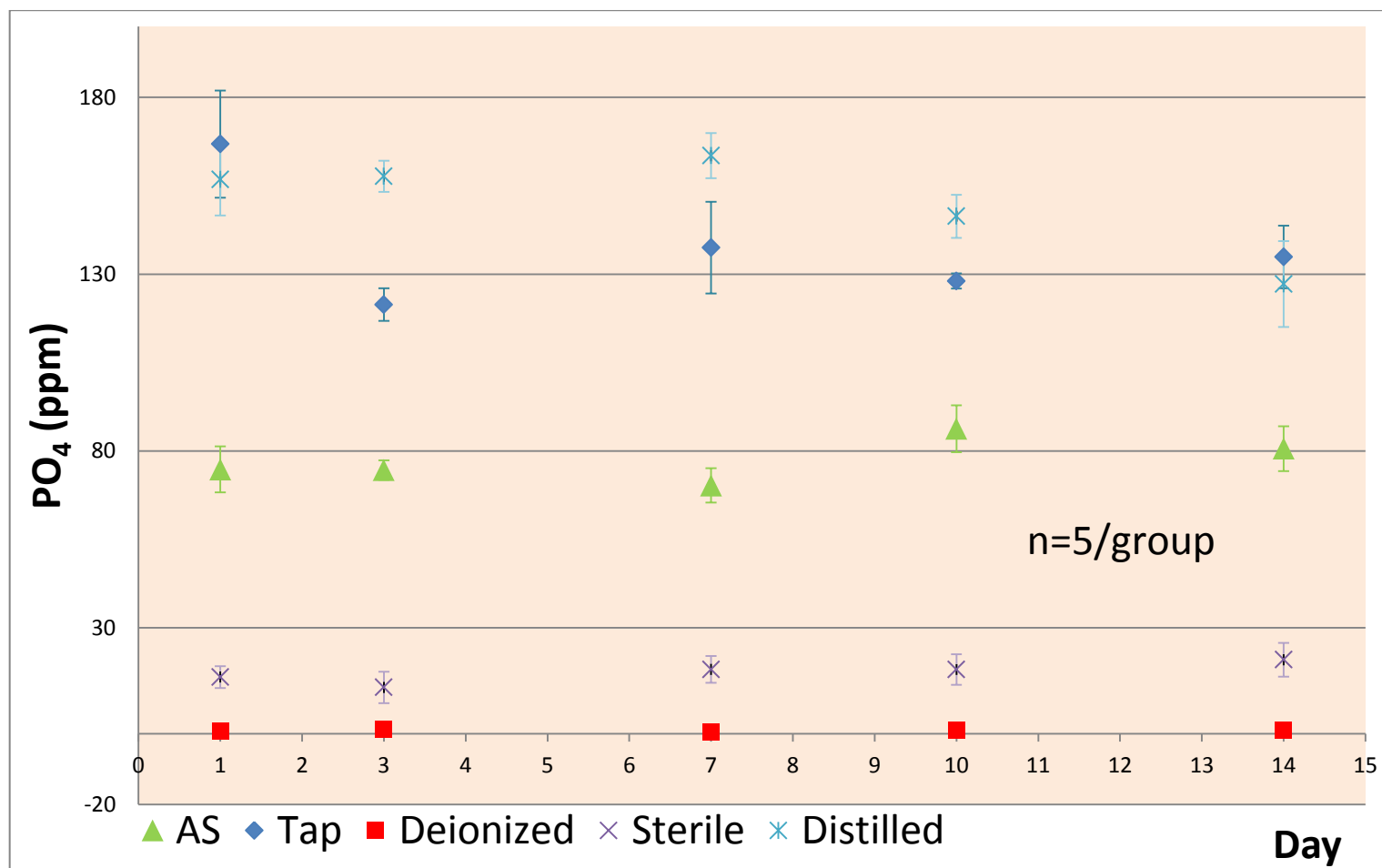


AS=artificial saliva, n=number

**Table 8-1 F standards for different types of storage mediums.**

Fstandard (ppm)	3	0.3	0.03	0.003
1 <sup>st</sup> measurement	3.01	0.29	0.03	0.002
2 <sup>nd</sup> measurement	2.88	0.31	0.02	0.003
3 <sup>rd</sup> measurement	2.96	0.28	0.03	0.004
Mean $\pm$ SD	2.95 $\pm$ 0.06	0.29 $\pm$ 0.01	0.02 $\pm$ 0.005	0.003 $\pm$ 0.001

Figure 8-2 PO<sub>4</sub> release from different types of storage medium over n=14 days.



AS=artificial saliva, n=number

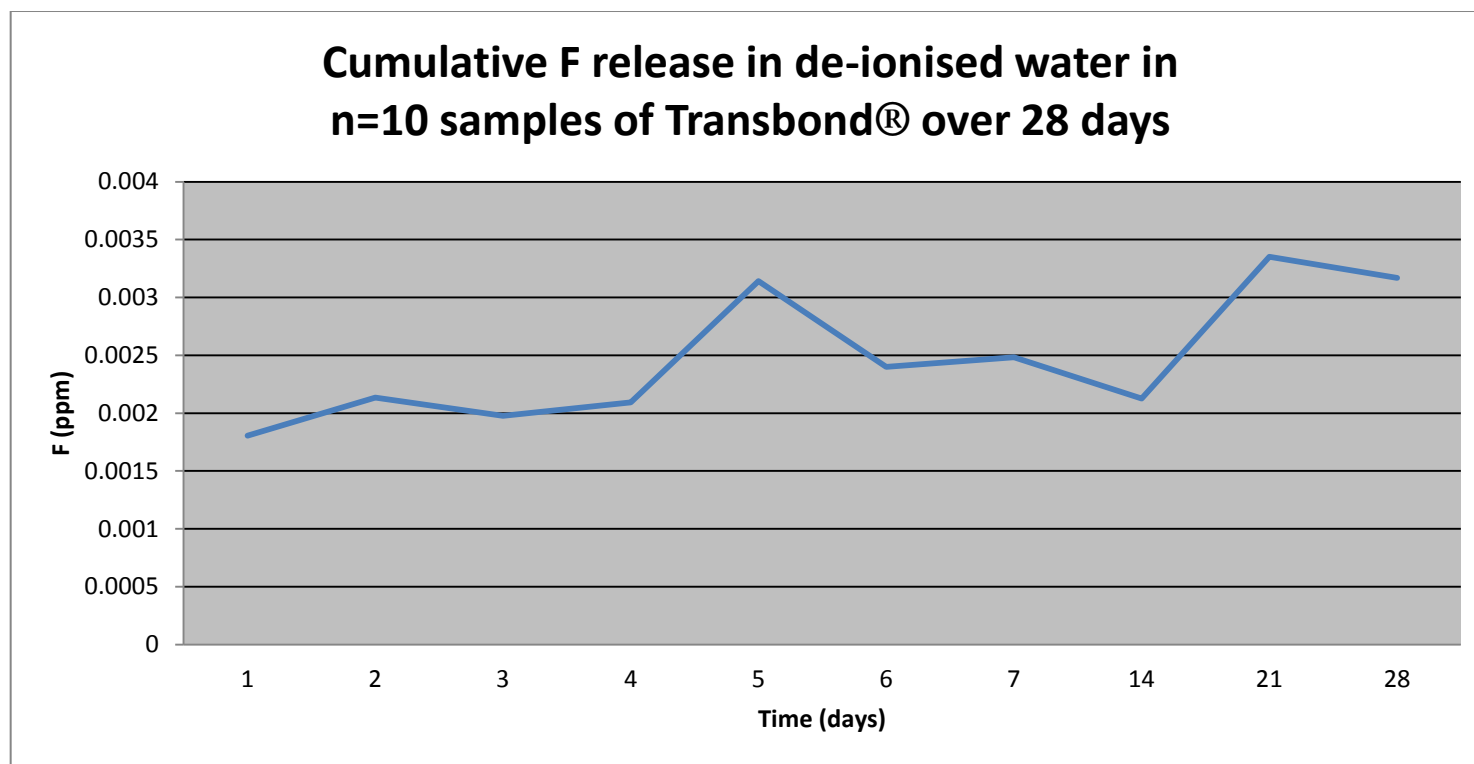
**Table 8-2 PO<sub>4</sub> standards used for different types of storage mediums.**

PO <sub>4</sub> standard (ppm)	500	250	100
1 <sup>st</sup> measurement	495.34	238.27	97.82
2 <sup>nd</sup> measurement	498.94	244.92	102.07
3 <sup>rd</sup> measurement	501.24	248.55	99.44
Mean ± SD	498.51 ± 2.98	243.91 ± 5.21	99.77 ± 2.15

### 8.1.2 Control material

The control material Transbond® was also tested for F and PO<sub>4</sub> release. The F and PO<sub>4</sub> standards are seen in Table 8-3 and Table 8-4 alongside the results seen in Figure 8-3 and Figure 8-4.

Figure 8-3 F release from control material (Transbond®) over 28 days.



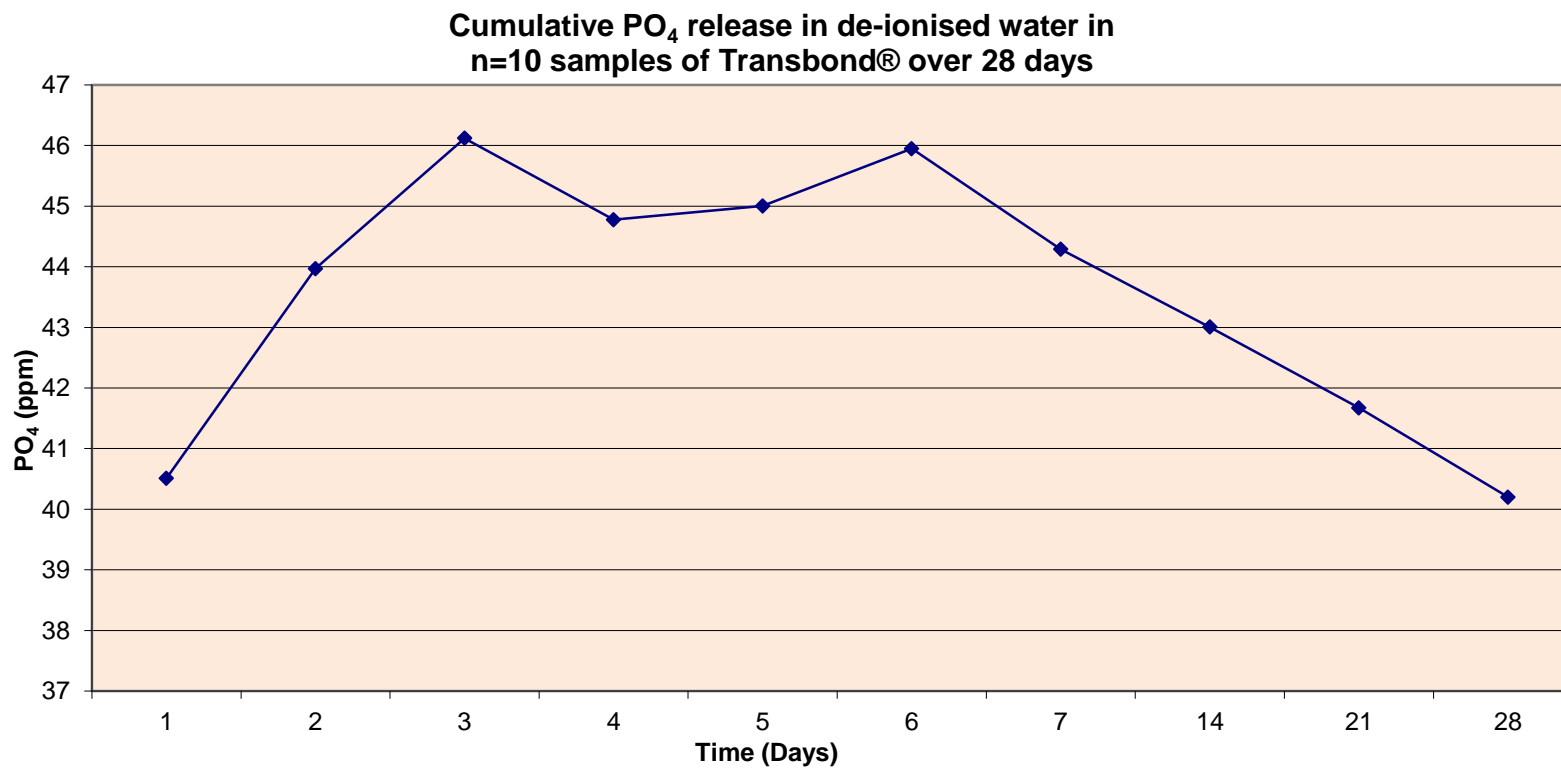
n=number

**Table 8-3 F standards for Transbond®.**

F standard (ppm)	3	0.3	0.03	0.003
1st measurement	3.01	0.28	0.03	0.002
2nd measurement	2.98	0.28	0.029	0.004
3 <sup>rd</sup> measurement	2.97	0.27	0.032	0.003
Mean $\pm$ SD	2.99 $\pm$ 0.02	0.28 $\pm$ 0.01	0.03 $\pm$ 0.00	0.003 $\pm$ 0.001



Figure 8-4 PO<sub>4</sub> release from control material (Transbond®) over 28 days.



n=number

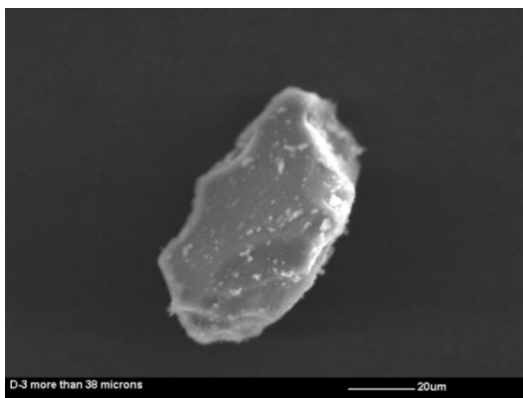
**Table 8-4 PO<sub>4</sub> standards used for Transbond®.**

PO <sub>4</sub> standard (ppm)	500	250	100
1st measurement	498.25	240.24	98.55
2nd measurement	496.54	246.85	103.21
3rd measurement	500.15	252.05	99.75
Mean $\pm$ SD	498.32 $\pm$ 1.80	246.38 $\pm$ 5.92	100.51 $\pm$ 2.42

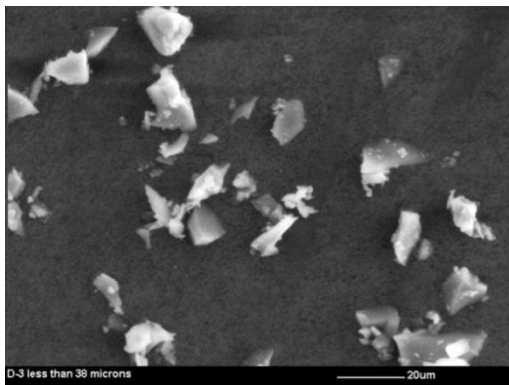
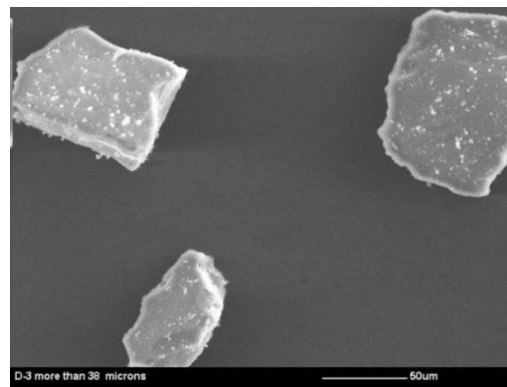
### 8.1.3 Assessment of powder particle size with scanning electron microscopy (SEM).

SRFGDs with different solubility (relative scales of 1, 3, 16 and 50) were changed into a powder form and fractionated using sieve stacks (30 $\mu$ m) producing a powder with a particle size of 38 $\mu$ m. The particle size was confirmed using SEM seen in Figure 8-5.

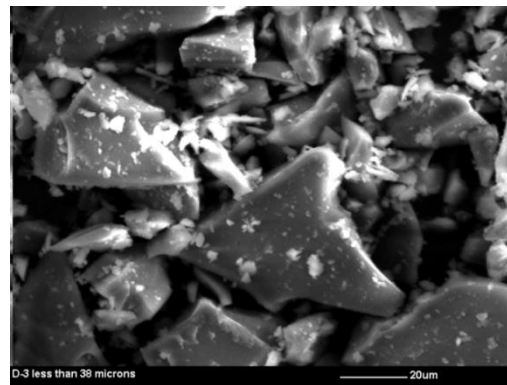
**Figure 8-5 SEM images of powder from SRFGDs with relative solubility 3.**



Particle size: >38  $\mu$ m



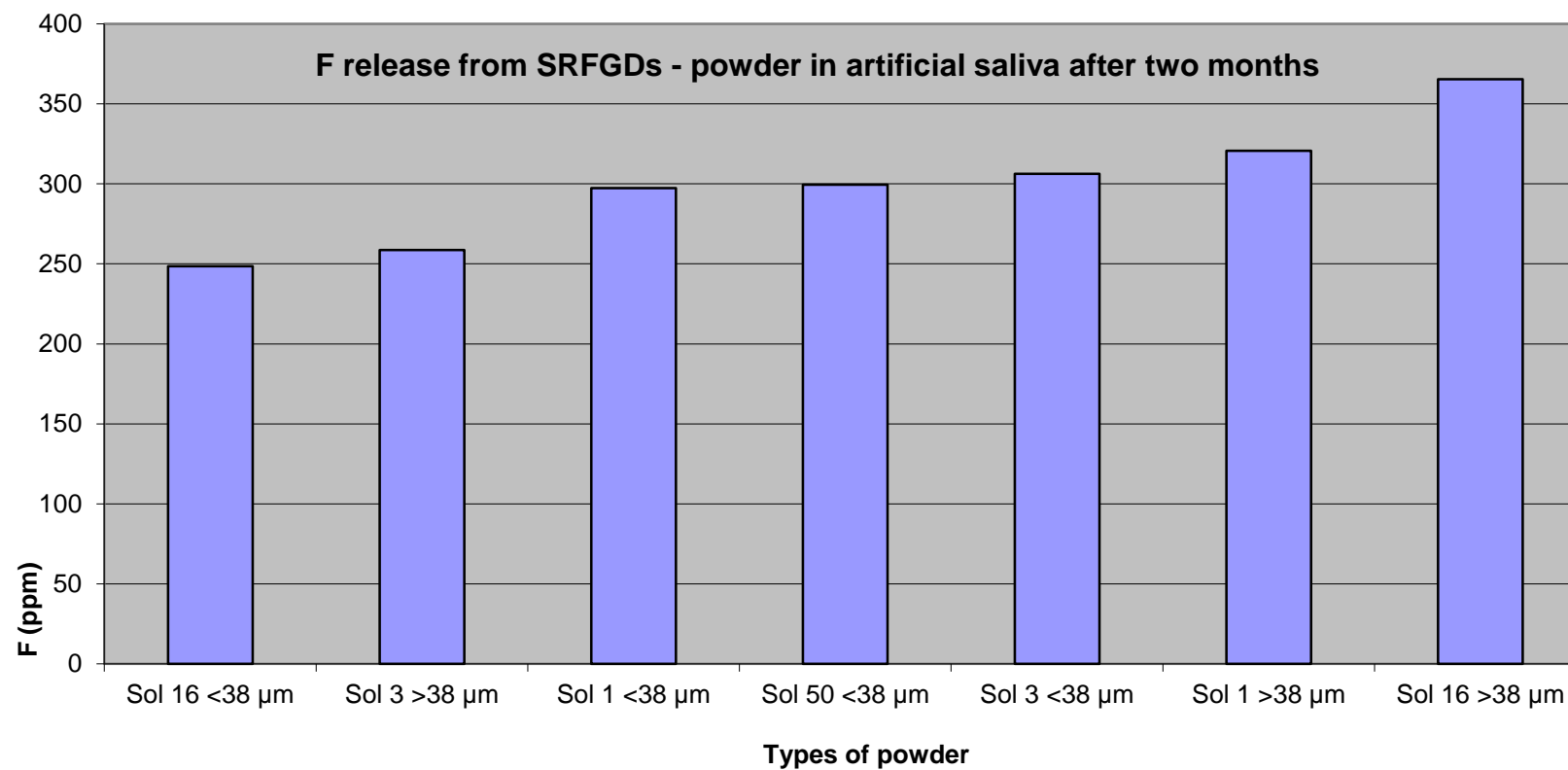
Particle size: <38  $\mu$ m



#### **8.1.4 F and PO<sub>4</sub> release from powder from SRFGDs**

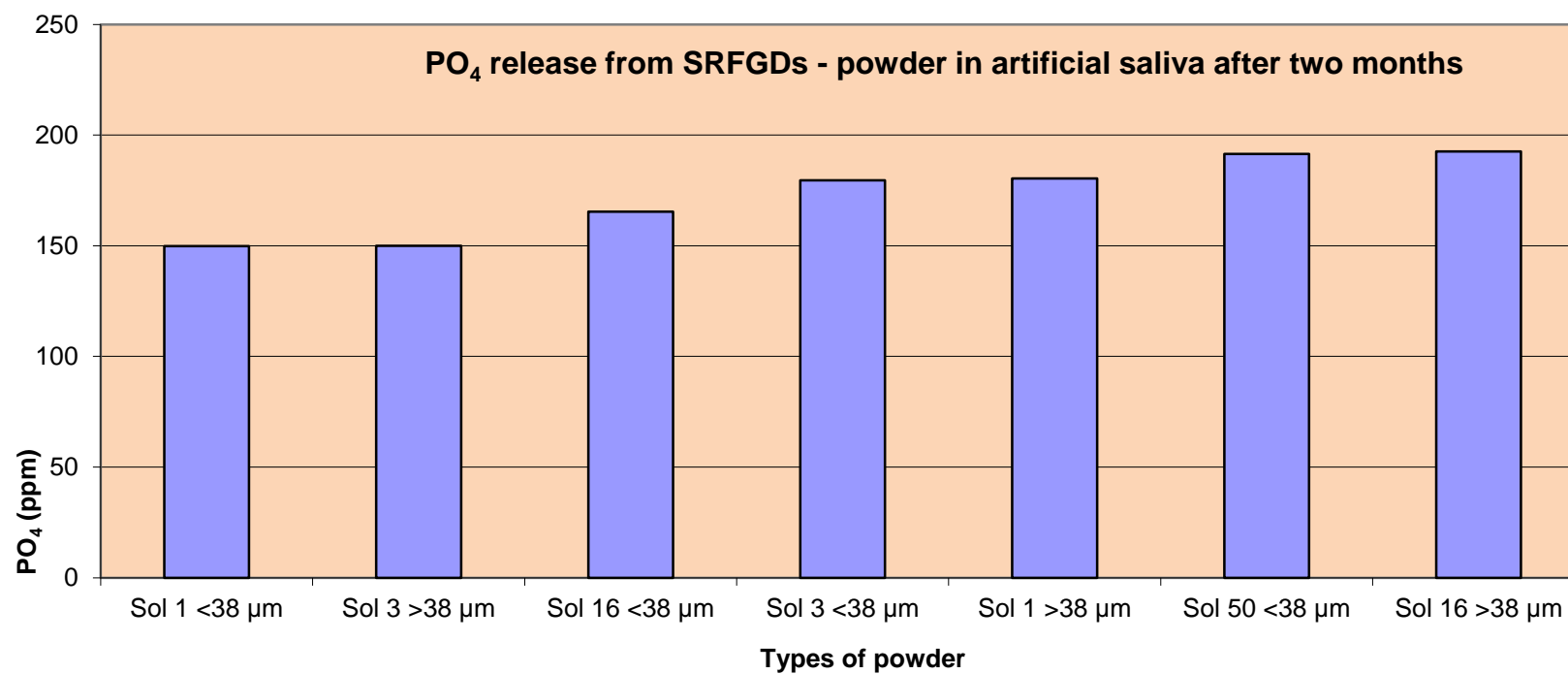
Powder from SRFGDs with different particle sizes was assessed with IC for F and PO<sub>4</sub> release. The results can be seen in Figure 8-6 and Figure 8-7.

Figure 8-6 F release from powder from SRFGDs with different solubility and particle size, in AS after two months.



Sol = relative solubility, n=5

Figure 8-7 PO<sub>4</sub> release from powder from SRFGDs with different solubility and particle size, in AS after two months.



Sol = relative solubility, n=5

### **8.1.5 F release from powder mixed with control material**

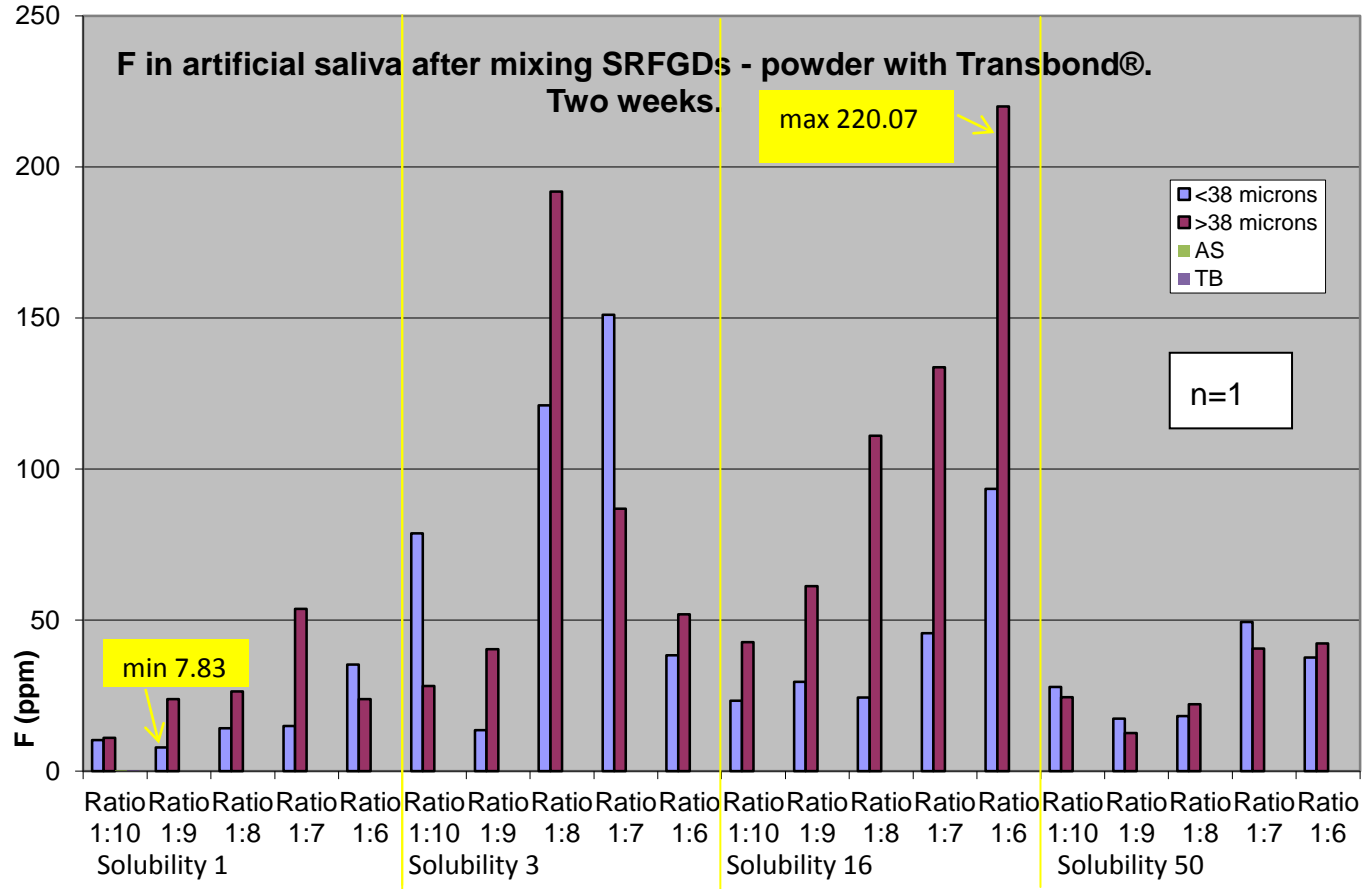
Powder from SRFGDs with different particle size and different solubility was mixed with the control material (Transbond®), giving 40 different combinations with one sample per combination. F release was assessed with IC at two weeks, four and six months. The results are seen in the Figure 8-8, Figure 8-9 and Figure 8-10.

Samples were left in 2ml of AS and after two weeks 200µl was analysed with IC. The findings as seen in Figure 8-8 show that the powder with the maximum F release (220.07 ppmF) had solubility of 16, ratio of powder to Transbond® 1:6 and particle size of more than 38µm.

The findings after four months as seen in Figure 8-9 show that the powder with the maximum F release (65.29 ppmF) had solubility of 16, ratio of powder to Transbond® 1:6 and particle size of less than 38µm.

The findings after six months as seen in Figure 8-10 show that the powder with the maximum F release (101.45ppmF) had solubility of 16, ratio of powder to Transbond® 1:8 and particle size of less than 38µm. A summary of the findings is seen in Table 8-5.

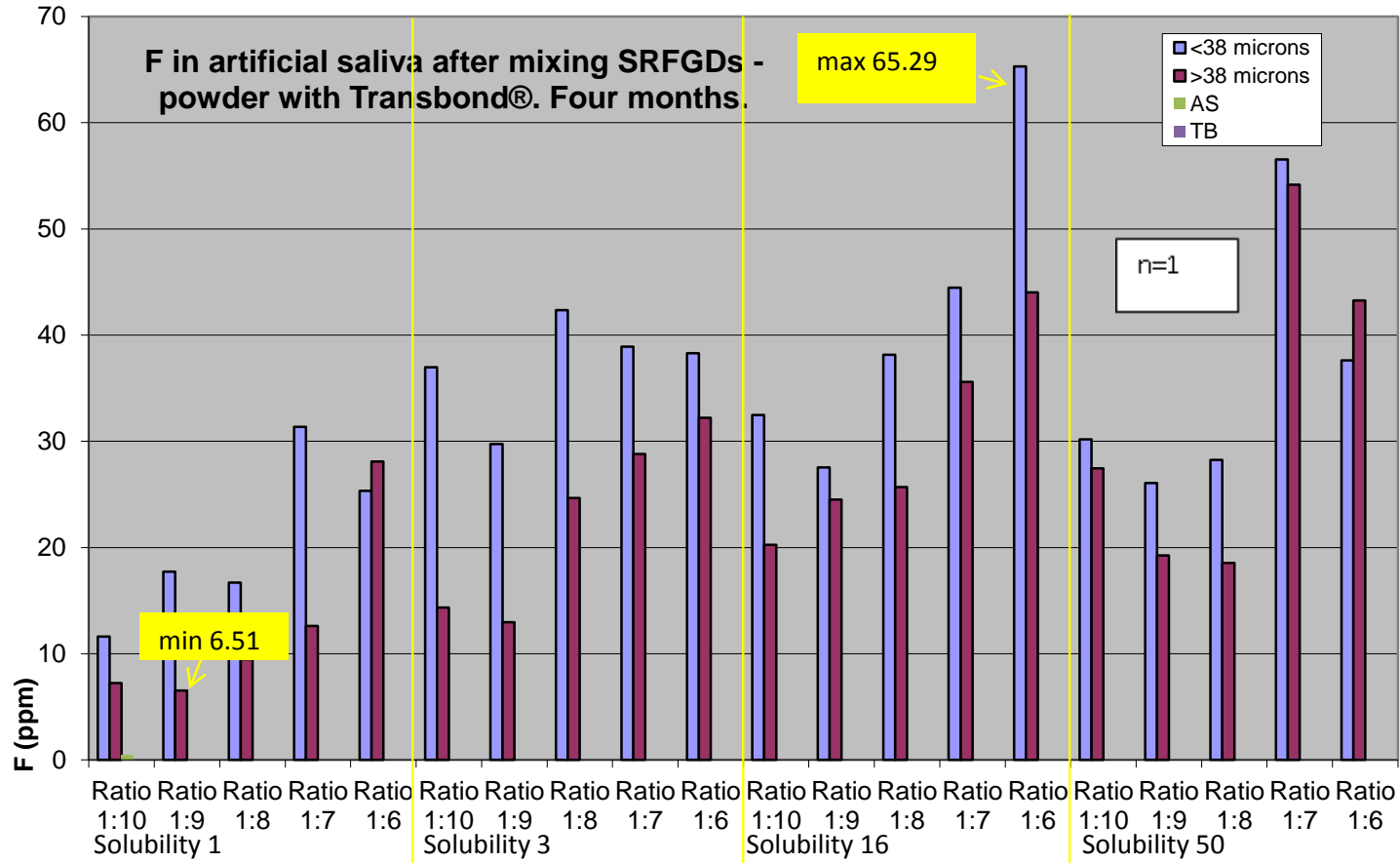
Figure 8-8 F release in AS after two weeks after mixing Transbond® with powder from SRFGDs.



Ratio = ratio of powder to Transbond®, AS= artificial saliva, TB = Transbond®, F release for AS and TB is zero therefore not shown on graph

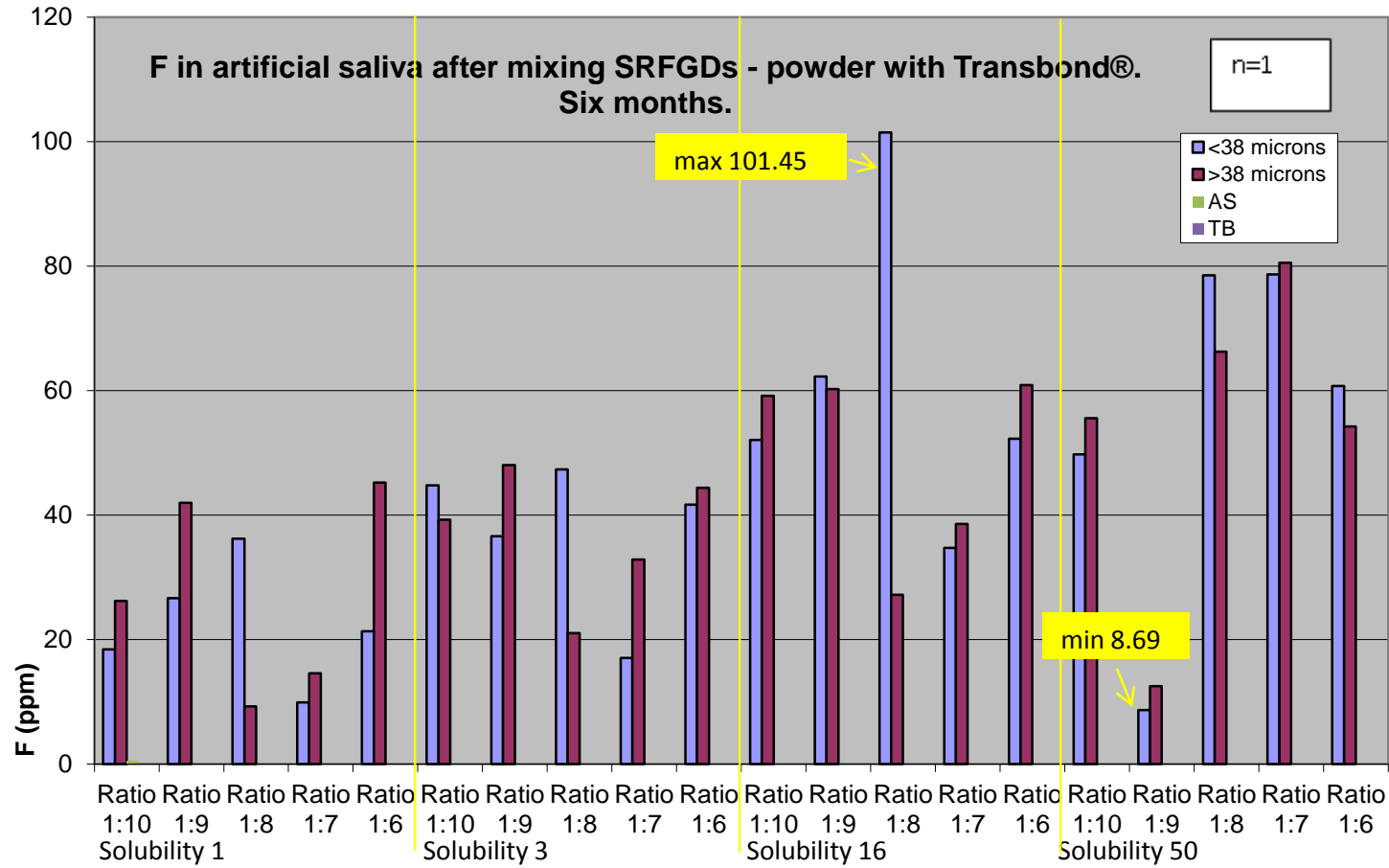


Figure 8-9 F release in AS after four months after mixing Transbond® with powder from SRFGDs.



Ratio = ratio of powder to Transbond®, AS= artificial saliva, TB = Transbond®, F release for AS and TB is zero therefore not shown on graph

Figure 8-10 F release in AS after six months after mixing Transbond® with powder from SRFGDs.



Ratio = ratio of powder to Transbond®, AS= artificial saliva, TB = Transbond®, F release for AS and TB is zero therefore not shown on graph

### **8.1.6 PO<sub>4</sub> release from powder mixed with control material**

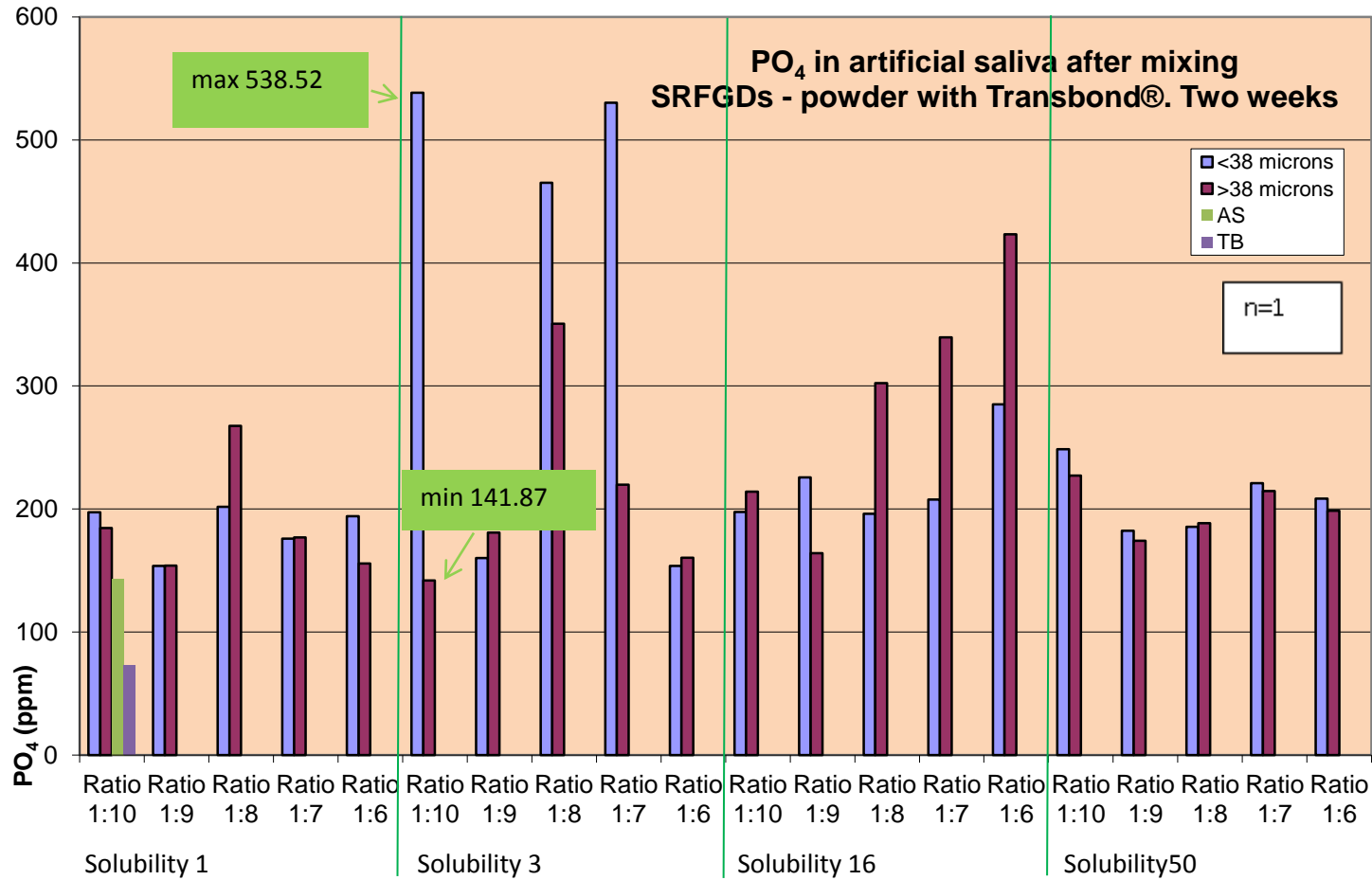
PO<sub>4</sub> release was also assessed with IC for 40 combinations with one sample per combination at two weeks, four and six months. The results are seen in Figure 8-11, Figure 8-12 and Figure 8-13.

Samples were left in 2ml of AS and after two weeks 200µl was analysed with IC. The powder with the lowest PO<sub>4</sub> release (141.87 ppmPO<sub>4</sub>) as seen in Figure 8-11 had solubility of 3, ratio of powder to Transbond® 1:10 and particle size of more than 38µm.

The powder with the lowest PO<sub>4</sub> release (55.52 ppmPO<sub>4</sub>) after four months as seen in Figure 8-12 had solubility of 50, ratio of powder to Transbond® 1:10 and particle size of less than 38µm.

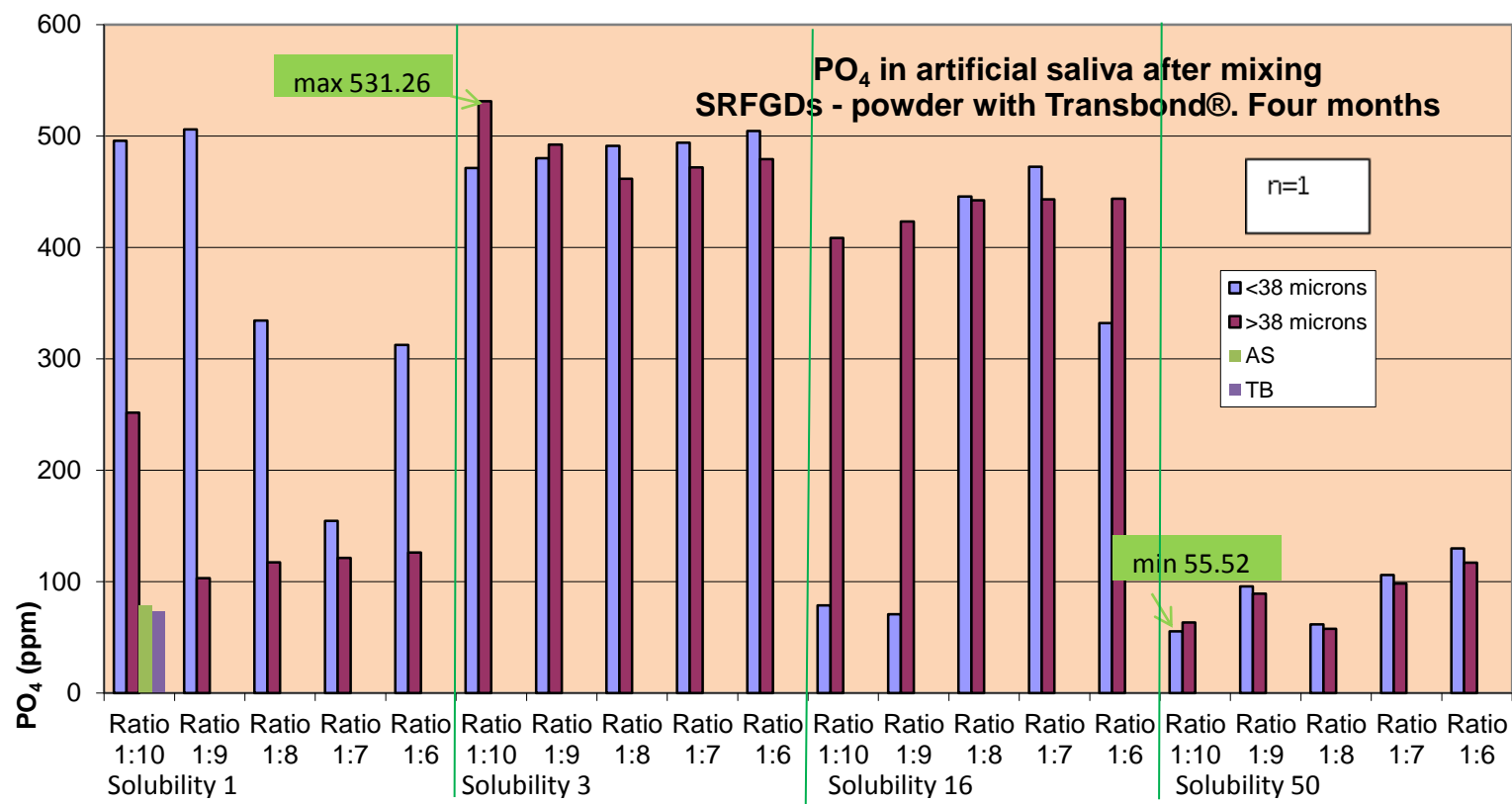
The powder with the lowest PO<sub>4</sub> release (75.51ppmPO<sub>4</sub>) after six months as seen in Figure 8-13 had solubility of 1, ratio of powder to Transbond® 1:8 and particle size of less than 38µm. A summary of the findings is seen in Table 8-5.

Figure 8-11 PO<sub>4</sub> release in AS after two weeks after mixing Transbond® with powder from SRFGDs.



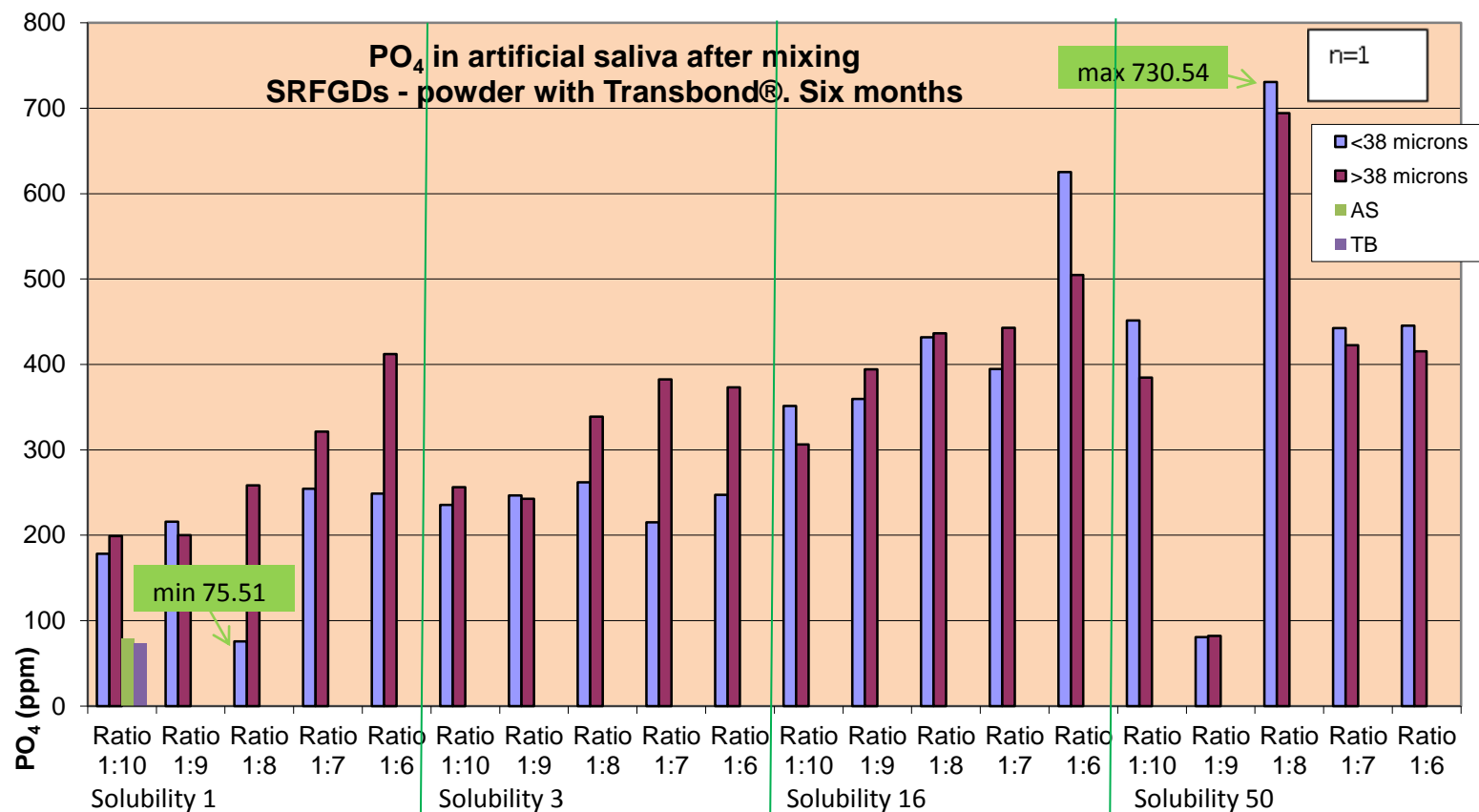
Ratio = ratio of powder to Transbond®, AS = artificial saliva, TB = Transbond®

Figure 8-12 PO<sub>4</sub> release in AS after four months after mixing Transbond® with powder from SRFGDs.



Ratio = ratio of powder to Transbond®, AS = artificial saliva, TB = Transbond®

Figure 8-13 PO<sub>4</sub> release in AS after six months after mixing Transbond® with powder from SRFGDs.



Ratio = ratio of powder to Transbond®, AS = artificial saliva, TB = Transbond®

A summary of the findings is seen in the following table.

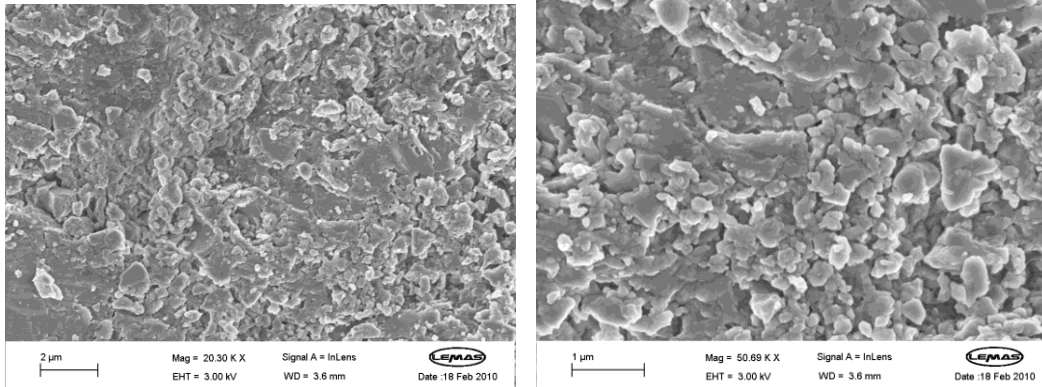
**Table 8-5 Summary of findings for F and PO<sub>4</sub> release after two weeks, four and six months.**

		Highest F (ppm)	Lowest PO <sub>4</sub> (ppm)
Six months	Release	101.45	75.51
	Solubility	16	1
	Ratio powder: Transbond®	1:8	1:8
	Particle size	<38µm	<38µm
Four months	Release	65.29	55.52
	Solubility	16	50
	Ratio powder: Transbond®	1:6	1:10
	Particle size	<38µm	<38µm
Two weeks	Release	220.07	141.87
	Solubility	16	3
	Ratio powder: Transbond®	1:6	1:10
	Particle size	>38µm	>38µm

### 8.1.7 SEM images of Transbond® mixed with powder from SRFGDs.

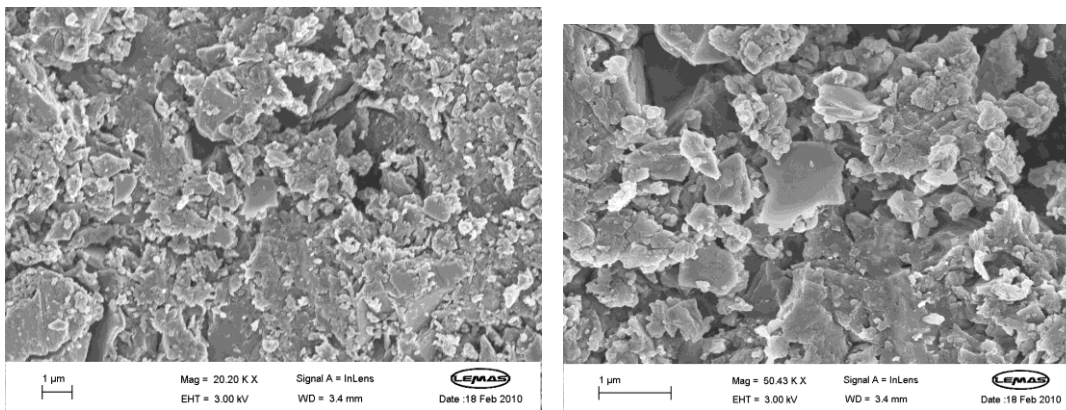
The powder with the highest F release and the lower PO<sub>4</sub> released was mixed with Transbond®. The test material seen in Figure 8-15 and Figure 8-16 and control material seen in Figure 8-14 were screened with SEM to assess their morphology.

**Figure 8-14 SEM images of Transbond® bonding material.**



Transbond® Magnification 20.30 Transbond® Magnification 50.69

**Figure 8-15 SEM images of powder (relative solubility 50, particle size <math><38\mu\text{m}</math>, ratio of bead to Transbond® 1:10) mixed with Transbond®.**

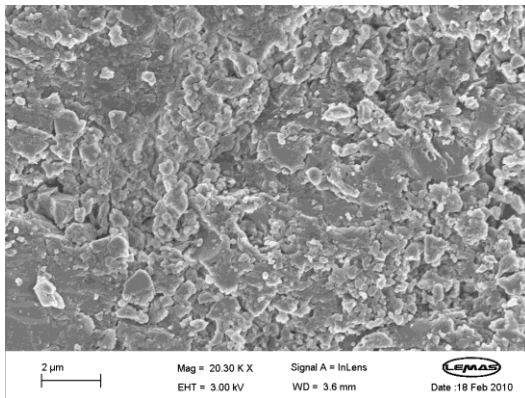


Magnification 20.20

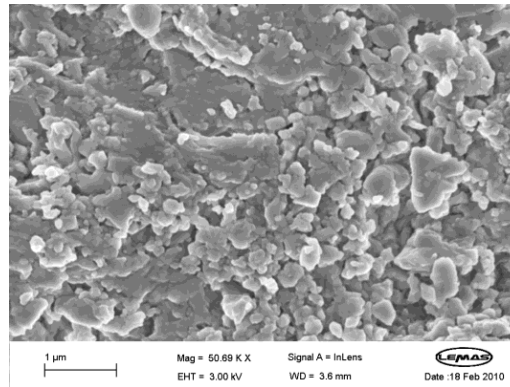
Magnification 50.43



**Figure 8-16 SEM images of powder (relative solubility 1, particle size >38 $\mu$ m, ratio of bead to Transbond® 1:6) mixed with Transbond®.**



Magnification 20.30



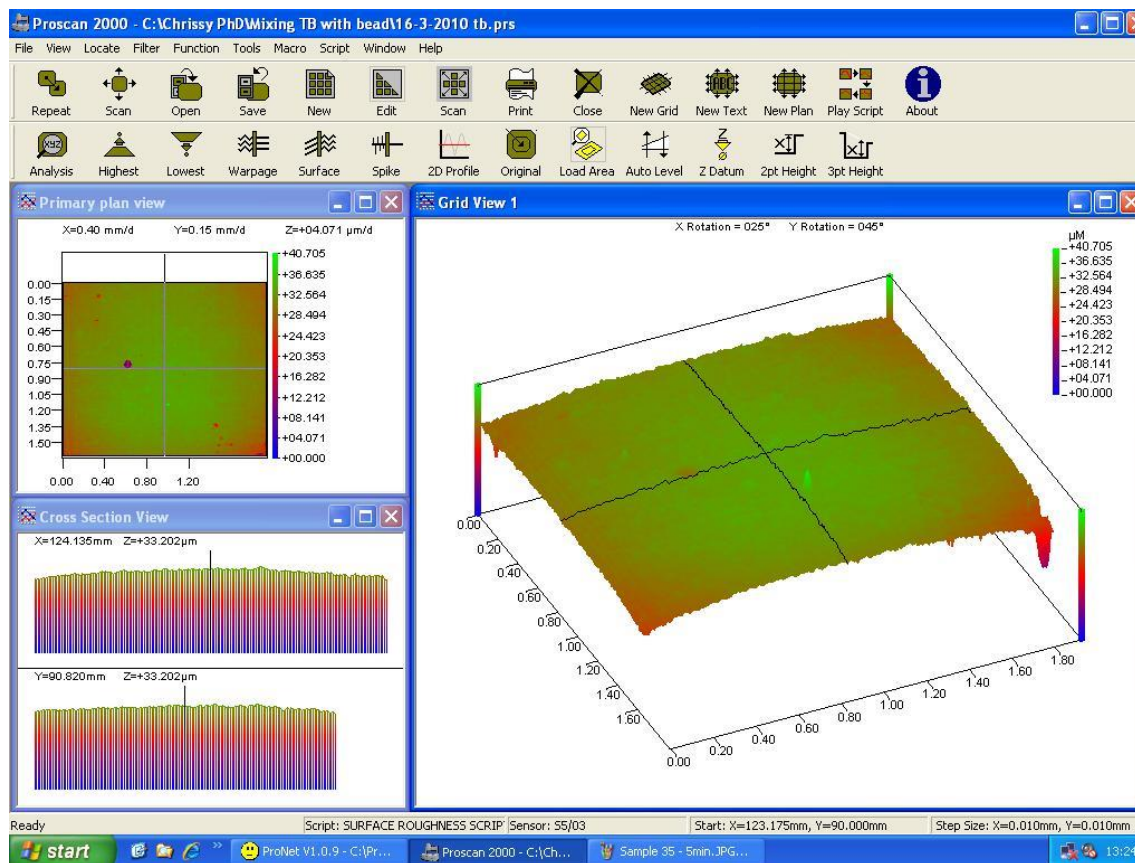
Magnification 50.69

### 8.1.8 Profilometry scan of Transbond® mixed with powder from SRFGDs.

The test and control materials were screened with profilometry to assess their morphology as seen in,

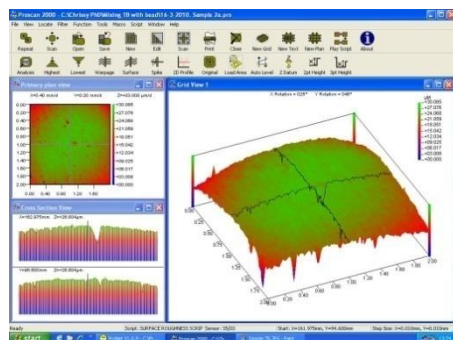
The powder with highest solubility, a particle size of less than  $38\mu\text{m}$  and the lowest ratio of 1:10 gave a different image following profilometry scan to the control material. Compared to Transbond® seen in Figure 8-17, all the test samples containing 1:10 ratio of SRFGDs to Transbond® appeared similarly flat seen in Figure 8-18, Figure 8-19, Figure 8-20, Figure 8-21 and Figure 8-22. The particle size of powder from SRFGDs of either less or more than 38 microns didn't alter much the profilometry scan. The powder of SRFGDs with relative solubility of 1 or 50 appeared to produce profilometry scans the closest to the one produced by Transbond® as seen in Figure 8-18 and Figure 8-22.

**Figure 8-17 Profilometry scan of Transbond® bonding material.**

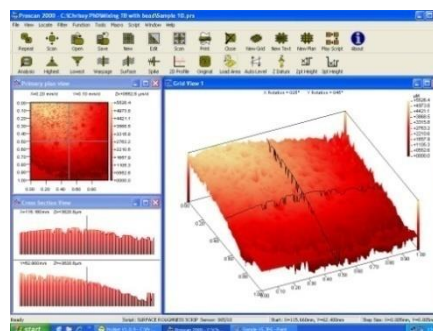


**Figure 8-18 Profilometry scan of powder with relative solubility 1 and particle size  $>38\mu\text{m}$ , mixed with Transbond®.**

Relative solubility 1, particle size  $>38\mu\text{m}$ , ratio of powder to Transbond® 1:6

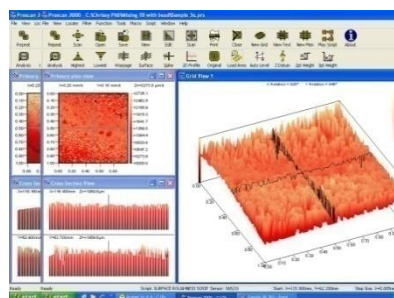


Relative solubility 1, particle size  $>38\mu\text{m}$ , ratio of powder to Transbond® 1:10

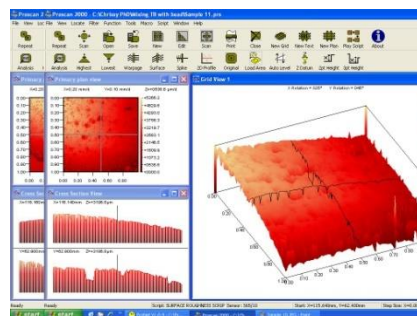


**Figure 8-19 Profilometry scan of powder with relative solubility 1 and particle size  $<38\mu\text{m}$ , mixed with Transbond®.**

Relative solubility 1, particle size  $<38\mu\text{m}$ , ratio of powder to Transbond® 1:6

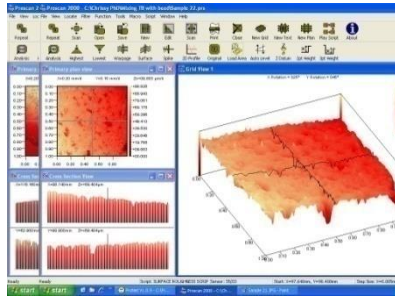


Relative solubility 1, particle size  $<38\mu\text{m}$ , ratio of powder to Transbond® 1:10

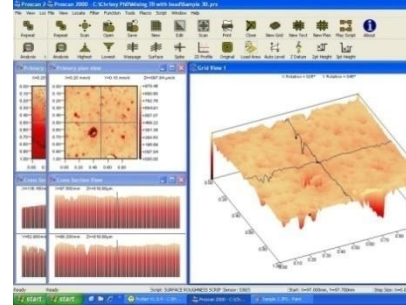


**Figure 8-20 Profilometry scan of powder with relative solubility 16 and particle size  $>38\mu\text{m}$ , mixed with Transbond®.**

Relative solubility 16, particle size  $>38\mu\text{m}$ , ratio of powder to Transbond®1:6

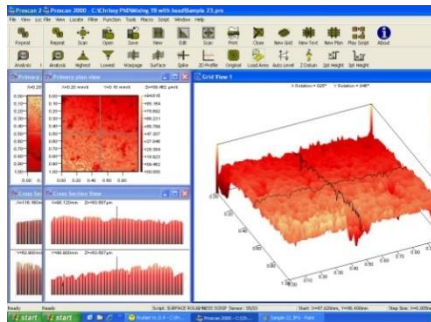


Relative solubility 16, particle size  $>38\mu\text{m}$ , ratio of powder to Transbond®1:10

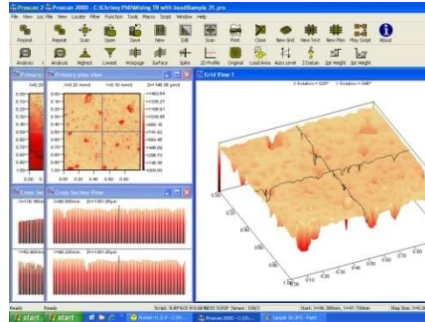


**Figure 8-21 Profilometry scan of powder with relative solubility 16 and particle size  $<38\mu\text{m}$ , mixed with Transbond®.**

Relative solubility 16, particle size  $<38\mu\text{m}$ , ratio of powder to Transbond®1:6

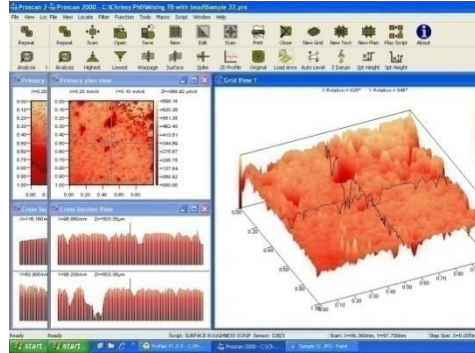


Relative solubility 16, particle size  $<38\mu\text{m}$ , ratio of powder to Transbond®1:10

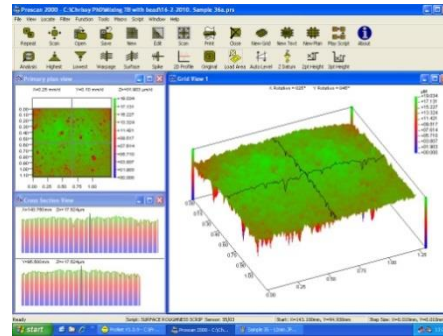


**Figure 8-22 Profilometry scan of powder with relative solubility 50 and particle size <math><38\mu\text{m}</math>, mixed with Transbond®.**

Relative solubility 50, particle size <math><38\mu\text{m}</math>, ratio of powder to Transbond®1:6



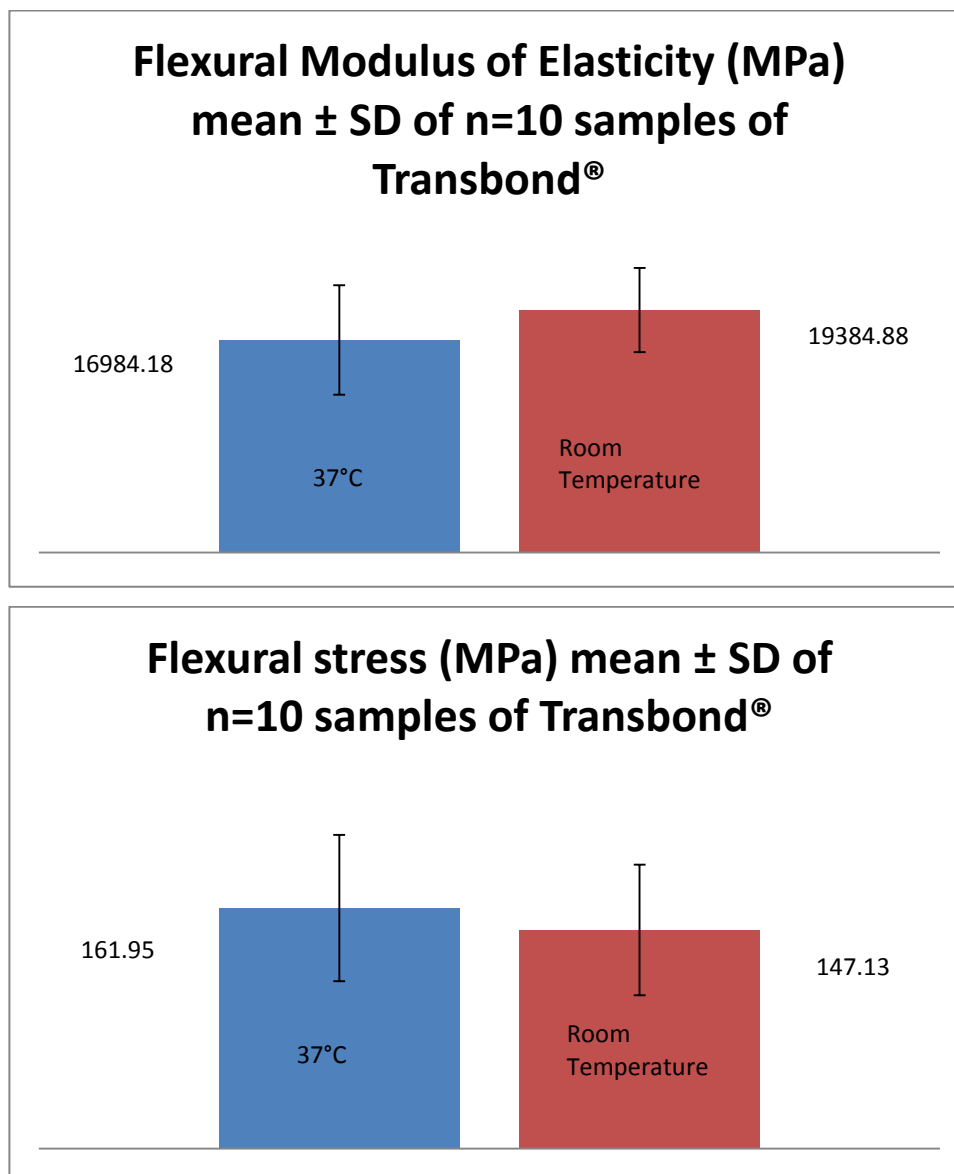
Relative solubility 50, particle size <math><38\mu\text{m}</math>, ratio of powder to Transbond®1:10



### 8.1.9 Bond strength of test and control (Transbond®) materials

In order to assess whether the bond strength of test and control material would be clinically acceptable I investigated whether storage temperature would produce a difference. Results in Figure 8-23 show no statistical significant difference for flexural modulus of elasticity and flexular stress in samples stored in controlled room temperature or 37°C to mimic oral conditions.

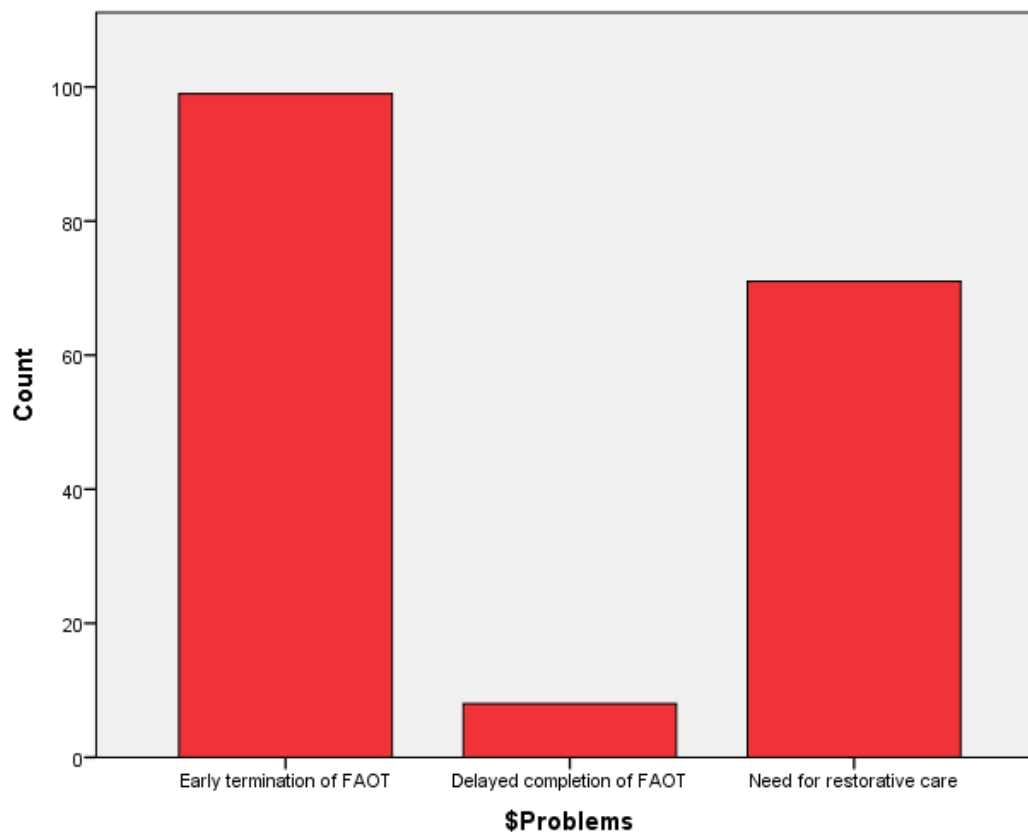
**Figure 8-23 Flexular modulus of elasticity and stress mean $\pm$ SD in n=10 samples of Transbond® bonding material.**



## 8.2 Questionnaire to orthodontist-members of the British Orthodontic Society

The response rate was 7.85% (115/1,464) and the fully completed questionnaires were 105/115 (91.30%). Majority of responders were females (55.2%) and the mean age for all responders was 48 years old and the median 47. The median year for obtaining orthodontic qualification was 1997. Based on the responses the median risk for developing WSLs during FAOT was estimated to be 20% and the mean 42.86%. Majority of responders (81.3%) would consider using the SRFGDs if proven effective clinically. Results on the multiple responses questions are shown in Figure 8-24, Figure 8-25, Figure 8-26, Figure 8-27, Figure 8-28, Figure 8-29, Figure 8-30, Figure 8-31 and Figure 8-32.

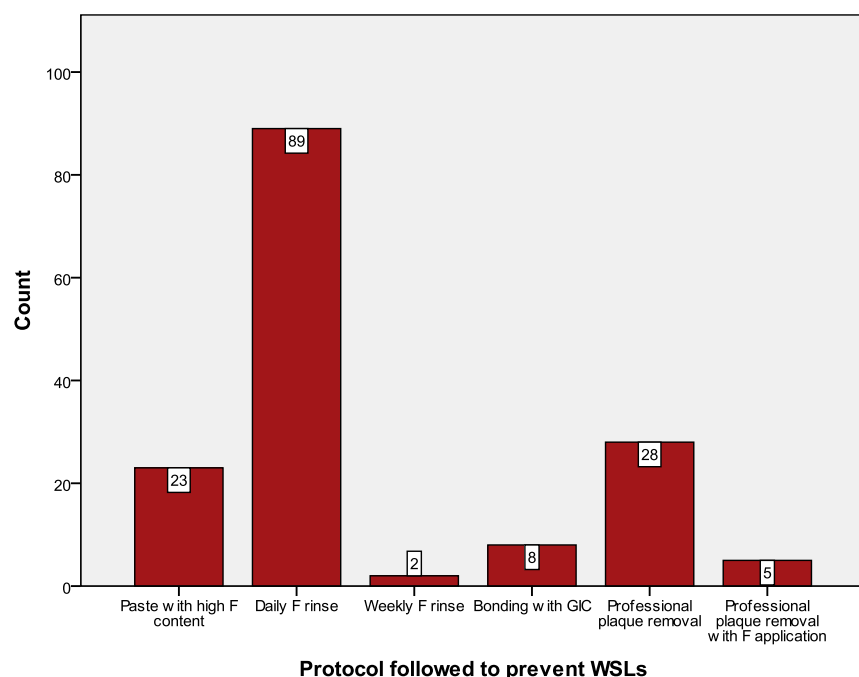
**Figure 8-24 Problems for patients with WSLs identified by dentists during FAOT.**



Count=number of dentists who have WSL problem(s) with patients  
 \$Problems=problems for patients with WSLs

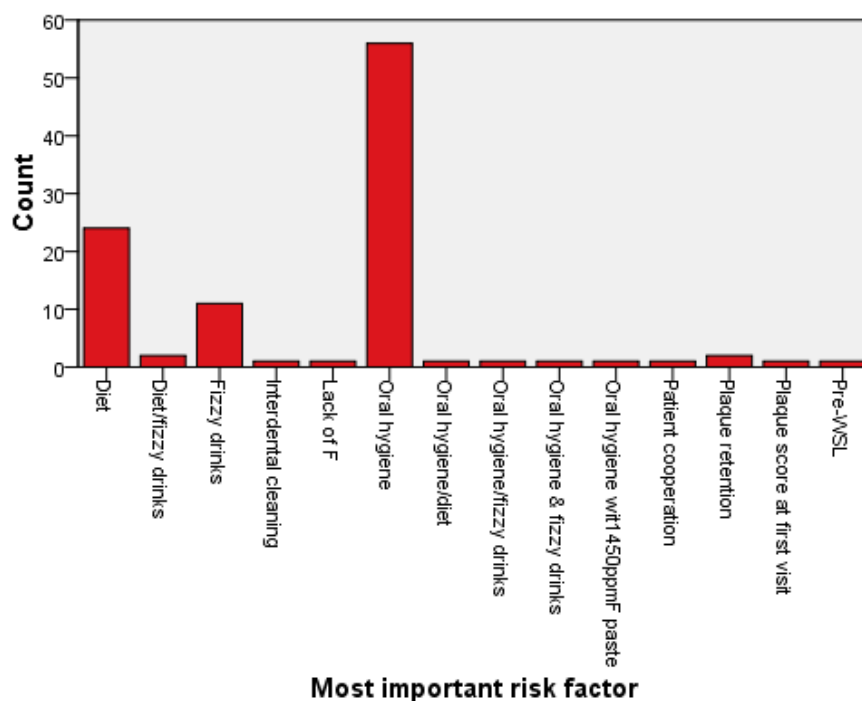


**Figure 8-25 Protocol followed to prevent WSLs during FAOT.**



Count=number of dentists who follow each protocol to prevent WSLs

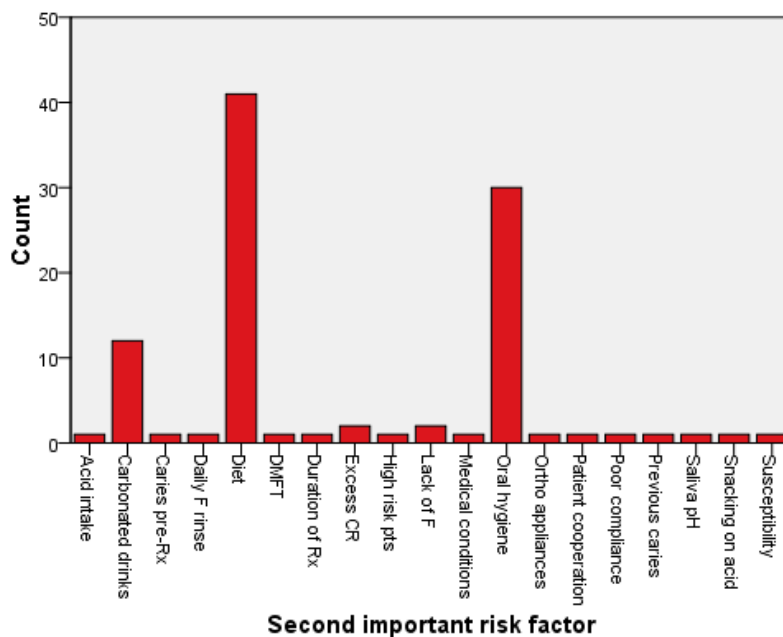
**Figure 8-26 The most important risk factor for developing WSLs identified by responders.**



Count=number of dentists who report the most important risk factor for developing WSLs

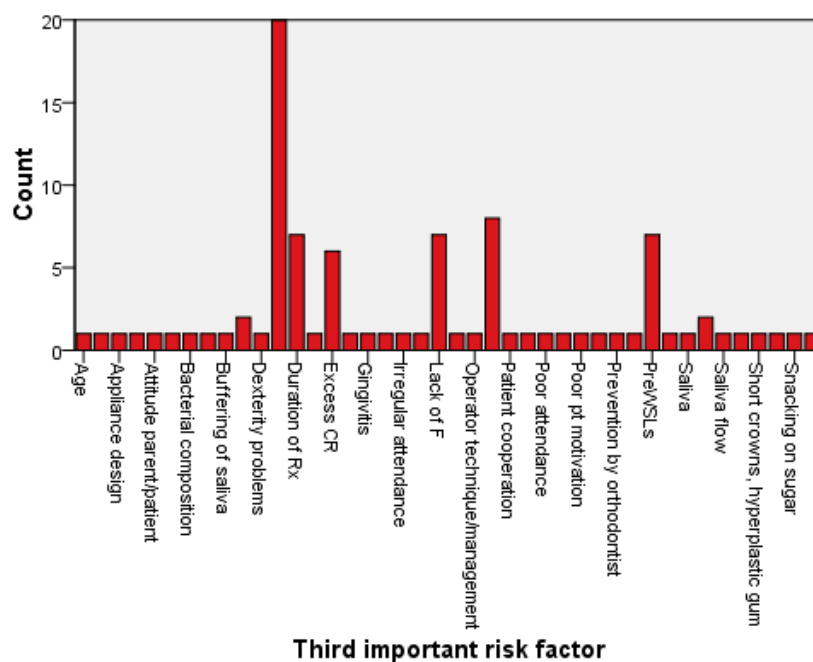


**Figure 8-27 Second most important factors for developing WSLs identified by responders.**



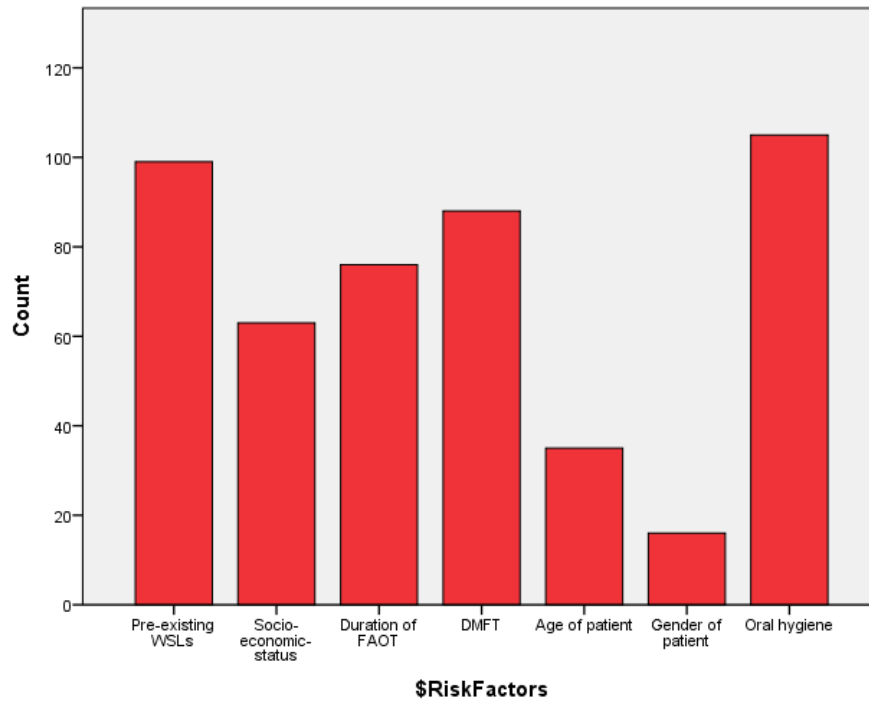
Count=number of dentists who report the second most important risk factor for developing WSLs

**Figure 8-28 Third most important factor for developing WSLs identified by responders.**



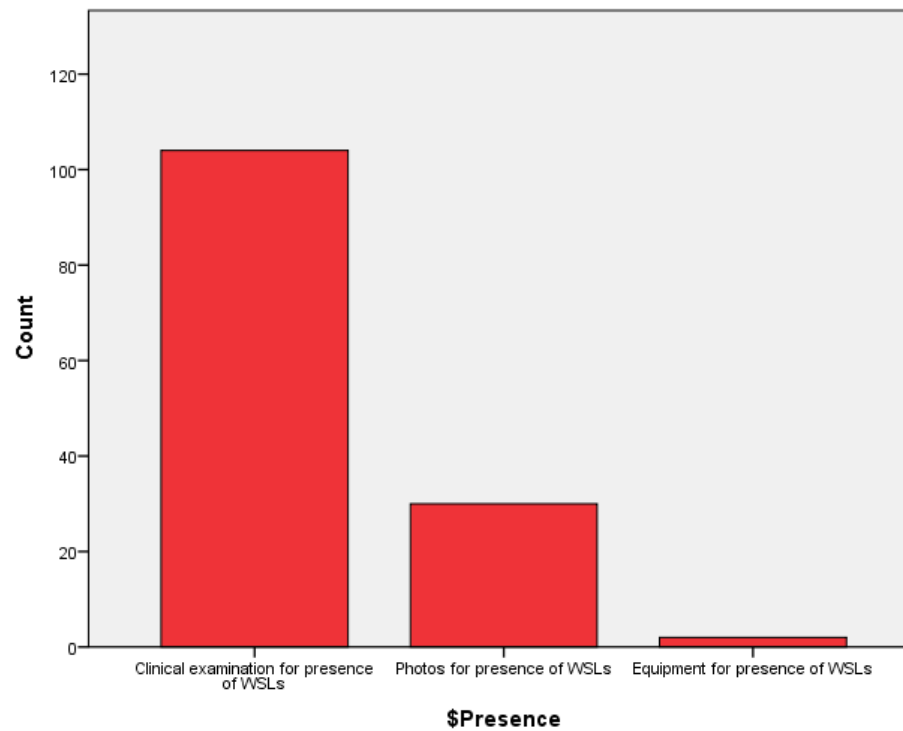
Count=number of dentists who report the third most important risk factor for developing WSLs

**Figure 8-29 Agreement with risk factors for developing WSLs identified in the literature.**



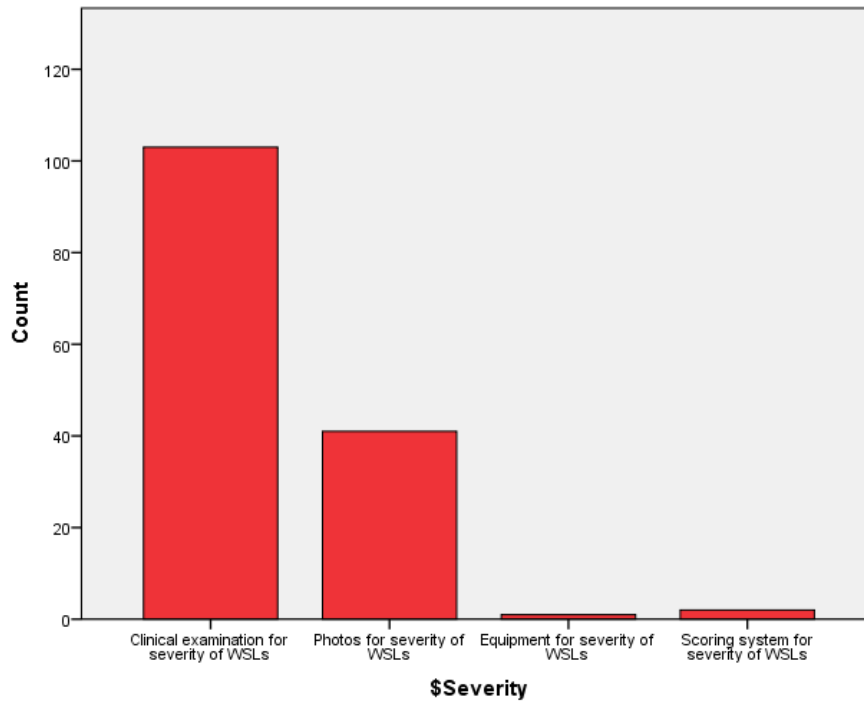
Count=number of dentists who agree with risk factor for developing WSLs

**Figure 8-30 Methods of diagnosis of WSLs as identified by responders.**



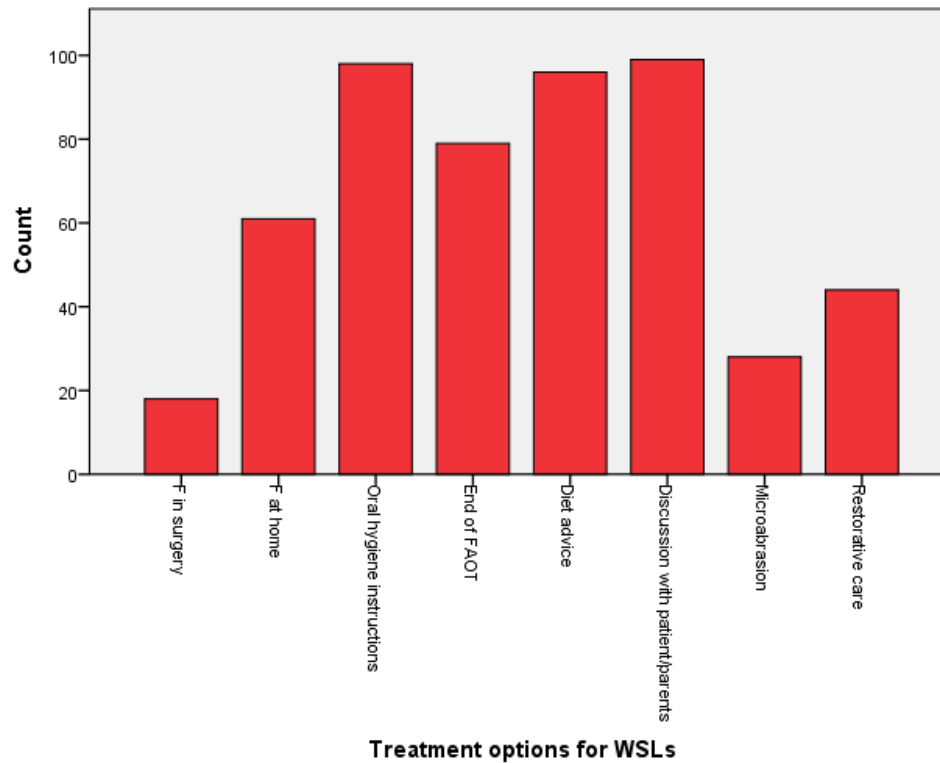
Count=number of dentists using different methods to detect presence of WSLs, \$Presence= methods of diagnosis of presence of WSLs

**Figure 8-31 Methods of assessment of severity of WSLs as identified by responders.**



Count=number of dentists using different methods to assess severity of WSLs, \$Severity=methods of assessment of severity of WSLs.

**Figure 8-32 Methods to arrest/treat WSLs during and/or after completion of FAOT as identified by responders.**



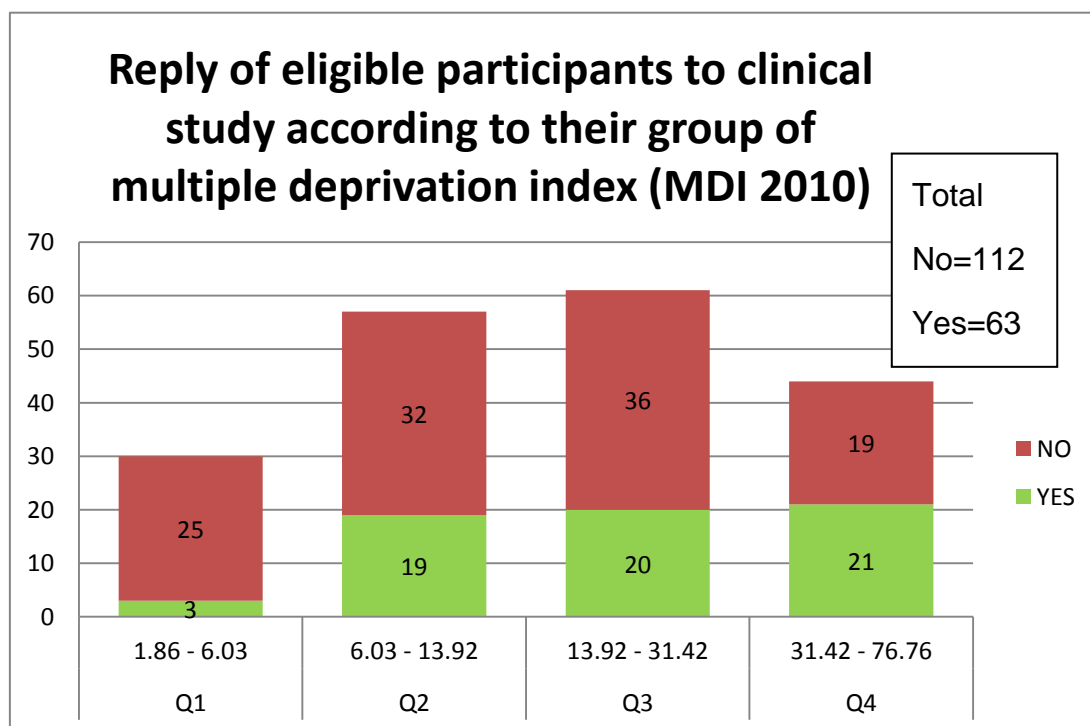
Count=number of dentists using different methods to arrest/treat WSLs

### 8.3 Socio-economic status (SES) of eligible participants in clinical study

After 10 months of recruitment, an interim report was produced and presented in an international conference for a total of 91 eligible participants who had been informed about the study. Amongst them 63/91 volunteered to participate in the clinical trial whereas 28/91 declined. For those who refused to participate, the majority (12/28 or 43%) were living in least deprived areas and the minority (4/28 or 14%) in most deprived areas. For those who agreed to participate, the results were almost the opposite. The majority (20/63 or 32%) belonged to the third quartile across the spectrum of the MDI (MDI 17.37-31.26) whereas the minority (10/63 or 16%) belonged to the least deprived group.

After two years of recruitment, a total of 175 eligible participants had been informed about the study; 112 refused to participate. Similar to findings from the interim report, majority of volunteers and the smallest number of refusals to participate were coming from people living in the most deprived areas. For those who agreed to participate the smallest number came from the least deprived areas. Seen in Figure 8-33, majority of refusals were coming from the second and third quartile across the spectrum of the MDI (MDI 6.03-13.92 & 13.92-31.42).

**Figure 8-33 MDI of eligible participants after two years of recruitment.**



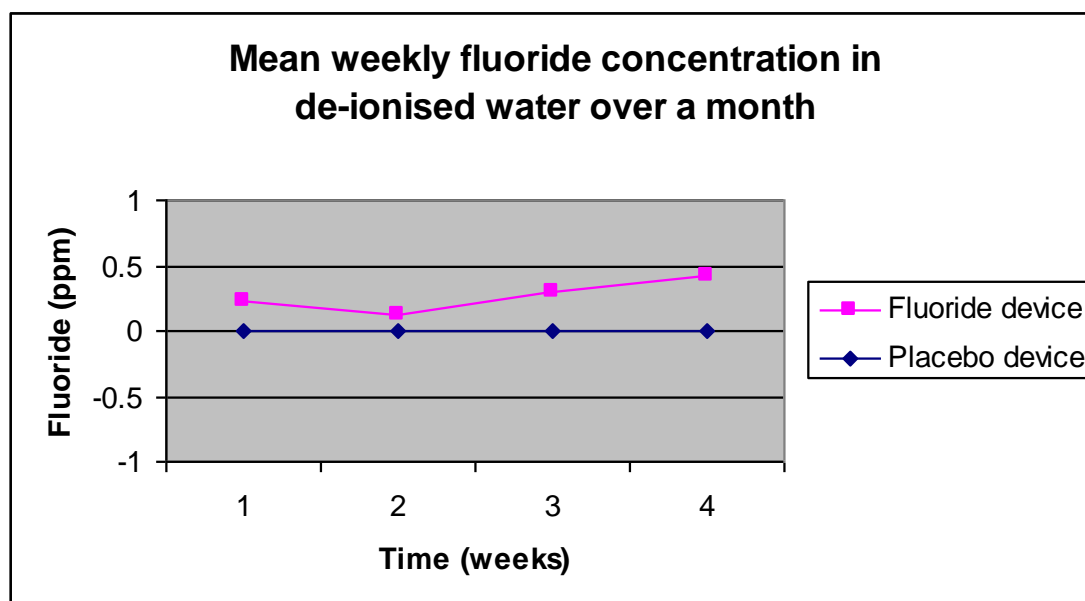
Q=quartile

## 8.4 Clinical study

### 8.4.1 F release from test and placebo bead

To confirm release of F, the test beads (one containing F and one placebo bead) together with their plastic holders were left in 5ml of de-ionised water for a period of one month. Every week for one month, .5ml of de-ionised water was measured for its F content using IC. The results are seen in Figure 8-34, confirming F release from the F devices and no F release from the placebo ones. F standard solutions containing 10, 1, .1 and .01 ppm F were used to test reproducibility of measurements.

Figure 8-34 Mean weekly F release from placebo and SRFGDs in de-ionised water for a month.



#### 8.4.2 Recruitment of volunteers for clinical trial

Based on the sample size calculation recruitment of  $n = 60$  volunteers for the clinical study began in November 2008 following favourable opinion of the Leeds Central Research Ethics Committee and it continued for two years.

In the flowchart of the study seen in Figure 8-35, a total 325 envelopes were posted to potential participants as identified through the booking system of the department; 150 were found not to fulfil inclusion criteria looking through their dental notes; the remaining 175 were eligible participants. From eligible participants  $n=63$  agreed to participate in the study thus the participation refusal rate was 64% (112/175). The study was completed by 40 participants thus the failure to complete rate was 23.8% (15/63). The CONSORT flow diagram is seen in Figure 8-36.

**Figure 8-35 Flow-chart of participants in clinical study.**

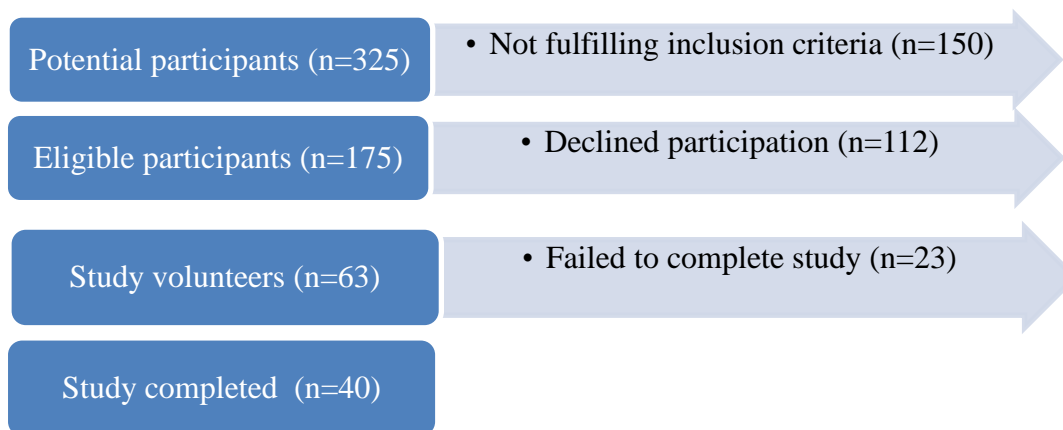
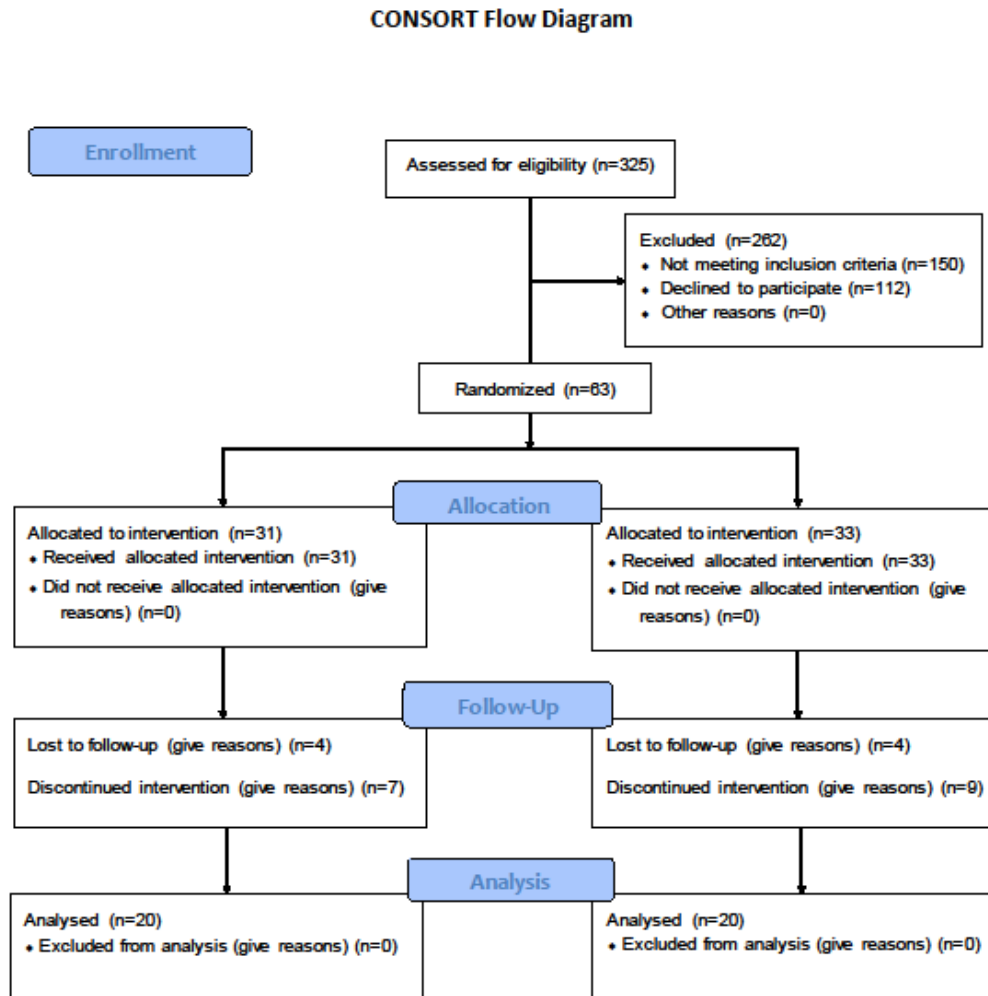


Figure 8-36 CONSORT clinical study flow diagram



### 8.4.3 Randomisation

The randomisation list was provided by a statistician and was produced by a computerised random number generator. The codes were broken by the supervisors at the end of the study and the list is found in the Appendix (Appendix No 13).

#### 8.4.4 Demographics of volunteers

The demographic characteristics of potential participants and volunteers are shown in Table 8-6, Table 8-7, Table 8-8 and Table 8-9.

**Table 8-6 Demographic characteristics of eligible participants who declined participation n=112.**

	Age (years)	MDI	Gender
Range	8-17	3.14-59.05	54 Males
Mean	13.57	19.17	58 Females
Median	13	13.2	

**Table 8-7 Demographic characteristics of study volunteers n=63.**

	Age (years)	MDI	Gender
Range	9 - 17	3.46 – 55.53	30 Males
Mean	12.93	22.65	33 Females
Median	13	20.00	

**Table 8-8 Demographic characteristics of volunteers who failed to complete study n=23.**

	Age (years)	MDI	Gender
Range	10 - 17	3.46 – 52.52	11 Males
Mean	12.95	24.94	12 Females
Median	13	29.11	

**Table 8-9 Demographic characteristics of volunteers who completed study n=40.**

	Age (years)	MDI	Gender
Range	9 – 17	4.12 – 55.53	19 Males
Mean	12.9	20.94	21 Females
Median	13	19.73	



#### **8.4.5 Participants screening failures**

Eight potential participants were screening failures as they did not fulfil inclusion criteria e.g. they had missing upper lateral incisors.

#### **8.4.6 Adverse events**

No adverse events were reported during the study.

#### **8.4.7 Volunteers lost to follow-up**

Eight volunteers were lost to follow up.

#### **8.4.8 Protocol deviation**

In two cases there was a protocol deviation with the SRFGDs placed unilaterally due to space shortage to fit the SRGFD onto the orthodontic wire.

#### **8.4.9 Volunteer withdrawals**

Sixteen participants withdraw from the study.

#### **8.4.10 Duration of treatment for volunteers who completed the study**

The duration of FAOT for volunteers who completed the study was from 5 to 39 months with a mean of  $16.60 \pm 1.18$  months and a median time of 17 months.

#### **8.4.11 DMFT(S)/dmft(s)**

At the start of the study the mean DMFT was  $0.79 \pm 1.6$  with a maximum value of 8.00. In the primary dentition the corresponding mean dmft was  $0.03 \pm 0.25$  with a maximum value of 2.00. The mean DMFS was  $2.46 \pm 5.73$  and a maximum value of 20.00. In the primary dentition the corresponding mean dmfs was  $0.03 \pm 0.25$  with a maximum value of 2.00.

#### 8.4.12 Plaque Index

The Löe Plaque Index has a range from 0-3 and it was used on teeth 16, 12, 24, 36, 32, 44 (FDI notation) at the start of the study. The mean index was  $0.62 \pm 0.35$ .

#### 8.4.13 Gingival Index

The Löe Gingival Index has a range from 0-3 and it was used on teeth 16, 12, 24, 36, 32, 44 (FDI notation) at the start of the study. The mean index was  $0.46 \pm 0.33$ .

#### 8.4.14 Satisfaction questionnaire to orthodontists

The return of the satisfaction questionnaire given to orthodontists at the end of the study was 79.3% (n=50/63) and the replies can be seen in the following table (Table 8-10).

**Table 8-10 Replies from orthodontists to satisfaction questionnaire post FAOT with SRFGDs.**

	Having the SRFGDs was easy	Having the SRFGDs was difficult	Placement of SRFGDs was easy	Placement of SRFGDs was time consuming	SRFGDs interfered with braces
Agree	34.92% (22/63)	63.49% (40/63)	44.44% (28/63)	17.46% (11/63)	60.31% (38/63)
Disagree	44.44% (28/63)	15.87 % (10/63)	44.44% (28/63)	66.66% (42/63)	39.68% (25/63)
Strongly disagree	20.63% (13/63)	20.63% (13/63)	11.11% (7/63)	15.87% (10/63)	n/a

#### 8.4.15 Satisfaction questionnaire to volunteers

The return of the satisfaction questionnaire given to volunteers at the end of the study was 57.81% (n=37/64). The replies can be seen in the following table (Table 8-11).

**Table 8-11 Replies from volunteers to satisfaction questionnaire post FAOT with SRFGDs.**

	Having the SRFGDs was easy	Having the SRFGDs was difficult	SRFGDs was uncomfortable	Different without SRFGDs
Yes	32.43% (12/37)	54.05% (20/37)	75.67% (28/37)	32.43% (12/37)
No	67.56% (25/37)	45.94 % (17/37)	24.32% (9/37)	40.5% (15/37)

#### **8.4.16 Breakage/loss of SRFGDs**

On two occasions there was loss of the SRFGDs whilst the orthodontic wire was changed.

#### **8.4.17 Incidence of WSLs**

Presence/absence of WSLs on the buccal surfaces of the maxillary anterior permanent teeth was assessed at the start of the study using hand-held QLF™ and digital photographs and at the end of the study using digital photographs only.

On a subject level amongst those who completed the study (n=40/63 or 63.49%) the number of subjects who had WSLs (prevalence) at the start was n=2/63 or 3.17% and at the end was n=15/40 or 37.5%. The number of subjects who developed WSLs during FAOT (incidence) was n=13/40 or 32.5%.

On a tooth level from 6x63=378 teeth examined at the start of the study 6x40=240 teeth were available for examination at the end. The six teeth examined were the maxillary permanent central, lateral incisors and canines. The number of teeth with WSLs at the start of the study (prevalence) was n=2/378 or 0.0053% and the number of teeth with WSLs at the end was n=28/240 or 11.67%. Teeth which developed WSLs during the course of the study (incidence) was 26/240 or 10.83%.

We used a Zero Inflated Poisson regression model to account for factors leading to appearance of WSL areas on individuals as well as factors that affect severity of the WSL presence. Measures of model fit are seen in Table 8-12, showing good fit of the model with p value (prob> LR) 0.000. WSL teeth count was used as the dependent variable and Code (placebo/SRFGDs), GI at the start of the study, PI at the start of the study, MDI, DMFT, DMFS as the independent variables. Results were adjusted for the effect of age, gender, outcome and duration.

**Table 8-12 Measures of model fit for the count of teeth with WSLs after completion of the study**

Measures of Fit for zip of WSLCOUNTpost

Log-Lik Intercept Only:	-64.470	Log-Lik Full Model:	-44.979
D(50):	89.958	LR(11):	38.982
		Prob > LR:	0.000
McFadden's R2:	0.302	McFadden's Adj R2:	0.101
ML (Cox-Snell) R2:	0.461	Cragg-Uhler (Nagelkerke) R2:	0.530
AIC:	1.841	AIC*n:	115.958
BIC:	-117.199	BIC':	6.592
BIC used by Stata:	143.818	AIC used by Stata:	115.958

**Table 8-13 Zero Inflated Poisson regression model on a subject level for the presence and severity of WSL. Coefficients and 95% C.I. transformed at the natural logarithm.**

```

Zero-inflated Poisson regression          Number of obs =          63
                                           Nonzero obs  =          19
                                           Zero obs     =          44

Inflation model = logit                  LR chi2(7)    =          30.65
Log likelihood = -44.97879                Prob > chi2   =          0.0001

```

WSLCOUNTpost	Coef.	Std. Err.	z	P> z	[95% Conf. Interval]	
WSLCOUNTpost						
Code	1.056139	.504896	2.09	0.036	.0665611	2.045717
Outcome	-.450532	.5590274	-0.81	0.420	-1.546206	.6451415
Age	-.0632938	.1312121	-0.48	0.630	-.3204648	.1938771
DMFT	.3305468	.2085294	1.59	0.113	-.0781634	.7392569
MDI2010	.059785	.0130157	4.59	0.000	.0342746	.0852953
GIpre	1.978042	.7819115	2.53	0.011	.4455239	3.510561
DMFS	-.0823581	.0542822	-1.52	0.129	-.1887493	.0240331
_cons	-2.469436	2.530165	-0.98	0.329	-7.428468	2.489596
inflate						
Duration	-.3272842	.1540246	-2.12	0.034	-.6291668	-.0254016
Gender	4.054905	2.374391	1.71	0.088	-.5988154	8.708625
GIpre	7.877972	3.552776	2.22	0.027	.9146601	14.84128
PIpre	4.915528	4.155787	1.18	0.237	-3.229665	13.06072
_cons	-8.755239	4.856172	-1.80	0.071	-18.27316	.7626827

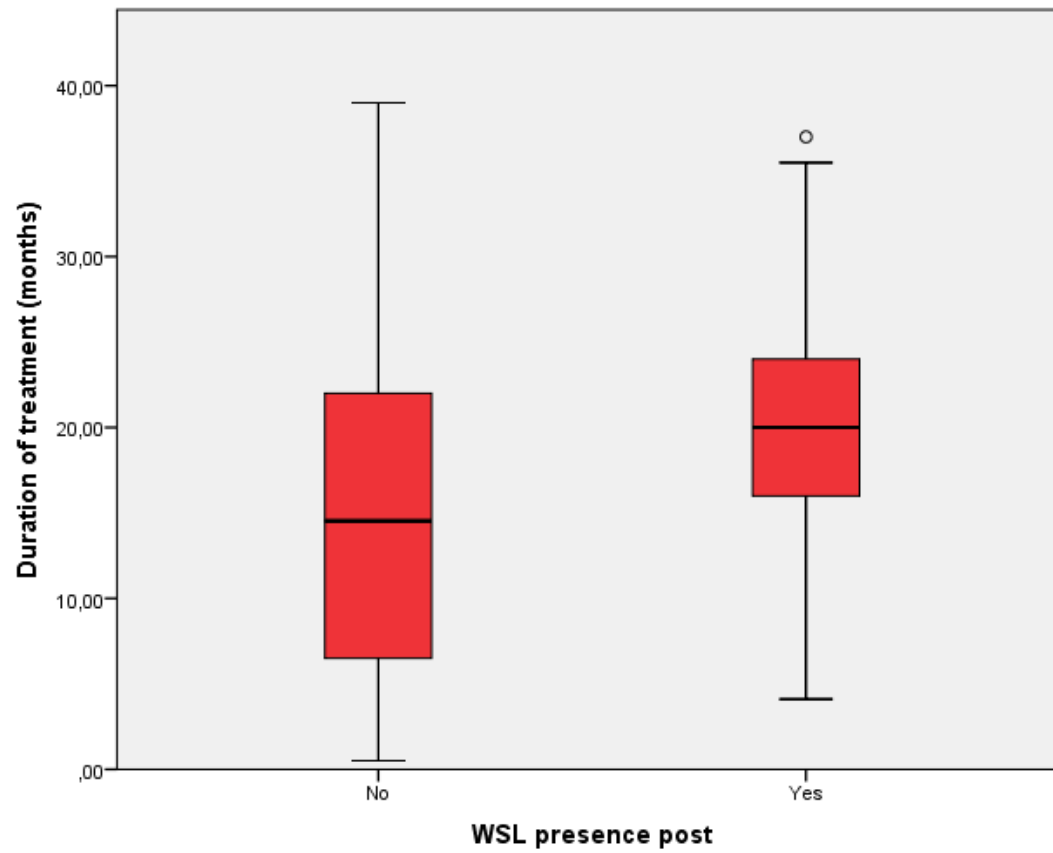
```

Vuong test of zip vs. standard Poisson:      z =          3.36  Pr>z = 0.0004

```

The second part of the analysis seen in Table 8-13, showed that for each unit increase in the duration of the treatment the odds of a patient to have no WSL teeth decrease by  $\exp(-0.3272842) = 0.72$  ( $p=0.034$ ). The 95% Confidence Interval for this estimate is (0.533, 0.975), seen Figure 8-37. The odds ratio is 0.72 with 95% Confidence Interval from 0.533 to 0.974. A closer inspection of the results leads to Figure 8-38, where it becomes quite clear that this inference is mostly due to the difference encountered within the withdrawn group of patients and not to the ones who completed the therapy. Unfortunately the sample size does not allow for the reliable estimation of a p-value for the described interaction and yields non statistically significant differences for the effect of duration on presence of WSL teeth within each group separately.

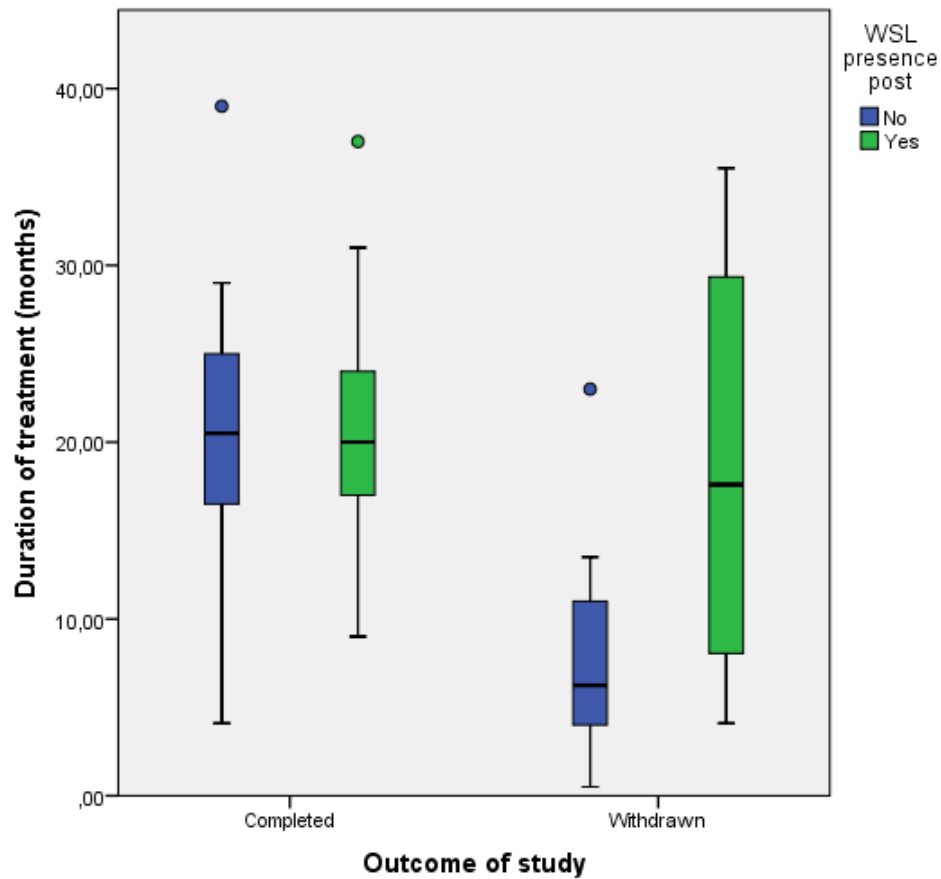
**Figure 8-37 Data distribution for presence of WSLs on a subject level according to duration of FAOT.**



No=subjects who did not develop WSLs during clinical study

Yes=subject who developed WSLs during clinical study

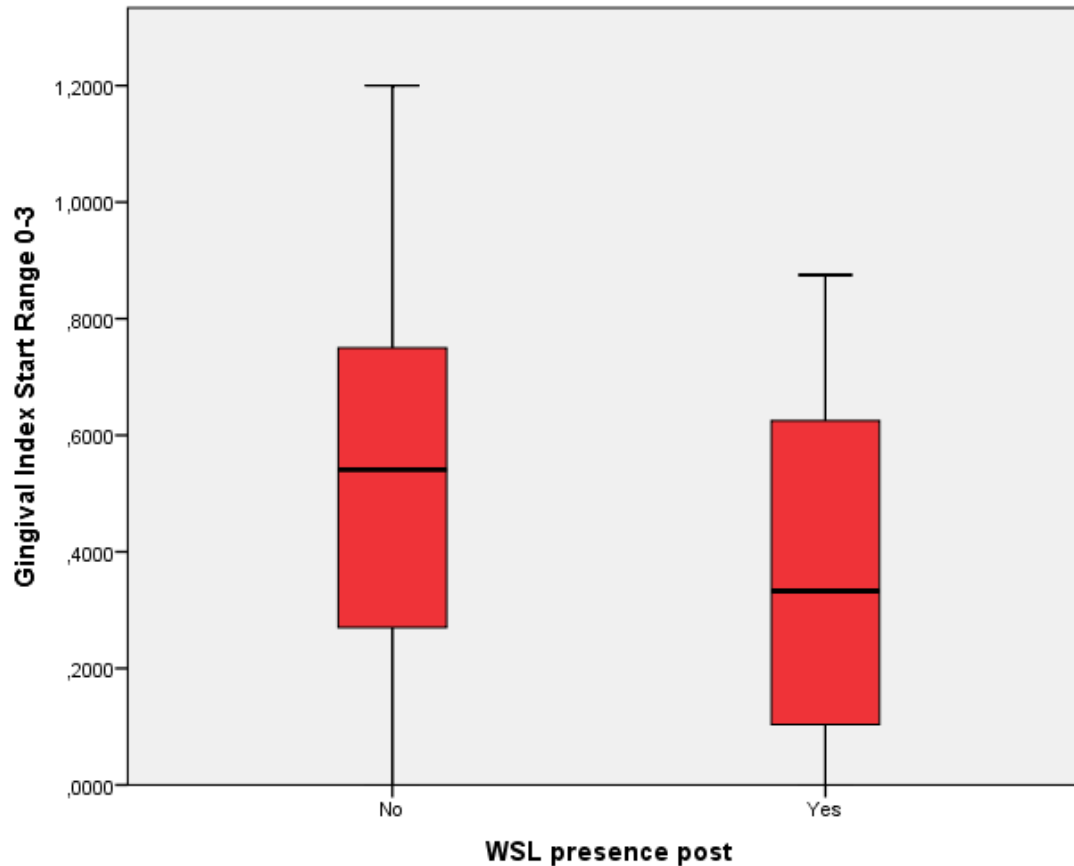
**Figure 8-38 Data distribution for presence of WSLs on a subject level according to duration of FAOT index by outcome of the study.**



Completed clinical study=40 subjects, withdrawn=23 subjects

As seen in Table 8-13 and in Figure 8-39, for each 0,1 unit increase in the GI at the start of the study, the odds of a patient to have no WSLs on his/her teeth increase by  $\exp(7,88) = 2450$  times ( $p=0.027$ ). That is, for a 0,1 unit increase in the GI at the start of the study, the odds of a patient to have WSL teeth increase by 245 times. The C.I. for this estimate is (0.914 – 14.841) seen in Table 8-13. The odds ratio for GI at the start of the study is 2638.52 with 95% C.I. from 2.49 to 2789240.79. The wide C.I. for this estimate is due to the small sample size so we cannot quantify the size of the effect.

**Figure 8-39 Data distribution for presence of WSLs on a subject level according to gingival index at the start of the study.**



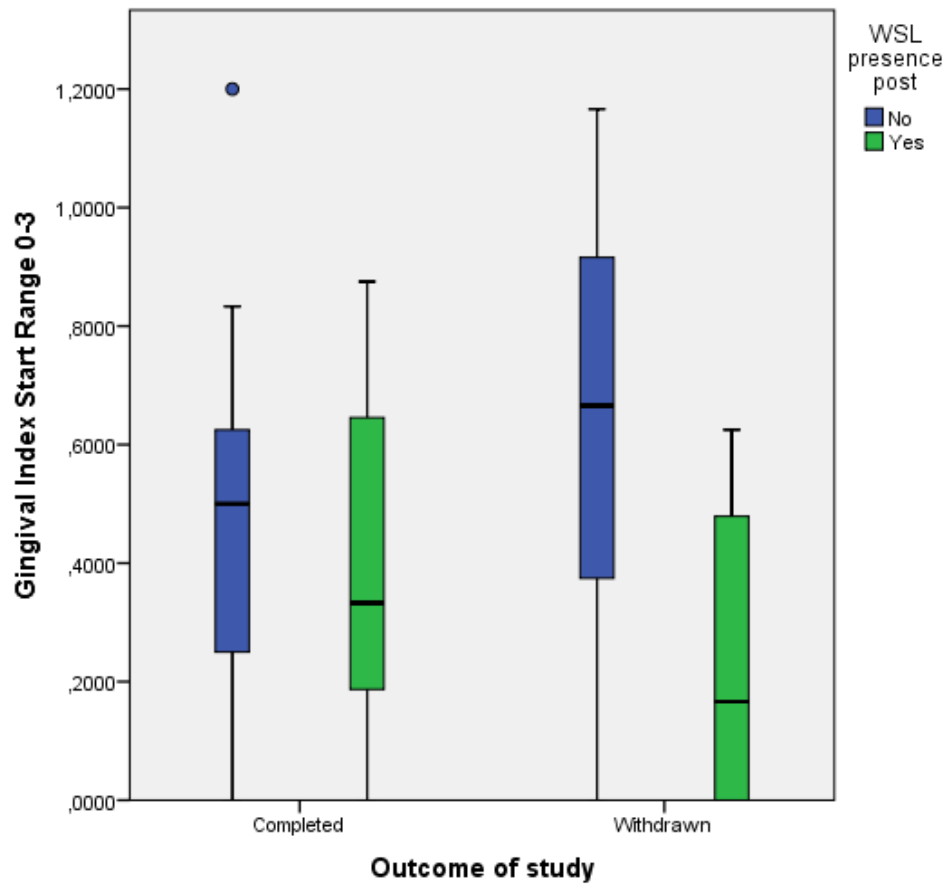
No=number of subjects who did not develop WSLs in the clinical study

Yes=number of subjects who developed WSLs in the clinical study

A closer inspection of the results leads to Figure 8-40, where it appears that this inference is mostly due to the difference encountered within the withdrawn group of patients and not to the ones who completed the study. Again, unfortunately the sample size does not allow for the reliable estimation of a p-value for the described difference and yields non-statistically significant differences for the effect of GI at the start of the study on presence of WSL teeth within each group separately. Therefore the GI index at the start of the study is of importance only for those who do not complete the treatment.



**Figure 8-40 Data distribution for presence of WSLs on a subject level according to gingival index by outcome of the study.**



Completed clinical study=40 subjects, withdrawn=23 subjects

#### **8.4.18 Kappa coefficient estimation for incidence of WSLs**

The intra-examiner reliability expressed as kappa score for presence/absence of WSLs assessed on digital photographs is estimated 0.82 showing good agreement (Randolph, 2008).

#### **8.4.19 Severity of WSLs**

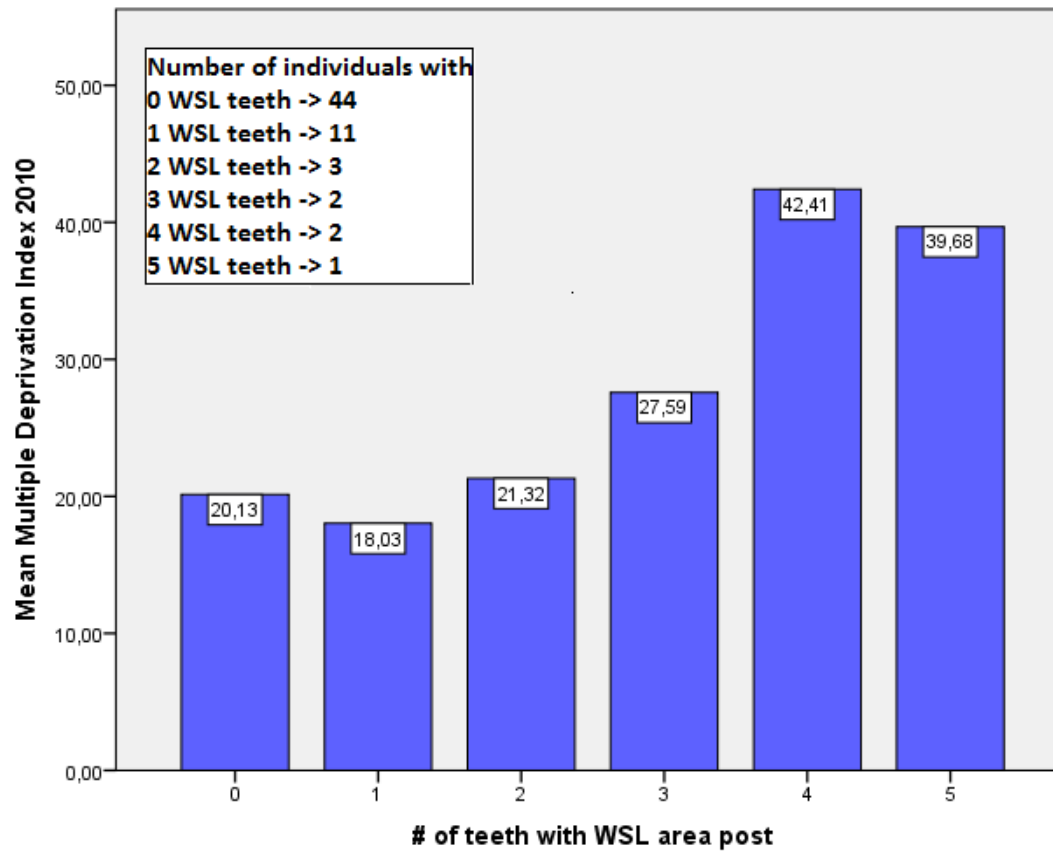
Although severity of WSLs was assessed using the surface area of the WSL in relation to the whole buccal surface area of the tooth and it was expressed as a percentage of the whole buccal area at the start and at the end of the study data distribution led to an analysis of the obtained sample of patients

with no statistical significant results in any case. Instead the number of teeth with WSL on each participant was used as a measure of the severity met.

According to the first part of the Zero Inflated Poisson regression seen in Table 8-13, MDI, GI at the start of the study and use of placebo/SRFGDs (Variable named Code) are variables statistically significant for the count of teeth with WSL in individuals.

If the MDI index of a patient was to increase by one the expected number of teeth with WSL would increase by a factor of  $\exp(0.059785) = 1.06$  (6%) while holding all other variables in the model constant (That is a 60% OR increase for a 10 unit MDI difference). Thus, the higher the MDI index, the more WSL teeth predicted ( $p < 0.001$ ), seen in the following figure (Figure 8-41). The odds ratio for this variable is 1.06 with 95% Confidence Interval from 1.035 to 1.089.

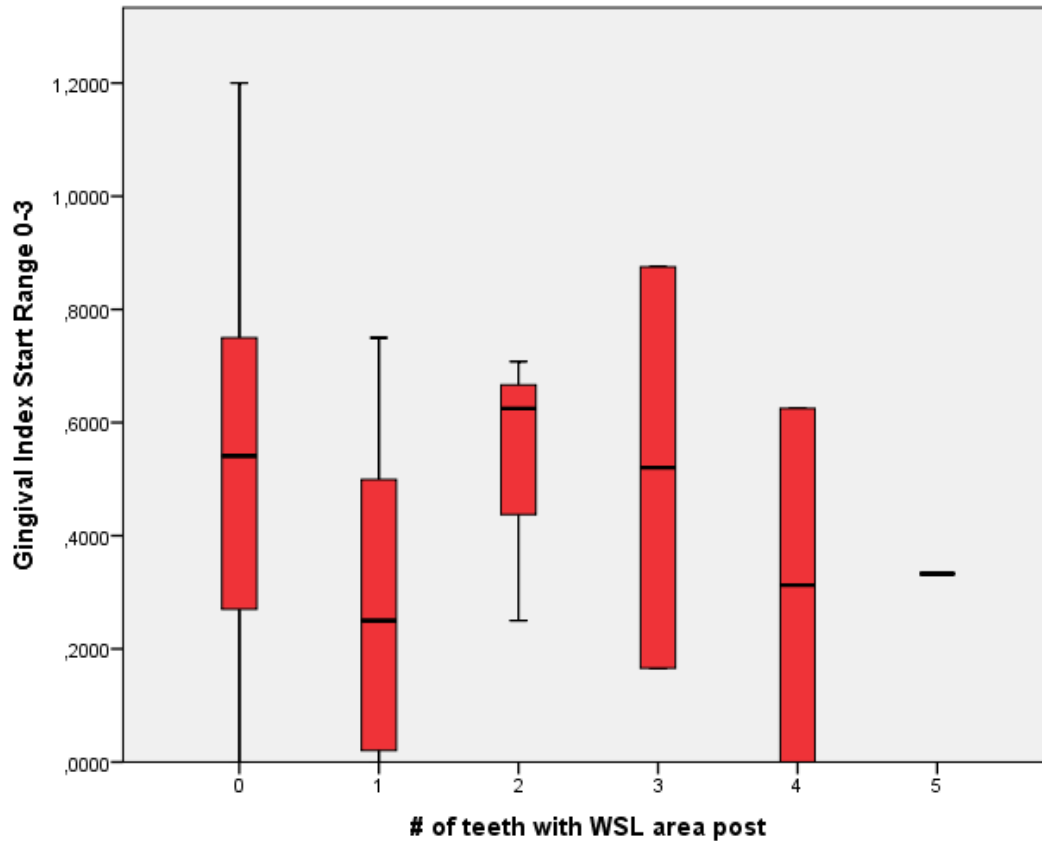
**Figure 8-41 Number of teeth with WSLs predicted as per Multiple Deprivation Index.**



#=number of teeth

If the GI at the start of the study of a patient was to increase by one the expected number of teeth with WSL would increase by a factor of  $\exp(1.978042) = 7.23$  times, while holding all other variables in the model constant. Thus, the lower the GI at the start of the study, the more WSL teeth predicted ( $p=0.011$ ) as seen in the following figure (Figure 8-42).

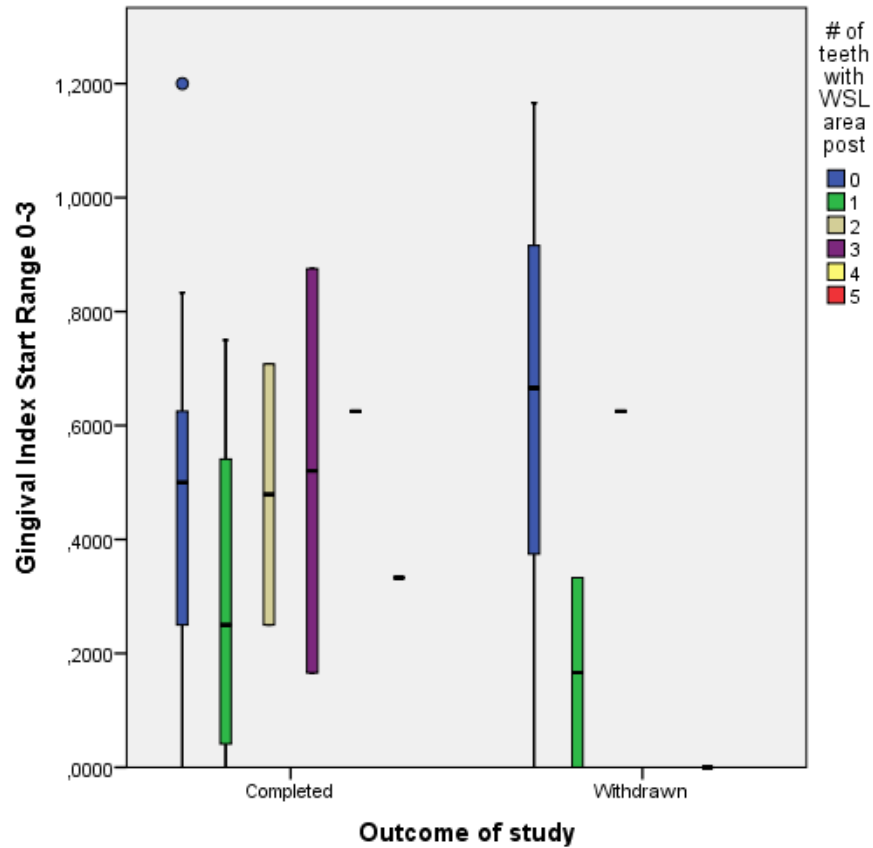
**Figure 8-42 Number of teeth with WSLs predicted according to GI at the start of the study.**



#=number of teeth

A closer inspection of the results leads to Figure 8-43, where it appears that this inference is mostly due to the difference encountered within the withdrawn group of patients and not to the ones who completed the therapy where differences seem to be non-significant. Again, unfortunately the sample size does not allow for the reliable estimation of a p-value for the interaction between outcome and GI at the start and yields non-statistically significant differences on the count of teeth with WSL presence. Therefore the GI index at the start of the study is of importance only for those who do not complete the treatment.

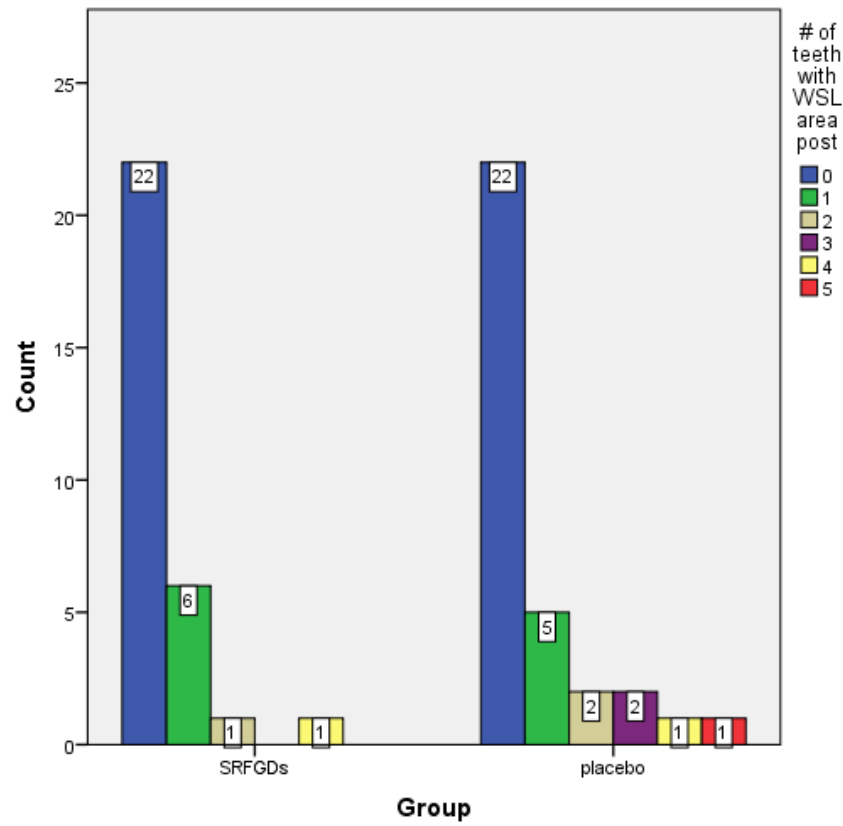
**Figure 8-43 Data distribution for presence of WSLs on a subject level according to gingival index by outcome of the study.**



Completed clinical study=40 subjects, withdrawn=23 subjects

The expected number of WSL teeth for a placebo patient is  $\exp(1.05639) = 2.88$  higher than the expected number of a SRFGDs patient while holding all other variables in the model constant ( $p=0.036$ ). The odds ratio for use of a placebo is 2.87 with 95% C.I. from 1.069 to 7.734. Thus more WSLs are expected for the placebo patients than for the patients receiving the treatment seen in the following figure (Figure 8-44).

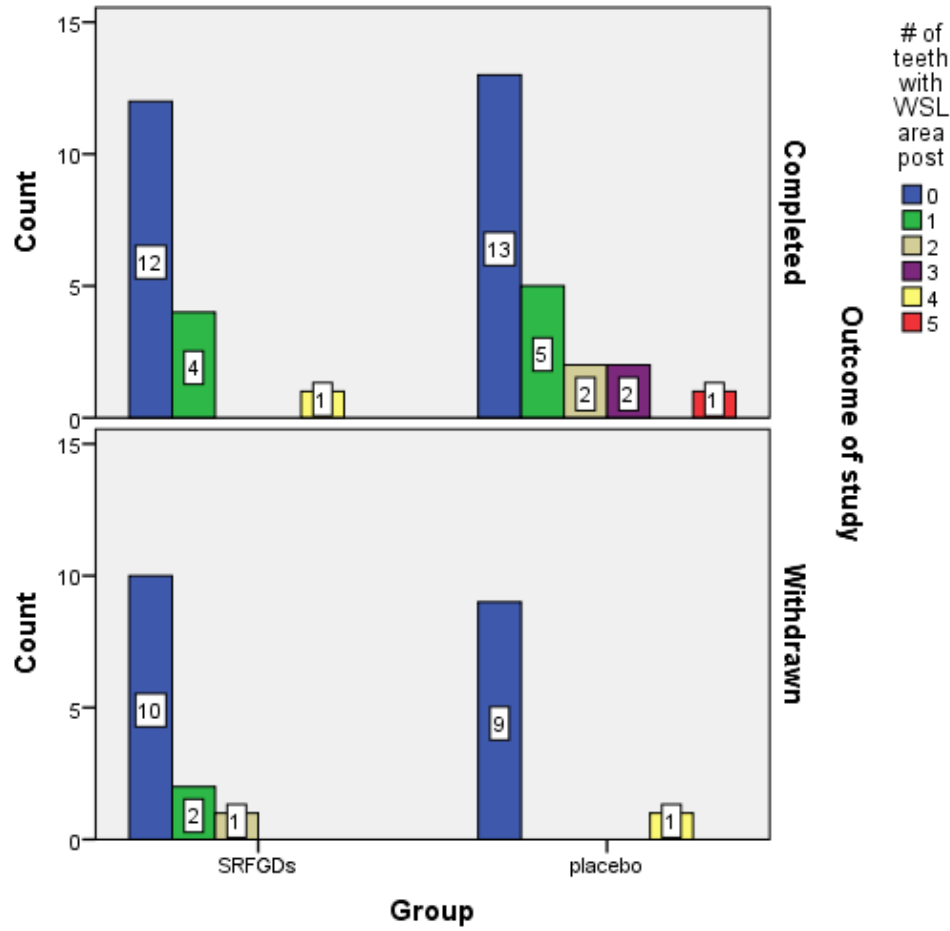
**Figure 8-44 Difference in presence of WSLs on a subject level between those who received treatment and those who received the placebo.**



Count=number of subjects, #=number

As shown in Figure 8-45, the result again seems to be deriving by the group of therapy completion and not by the withdrawn one.

Figure 8-45 Difference in presence of WSLs on a subject level between those who received treatment and those who received the placebo.



Completed=40 subjects, withdrawn=23 subjects, Count=number of subjects, #=number

## 9 Discussion

### 9.1 *In-vitro* exploratory study

The aim of the exploratory study was to assess primarily F and PO<sub>4</sub> release with IC but also bond strength of a bonding material produced by mixing a composite resin (Transbond®) with SRFGDs in powder form.

The first step was to check the materials for any background F and PO<sub>4</sub> release that could mask the results. It was confirmed that Transbond® had minimal F release (1.27ppm was the maximum F release) and PO<sub>4</sub> (51.62ppm was the maximum PO<sub>4</sub> release) over 28 days as seen in Figure 8-3 and Figure 8-4. The storage medium chosen was de-ionised water in order to enhance ion release from the material over time. The samples were prepared using a PTFE mould seen in Figure 7-1 to ensure similar surface area.

The storage medium for the study had to be clearly defined, not compromising ion release from test and control materials and if possible to mimic intra-oral conditions or at least not to create an environment where materials would behave completely differently than in the mouth. For these reasons AS and different types of water namely de-ionised, distilled, tap and sterile waters were assessed for their F and PO<sub>4</sub> release. Samples were prepared in an identical way and after 14 days the results showed that AS were more stable for PO<sub>4</sub> release compared to tap and distilled water as seen in Figure 8-2. Also PO<sub>4</sub> release from distilled and de-ionised water was closer to zero as expected. In terms of F release as seen in Figure 8-1, the AS was again stable and showed the highest F release with a range of 0.52-0.65 ppmF with all other storage mediums showing F release of closer or less than 0.1 ppmF. Choosing storage mediums with minimal ion release (either F and/or PO<sub>4</sub>) would enhance ion release since the material would appear to be supersaturated in an “empty” liquid storage medium. On the other hand release levels were low compared to SRFGD either in a bead or powder forms therefore would provide a minimal background level that would not overlap with F and PO<sub>4</sub> from test materials.



### 9.1.1 F and PO<sub>4</sub> release from powder from SRFGDs in AS

All different types of powder were assessed for their F and PO<sub>4</sub> release prior to mixing with Transbond®. The ideal was to combine the highest F release with the lowest PO<sub>4</sub> release in order to meet both objectives. The primary objective was to enhance F presence and prevent demineralisation but also minimise PO<sub>4</sub> release which shows degradation of the test material as it is a PO<sub>4</sub> based glass. Cumulative release after two months showed that powder with solubility 16 and a particle size of more than 38µm had the highest F release (365.28 ppmF) and also the highest PO<sub>4</sub> release (192.64 ppmPO<sub>4</sub>). This powder would have an enhanced F presence but at the expense of material loss. The powder with the lowest PO<sub>4</sub> release had solubility of 1 and a particle size of less than 38µm (149.90 ppmPO<sub>4</sub>) whereas its F release was 297.27 ppmF *i.e.* 18.6% less than the highest. The powder with solubility of 1 and a particle size of more than 38µm would better meet both objectives as it had the second highest F release, with a difference of 12.30% (320.64 ppmF) and PO<sub>4</sub> release (180.48 ppmPO<sub>4</sub>) increased by 16.94% more compared to the lowest as seen in Figure 8-6 and Figure 8-7.

### 9.1.2 Bond strength of test and control materials

A study investigated differences for flexural modulus of elasticity and flexural stress in samples stored at a controlled room temperature or 37°C. Results showed higher flexural stress at 37°C by 9.1% and lower flexural modulus of elasticity by 12.38% as seen in Figure 8-23. Both differences were considered not clinically significant therefore storage of samples in AS in controlled room temperature was acceptable.

### 9.1.3 Summary of *in-vitro* exploratory study

With all the limitations of an exploratory study in order to develop a methodology to test a new material for its bond strength, F and PO<sub>4</sub> release, a number of parameters were investigated. Storage medium and room temperature for storage of material samples need to be controlled so as not to alter the performance of the material. This way the methodology is reproducible and it enhances the strength of the *in-vitro* environment compared to clinical studies which are to test the outcome in question without any confounding variable.

The PFTE mould was chosen to produce samples with identical shape and size whether tested for F, PO<sub>4</sub> release, flexural modulus of elasticity or flexural stress. The concern was again reproducibility and well defined and controlled settings.

Samples were scanned with profilometry and SEM in order to obtain a close up “image” of their surface morphology to assess for “breaks” as they had been reported in cases on enamel sealants (Frazier et al., 1996).

IC has a detection limit of 0.001ppm (or 1ppb) in liquids free of organic material. It is possibly the method of choice to assess both F and PO<sub>4</sub> release as it can detect all ions. There was a marked decrease in F and PO<sub>4</sub> release from SRFGDs in the form of powder left in AS or “blocked” in composite resin of the control material.

Investigation of all 40 different types of powder solubility, ratio of powder to control material and particle size over six months gave an insight as to how the test material would perform. One sample for each of the 40 different types was investigated therefore no conclusions could be made. The next step would be to increase the sample size for all 40 different combinations to at least n=5 giving a total of n=200 samples to investigate. The alternate would be to increase the sample size only for the combinations showing promising results from the exploratory study. The most promising findings came from the powder with solubility of 1 and a particle size of more than 38µm. It showed the second highest F release, with a difference of 12.30% (320.64 ppmF) and PO<sub>4</sub> release (180.48 ppmPO<sub>4</sub>) increased by 16.94% more compared to the lowest. When powder was mixed with Transbond® the most promising results after six months were obtained when powder with particle size of less than 38µm was mixed at a ratio of 1:8 with Transbond® with solubility of either 1 or 16. These three different types of powder would need to be tested on a larger scale to detect F and PO<sub>4</sub> release after mixing with composite resin and also bond strength of the test material bonded onto human premolar teeth.

## **9.2 Questionnaire to orthodontist-members of the British Orthodontic Society**

The response rate to the questionnaire was very low (7.85%) even though it was an electronic questionnaire emailed twice via the British Orthodontic Society and it concerned a clinical risk during every day practice of FAOT. The median year for obtaining orthodontic qualification for responders were

1997, that is 5 years after the last review on prevalence/incidence of WSLs (Mitchell, 1992b). Early termination and need for restorative care were the most common problems reported which indicated either early diagnosis of WSLs and early action or late diagnosis of WSLs with all the consequences thereof. F was at the centre of prevention of WSLs either as daily mouth rinse or tooth paste, alongside professional plaque removal. Oral hygiene, diet and duration of FAOT were believed to be important factors for developing WSLs. The orthodontist may not have direct control though as use of F, oral hygiene and diet depends on compliance and the behaviour of the individual whereas professional plaque removal may be performed by other members of the dental team, leaving the duration of FAOT to be under the direct supervision of the orthodontist. For majority of risk factors as identified in the literature responders agreed that they contribute to development of WSLs. Few responders though agreed that the age and gender of the patient played a significant role. Clinical examination with or without photographs were the main tools for presence and severity of WSLs without a specific methodology employed. In the unfortunate situation where WSLs did develop the majority of responders would discuss the problem with the patient/parent(s) offering diet and oral hygiene advice. Their next step though would be to end FAOT and allow self-healing rather than employ microabrasion or F application, either at home or in surgery.

### **9.3 Socio-economic status of eligible participants in clinical study**

The MDI is part of the English Indices of Deprivation 2010 available from the Department for Communities and Local Government, Indices of Deprivation 2010 and it is used to assess the socio-economic-status of eligible participants. The 2010 Index has a range of 1.86-76.76 at Lower Layer Super Output Area and it was constructed by combining the seven transformed domain scores, using the following weights:

- Income (22.5%)
- Employment (22.5%)
- Health and Disability (13.5%)
- Education, Skills and Training (13.5%)
- Barriers to Housing and Services (9.3%)
- Crime (9.3%)
- Living Environment (9.3%)

Majority of eligible participants who volunteered to participate in the study and the smallest number of refusals lived in the most deprived areas according to the MDI. Eligible participants living in the least deprived areas formed the smallest proportion of volunteers to the study. Majority of refusals came from eligible participants living in areas with a MDI from the second and third quartile across the range. Participation in a research study is voluntary and this fact by nature can bring imbalance to the study sample which could introduce bias. In this study this imbalance was investigated because socio-economic-status has a strong relationship with provision of FAOT (Germa et al., 2010) and because of the number of eligible participants refusing participation was high (64% or 112/175). For future studies it is probably unethical to include socio-economic status amongst the inclusion criteria but a statistical sampling technique may need to be employed rather than having a convenience sample.

## **9.4 Clinical study**

### **9.4.1 Recruitment of volunteers for clinical study**

Recruitment of volunteers lasted for almost two years with a total of 325 envelopes posted to potential participants. Almost half of them ( $n=150/325$  or 46.1%) did not fulfil inclusion criteria and this high percentage highlights the need for development of protocols for effective identification via hospital electronic records or for less strict inclusion criteria. Better electronic record keeping or logging patients diagnosis would help exclude or include groups of patients. In this study patients with missing maxillary lateral permanent incisors were excluded and since in a hospital many such hypodontia cases would have been referred it would have been useful to be able to identify such cases electronically and avoid inviting them to participate in a study where they cannot take part anyway.

As discussed previously many declined participation ( $n=112/175$  or 64%) which was of great concern and possibly a weakness of this study. However participation in a research study is voluntary, however it may be more difficult to recruit children for clinical research studies in another department. The voluntary nature of recruitment may produce an unbalanced sample which could introduce bias to the study. In this study participants and those who declined participation came from different socio-economic background. Majority of volunteers and the smallest number of refusals to participate were coming from people living in the most deprived areas. For those who agreed to participate the smallest number came from the least deprived areas. This finding may be of interest for recruitment in future studies but also to be quantified whether it could have a significant impact on the sample.

### **9.4.2 Randomisation**

Patients were randomised based on their gender because it was believed that gender played a role in seeking FAOT, oral hygiene and there was no clear outcome as to whether it should be considered a risk factor for development of WSLs.

### **9.4.3 Demographics of eligible participants, volunteers and volunteers who failed to complete the study**

There was no great difference in mean and median value for age and MDI index with almost equal number of boys and girls who completed the study (n=40/60 or 66.67%). The final study group which was analysed appeared to be balanced for these three variables and the same applied for the group who failed to complete the study (n=23/60 or 38.33%). For those who declined participation majority were females (58 compared to 54 males) and they were slightly older by 0.64 months but still the median age was 13 years old for all groups.

### **9.4.4 Participants screening failures, adverse events and protocol deviation**

Inclusion criteria were not fulfilled in only eight cases from those who volunteered to participate. It is the norm to accept cases with high index of orthodontic treatment needs in a hospital setting as these would include hypodontia cases with maxillary lateral permanent incisors frequently missing. On the other hand these teeth seem to be frequently affected by WSLs and due to their location they are of aesthetic concern and value in terms of prevention of WSLs. In two cases there was a protocol deviation due to space shortage to fit bilaterally the glass bead in non-extraction cases. Still one bead was placed so the study was not suspended.

### **9.4.5 Volunteers lost to follow-up and withdrawals**

Eight volunteers were lost to follow-up either because they stopped FAOT or moved out of the area. The remaining 16 volunteers who withdrew from the study felt that the SRFGDs were uncomfortable or they were “fed up” as it was frequently quoted. Volunteers mainly withdrew from the study either at the beginning or towards the end of the study; this could possibly indicate difficulties with FAOT itself as there is an adjustment period at the start of FAOT and patients may well have been overwhelmed or at the end of FAOT when patients were tired from a long course of treatment.

#### **9.4.6 Duration of treatment for volunteers who completed the study**

The mean duration of FAOT was  $16.60 \pm 1.18$  months with a wide range of five to 39 months. The median time (17 months) was not different to the mean time so it is clear that the five months duration of FAOT was an outlier.

#### **9.4.7 DMFT(S)/dmf(s)**

In general these indices were low at the start of the study and few subjects had the highest values. During FAOT in some cases extractions are requested increasing the DMFT by the end of the study however these extractions are not a result of caries and should not be included in the post-FAOT DMFT(S) measurement.

#### **9.4.8 Plaque and Gingival Index**

The mean plaque and gingival index had a range from 0-3 and at the start of the study it was low;  $0.62 \pm 0.35$  for the plaque and  $0.46 \pm 0.33$  for the gingival index. This is probably expected as the participants were about to start FAOT and control of oral hygiene is of paramount importance.

#### **9.4.9 Satisfaction questionnaire to orthodontists**

There was a quite good return rate from orthodontists of 79.3% ( $n=50/63$ ) and even though there was a small number of orthodontists who replied ( $n=7$ ) they treated 63 different cases therefore for each treated case the orthodontist was given a questionnaire. Results showed that orthodontists overall were not satisfied with the SRFGDs as they felt it interfered with the orthodontic wire and brackets by 60%. Time was not a problem as 80% either disagreed or strongly disagreed that they were time consuming. Placing SRFGDs seemed to be a difficult task for 55% of them. There were mixed results whether it was easy or difficult to have SRFGDs as part of their fixed appliance but that would probably depend on the case; for example a crowded dentition treated on a non-extraction basis would probably provide just sufficient space for the SRFGDs. Majority of orthodontists who treated volunteers in the study were under specialist clinical training thus lacking clinical experience, which may also contribute to the difficulties faced by them.

#### **9.4.10 Satisfaction questionnaire to volunteers**

There was a good return rate from volunteers by 57.81% (n=37/64). Overall volunteers found it difficult (46%) to have the SRFGD in their mouth alongside their fixed appliance and it felt uncomfortable (76%). Interestingly though they felt that it would have been no different if the SRFGDs were not present (41%) therefore it may be that the fixed appliance could have been an equally contributing factor in their overall dissatisfaction.

#### **9.4.11 Breakage/loss of SRFGDs**

The limited number of losses and/or breakages of SRFGDs whilst the orthodontic wire was changed clearly indicate that the design of the appliance proved to be effective.

#### **9.4.12 Photographic technique for assessment of WSLs**

The photographic technique used followed the protocol of previous study (Kanthathas et al., 2005b). Staff members of the photography department were familiar with cross polarizing technique and used standard equipment in the photography laboratory like wall mount camera holders to take the photos. Photos were stored in JPEG format which allows lossy compression typically at a scale of 10:1 for digital images. This lossy compression means that some original image information is lost and cannot be restored, possibly affecting image quality.

Assessment of presence and severity of WSLs was performed using digital 32-bit coloured photographs and measuring the mean grey value on a pixel level using Adobe Photoshop® software as shown in Figure 7-8 to Figure 7-16. Alternative methods to enhance data management based on their grey value would be to use black and white photos combined with a reference greyscale index. This approach would enhance standardisation and reproducibility of the technique for other researchers and/or examiners of the photos. Photos could also be stored and processed as 12-bit JPEG image provided it is a greyscale photo. Storage of photos in TIFF (Tagged Image File Format) format would use no compression and as a result files can be edited without losing image information and image quality.



### 9.4.13 Incidence of WSLs

The six teeth examined for presence/absence of WSLs were the maxillary permanent central, lateral incisors and canines. The choice was based on the facts that these teeth are of most aesthetic concern due to their location and are frequently affected by WSLs as seen in Table 2-4.

On a subject level amongst those who completed the study ( $n=40/63$  or 63.49%) the number of subjects who developed WSLs (incidence of WSLs) was 32.5% or  $n=13/40$ . The number of subjects with WSLs at the start of the study was  $n=2/63$  or 3.17% and at the end was  $n=15/40$  or 37.5%. Previous review has reported a range for patients from 2-96% (Mitchell, 1992b) whereas material published from 1992 report a range from 10.7 - 73% as seen in Table 2-3.

On a tooth level from  $6 \times 63 = 378$  teeth examined at the start of the study  $6 \times 40 = 240$  teeth were available for examination at the end. The number of teeth with WSLs at the start of the study were  $n=2/378$  or 0.0053% and at the end was  $n=28/240$  or 11.67%. The number of teeth which developed WSLs (incidence of WSLs) during the course of the study was  $n=26/240$  or 10.83%. Previous review has reported a range from 0-24% (Mitchell, 1992b) and material published thereafter reported a range from 1.9-76.8% of teeth as seen in Table 2-3.

Findings from this study appear to be low both on a subject level (32.5%) and 10.83% on a tooth level. Previous studies investigating WSLs on maxillary permanent teeth showed different results. In many studies (Banks and Richmond, 1994, Marcusson et al., 1997, Millett et al., 1999, Wenderoth et al., 1999, Banks et al., 2000, Ogaard et al., 2001) WSLs were scored using a variety of indices described in Table 2-1, for example index by Gorelick in 1982, Enamel Decalcification Index by Banks and Richmond 1994, Geiger 1988. These indices have a range from 0-3 and WSLs are assessed as none, mild/moderate, severe leaving results open to discussion as to what is the difference between a mild, moderate and a severe WSL. Two studies (Trimpeneers and Dermaut, 1996, Tobin, 2001) categorised WSLs as being either present or absent. In a split mouth design study 12.7% of teeth in the control group developed WSLs (Trimpeneers and Dermaut, 1996). In the other study with two parallel groups 19% of teeth in the control

group developed WSLs (Tobin, 2001). Findings from this study was 10.83% on a tooth level, much lower compared to aforementioned studies.

Possible explanations for findings in this study are strict acceptance criteria for provision of FAOT in a hospital setting despite the increased orthodontic treatment needs however majority of previous studies though have been undertaken in a hospital setting. Another possible factor could be the methodology of diagnosis of WSLs using cross-polarised digital photographs which masks flash reflection (Robertson and Toumba, 1999) and improves visualisation of enamel defects (Willmot et al., 2000). The only other clinical study using cross-polarised digital photographs produced an interim report (Tobin, 2001). *In-vitro* investigation with brackets in place (Livas et al., 2008) reported that flash masking and 20° angle were suggested to reduce flash reflection (Benson et al., 2000). The need for equipment and possible training as it is difficult to focus as there is restricted flash output (Fleming et al., 1989) may be a prohibiting factor for such a method to be used in every day clinical practice.

It was not the aim of this study to investigate all possible risk factors for development of WSLs nor to detect and quantify their relationship if there was any. Decision was made not to investigate them all as it would complicate the study protocol. Demographic data and data that was part of the patient standard clinical examination e.g. DMFT were collected. Statistical analysis showed that there was a statistically significant difference between the placebo and the test group in development of WSLs during FAOT ( $p=.036$ , with 95% C.I. .066 - 2.04). The expected number of WSL teeth for a patient having a placebo is  $\exp(1.05639) = 2.88$  higher than the expected number of a SRFGDs patient while holding all other variables in the model constant ( $p=.036$ ). The odds ratio is 2.87 with 95% C.I. of 1.068 – 7.734. That means that use of SRFGDs could reduce the risk for development of WSLs on a tooth level by an additional 2.88 times. That is on condition that standard protocol of brushing with 1,450 ppmF tooth-paste twice daily alongside daily use of 225 ppmF mouth-rinse is followed.

A number of other variables appeared to be statistically important. For each unit increase in the duration of the treatment (range of 5-39 months) the odds of a patient not to have WSL teeth decrease by  $\exp(-0,3272842) =$

0,72 times ( $p=0.034$ ) with odds ratio of 0.72 and 95% C.I. of 0.533 to 0.974. Therefore the duration of FAOT increases the chances for a patient to develop WSLs. Duration of FAOT of more than 17 months (Marcusson et al., 1997) or 24 months (Geiger et al., 1988) has also been identified as a risk factor in other studies. Duration of FAOT in this study had a range of 5-39 months with a mean of  $16.60 \pm 1.18$  months and a median time of 17 months similar to the study by Marcusson et al. 1997.

The MDI also appeared to increase the chances of developing WSLs during the course of FAOT with odds ratio of 1.06 and 95% C.I. of 1.034 – 1.089, thus an increase in the MDI score makes it more likely to develop WSLs.

If the GI index (range 0-3) of a patient at the start of the study was to increase by one the expected number of teeth with WSL would increase by a factor of  $\exp(1.978042) = 7.23$  times, while holding all other variables in the model constant. Thus, the higher the GI index at the start of the study, the more WSL teeth predicted ( $p=0.011$ ) with odds ratio of 2638.52 and 95% C.I. from 2.495 to 2789240.794. Increased GI index at the start of FAOT makes it more likely for WSLs to develop. The small sample size in this study did not allow reliable quantification of the effect of this variable hence the wide range for 95% C.I.. This index was also found to be a significant risk factor for development of WSLs in other studies (Zachrisson and Zachrisson, 1971a, Ogaard et al., 2001). It is only logical that plaque induced gingival inflammation would be closely associated to development of WSLs the precursor state of dental decay.

#### **9.4.14 Kappa coefficient estimation for incidence of WSLs**

The primary outcome was presence of WSLs assessed by one examiner using cross-polarised digital photographs and the kappa score of 0.821 showed very good intra-examiner reliability. There is no clear answer as to how many examiners should examine data and whether the same examiners should examine the data at the start and at the end of the study. The median duration of FAOT in this study was 17 months with  $n=63$  volunteers to be followed for the duration of the clinical study and that posed a risk of not having the same examiners. In case of a single examiner he/she

needs to be calibrated against a standard whereas in cases of multiple examiners they need to be calibrated against a standard and against each other. As a result the number of examiners multiplies the standard error produced by each examiner as well. For these reasons the decision was made to have one examiner to perform all assessments - the principal investigator - and since the study had a double-blind design there was no bias introduced.

#### **9.4.15 Severity of WSLs**

The data distribution from changes in area of WSLs would not allow statistical analysis therefore as a measure of severity the number of WSLs on each participant was used. A possible explanation might be the effect of polishing teeth after removal of bracket and bonding material. Use of polishing burs would not only remove bonding material but may also remove the outer surface of enamel hence altering WSLs if there are any. Based on that outcome the Zero Inflated Poisson regression model showed that MDI, GI at the start of the study and use of placebo/SRFGDs all predicted future development of WSLs. It is important to note that even though 40/63 subjects completed the study the effect of those who failed to complete the study (n=23/63 or 36.5%) was also investigated in the statistical model. Sample size though was small for the two groups (those who completed and those who failed to complete the study) therefore reliable p-value estimation was not possible. Patient who had more WSLs at the end of the study would benefit more from a SRFGDs, in other words patients more prone to development of WSLs would benefit. The question remains how best to identify such patients.

## 9.5 Conclusions

### 9.5.1 Conclusions from clinical study:

- Incidence of WSLs on a subject level was 32.5% (n=13/40). The number of subjects with WSLs at the start of the study was n=2/63 or 3.17% and at the end was n=15/40 or 37.5%.
- Incidence of WSLs on a tooth level was n=26/240 or 10.83%. The number of teeth with WSLs at the start of the study were n=2/378 or 0.0053% and at the end was n=28/240 or 11.67%.
- There was a statistically significant difference between the placebo and the SRFGDs group in development of WSLs during FAOT. The expected number of WSL teeth for a patient having a placebo is  $\exp(1.05639) = 2.88$  higher than the expected number of a SRFGDs patient while holding all other variables in the model constant (p=.036, with 95% C.I. of 0.06 - 2.04 and odds ratio 2.88 with 95% C.I. of 1.06 – 7.73).
- The higher the MDI index, the more WSL teeth predicted (p<.001, with 95% C.I. of .03 - .08) with odds ratio 1.06 and 95% C.I. of 1.034 – 1.089. If the MDI index (range 1.86 – 76.76) was to increase by one the expected number of teeth with WSL would increase by a factor of  $\exp(0.059785) = 1.06$  (6%) while holding all other variables in the model constant.
- The higher the GI index at the start of the study, the more WSL teeth predicted (p=0.027, with 95% C.I. of .91- 14.84) and odds ratio of 7.87 with 95% C.I. of 2.496 – 2789240.794. The wide C.I. results from the small sample size so even though an effect was detected it was not possible to quantify this effect. If the GI index (range 0-3) of a patient at the start of the study was to increase by 0.1, the expected number of teeth with WSL would increase by a factor of  $\exp(7.88) = 245$  times, while holding all other variables in the model constant.
- For each unit increase in the duration of the treatment (range of 5-39 months) the odds of a patient not to have teeth with WSLs decrease by  $\exp(-0,3272842) = 0.72$  times (p=.034, with 95% C.I. of -.62 to -0.2) and odds ratio of 0.72 with 95% C.I. of 0.533 – 0.974.

- There was a high percentage of eligible participants who did not volunteer to participate in the study (n=112/175 or 64%).
- From those who volunteered to participate in the study n=40/63 (63.49%) managed to complete it. From those who failed to complete the study (n=23 or 36.5%), eight were lost to follow-up and 16 withdrew from the study.
- Majority of volunteers and the smallest number of refusals were from people living in the most deprived areas around the hospital setting in Leeds, West Yorkshire, U.K. This may well have introduced bias to the study as it has produced an unbalanced sample in terms of their socio-economic background.

#### **9.5.2 Conclusions from electronic questionnaire emailed to members of the British Orthodontic Society:**

- The response rate was 7.85% (115/1,464) and the fully completed questionnaires were 105/115 (91.30%).
- The median risk for development of WSLs was estimated by responders to be 20% and the mean risk 42.86%.
- Early termination and need for restorative care were the main problems associated with WSLs.
- Responders would primarily use clinical examination and secondly photographs to diagnose and quantify severity of WSLs.
- F was at the core of the prevention protocol adopted by responders.
- Poor oral hygiene, diet and duration of FAOT were believed to be the main three risk factors for development of WSLs.
- Responders agreed with many risk factors identified in the literature (pre-existing WSLs, socio-economic status, duration of FAOT, DMFT and oral hygiene).
- Responders did not agree that age and/or gender of the patient are risk factors for development of WSLs.
- A variety of methods would be considered by responders to treat/arrest WSLs with the most popular ones being discussion with patient/parent, oral hygiene instructions and diet advice.

### 9.5.3 Conclusions from *in-vitro* study:

- AS was the most appropriate storage medium compared to different types of waters to assess F and PO<sub>4</sub> release from bonding materials.
- AS had a background F release of 0.61±0.08 ppm F (mean±SD) and PO<sub>4</sub> release of 70.25±4.84 ppm PO<sub>4</sub> (mean±SD) over two weeks.
- F and PO<sub>4</sub> release from powder from SRFGDs mixed with a CR control bonding material (Transbond®) after six months showed that some types of powder showed higher F release and similar PO<sub>4</sub> release compared to artificial saliva.
- Compared to artificial saliva, three types of powder from SRFGDs showed promising results in terms of high F release and low PO<sub>4</sub> release.
  - a. Powder with particle size of less than 38µm, mixed at a ratio of 1:8 with Transbond® and relative solubility of 1. After six months showed mean F release of 36.21 ppm F and mean PO<sub>4</sub> release of 75.51 ppm PO<sub>4</sub>.
  - b. Powder with particle size of less than 38µm, mixed at a ratio of 1:8 with Transbond® and relative solubility of 16. After six months showed mean F release of 101.45 ppm F and mean PO<sub>4</sub> release of 431.62 ppm PO<sub>4</sub>.
  - c. Powder with relative solubility of 1 and particle size of more than 38µm, showed high F (320.64 ppm F) and low PO<sub>4</sub> release (180.48 ppm PO<sub>4</sub>) after two months.
- Room temperature showed no difference for storage of materials tested for flexural modulus of elasticity and flexural stress in order to assess their bond strength *in-vitro*.

## 9.6 Rejection of null hypotheses

From the aforementioned we failed to reject the first null hypotheses whereas there was a difference in the severity of WSLs assessed by the number of teeth with WSL(s) on each participant having SRFGDs compared to a placebo device.

## 9.7 Future studies

- Based on the inclusion criteria for the clinical study eligible participants should be identified in a more effective manner in an appropriate setting to maximise participation in the clinical study.
- Specialists in orthodontics should test the SRFGDs rather than clinicians in training.
- The duration of FAOT and the MDI of volunteers should be documented as it appears to increase the risk of developing WSLs.
- GI at the start of the clinical study could be part of the inclusion/exclusion criteria as it appears to increase the risk of developing WSLs. However low GI may not be a requirement to provide FAOT and this may complicate recruitment.
- MDI cannot be part of the inclusion/exclusion criteria as it would be unethical to exclude potential participants due to their socio-economic status as dictated by their MDI.
- MDI needs to be documented in order to assess whether an unbalanced study sample has been obtained and if possible to assess and quantify risk of bias, if any.
- Other risk factors for development of WSLs which have not been investigated in this study may need to be documented. These factors were frequency of tooth-brushing and use of F, *mutans streptococci* counts in plaque, *lactobacilli* counts in saliva, diet, gingivae clinical attachment and compliance with use of F mouth-rinse. This way they can be incorporated into a model for statistical analysis that can provide more clinically meaningful results since these confounding variables will be kept constant in the statistical model.



- Another method needs to be employed to increase number of responders to electronic questionnaires. A postal questionnaire may be more expensive but it may increase response rate.
- Future studies should investigate a larger number of samples based on a sample size calculation to assess F, PO<sub>4</sub> release and bond strength of orthodontic brackets bonded onto human teeth *in-vitro*.

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**11 Glossary of acronyms**

APF	acidulated phosphate fluoride
AS	artificial saliva
CFLM	confocal laser microscopy
CFU	colony forming units
CHX	chlorhexidine
CI	confidence interval
CR	composite resin
F	fluoride
FAOT	fixed appliance orthodontic treatment
F-CR	fluoride containing composite resin
GI	gingival index
GIC	glass-ionomer cement
IC	ion chromatography
ICC	intra class correlation
JPEG	joint photographic expert group
MDI	multiple deprivation index
MFP	mono-fluoro-phosphate
MH	micro-hardness
MR	micro-radiography
MW	mouth-wash
PF	preventive fraction
PI	plaque index
PLM	polarised light microscopy
QLF™	quantitative light fluorescence
RM-GIC	resin modified glass-ionomer cement
SD	standard deviation
SE	standard error

SEM	scanning electron microscopy
SnF <sub>2</sub>	stannous fluoride
SRFGD(s)	slow-release fluoride glass device(s)
TB	tooth-brushing
TIFF	tagged image file format
TMR	transverse micro radiography
TP	tooth-paste
UTM	universal testing machine
WSL(s)	white spot lesion(s)

## 12 Appendices

1. Research Ethics Committee Letter
2. Research and Development Approval Letter
3. Declaration of the end of the study
4. Invitation Letter for Participation in a Research Study
5. Parental Information Sheet About The Research
6. Child Information Sheet aged 6-10 years old
7. Young People Information Sheet aged 11-15 years old
8. Letter to Doctor/Dentist
9. Consent Form
10. Case Record Form (CRF)
11. Satisfaction Questionnaire for Orthodontists
12. Satisfaction Questionnaire for Participants
13. Randomisation codes
14. Questionnaire on WSLs emailed to members of British Orthodontic Society
15. Abstract for International Association of Dental Research (IADR) Meeting in Barcelona, Spain July 2010
16. Poster presentation for International Association of Dental Research (IADR) Meeting in Barcelona, Spain July 2010

## 12.1 Research Ethics Committee Letter

### Leeds (Central) Research Ethics Committee

Room 5.2, Clinical Sciences Building  
St James's University Hospital  
Beckett Street  
Leeds  
West Yorkshire  
LS9 7TF

Telephone: 0113 2065652  
Facsimile: 0113 2066772

29 April 2008

Miss Chrysoula Tatsi  
PhD Student  
Leeds Dental Institute  
Clarendon Way  
Leeds  
West Yorkshire  
LS2 9LU

Dear Miss Tatsi

**Full title of study:** Slow Release Fluoride Glass Devices in the prevention of enamel demineralization during fixed appliance orthodontic treatment. A randomised double-blind controlled clinical trial.  
**REC reference number:** 08/H1313/6

Thank you for your letter of 23 April 2008, responding to the Committee's request for further information on the above research.

The further information has been considered on behalf of the Committee by Mr Long.

#### Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation.

#### Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form.

#### Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

#### Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application		01 January 1900
Investigator CV		20 December 2007

Protocol	1	18 January 2008
Compensation Arrangements		24 September 2007
Questionnaire: Satisfaction questionnaire for Orthodontists	1	18 January 2008
Questionnaire: Satisfaction questionnaire for children aged 6-10 years and young people aged 11-15 years	2	27 February 2008
Letter of invitation to participant	1	18 January 2008
GP/Consultant Information Sheets	1	18 January 2008
Participant Information Sheet: Children aged 6-10 years	1	18 January 2008
Participant Information Sheet: Parents	1	18 January 2008
Participant Information Sheet: Young people aged 11-15 years	2	27 February 2008
Participant Consent Form	1	18 January 2008
Response to Request for Further Information		27 February 2008
Response to Request for Further Information		23 April 2008
Email from Clinical Trials Unit, MHRA		21 April 2008
Email from Regulatory Affairs Manager (Medical Devices), MHRA		26 February 2008
Poster	1	18 January 2008
Poster	1	18 January 2008
Assent form for children	1	18 January 2008
CV for Prof Toumba		

#### R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from <http://www.rdforum.nhs.uk/rdform.htm>.

#### Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

#### After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

Here you will find links to the following

- a) Providing feedback. You are invited to give your view of the service that you have received from the National Research Ethics Service on the application procedure. If you wish to make your views known please use the feedback form available on the website.
- b) Progress Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
- c) Safety Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
- d) Amendments. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

- e) End of Study/Project. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email [referencegroup@nationalres.org.uk](mailto:referencegroup@nationalres.org.uk).

08/H1313/6	Please quote this number on all correspondence
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With the Committee's best wishes for the success of this project

Yours sincerely

**Dr Margaret L Faull**  
Chair

Email: [ann.prothero@leedsth.nhs.uk](mailto:ann.prothero@leedsth.nhs.uk)


Enclosures:           Standard approval  
                              Site approval form

Copy to:                Ms Clare Skinner, University of Leeds  
                              R&D Department, Leeds Teaching Hospitals NHS Trust

Leeds (Central) Research Ethics Committee					
LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION					
<i>For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.</i>					
<b>REC reference number:</b>	08/H1313/6	<b>Issue number:</b>	1	<b>Date of issue:</b>	29 April 2008
<b>Chief Investigator:</b>	Miss Chrysoula Tatsi				
<b>Full title of study:</b>	Slow Release Fluoride Glass Devices in the prevention of enamel demineralization during fixed appliance orthodontic treatment. A randomised double-blind controlled clinical trial.				
<i>This study was given a favourable ethical opinion by Leeds (Central) Research Ethics Committee on 29 April 2008. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.</i>					
<i>Principal Investigator</i>	<i>Post</i>	<i>Research site</i>	<i>Site assessor</i>	<i>Date of favourable opinion for this site</i>	<i>Notes <sup>(1)</sup></i>
Miss Chrysoula Tatsi	PhD Student	The Leeds Teaching Hospitals NHS Trust	Leeds (Central) Research Ethics Committee	29/04/2008	
Approved by the Chair on behalf of the REC:					
..... (Signature of Chair/Co-ordinator)					
(delete as applicable)					
..... (Name)					



## 12.2 Research and Development Approval Letter

The Leeds Teaching Hospitals   
NHS Trust

15/05/2008

Miss Chrissoula Tatsi  
Flat 3  
132 Otley Road Headingley  
Leeds LS16 5JX

Research & Development Directorate  
A/B Corridor, Old Site  
**The General Infirmary at Leeds**  
Great George Street  
Leeds  
West Yorkshire  
LS1 3EX  
Tel: 0113 392 2878  
Fax: 0113 392 6397  
www.leedsteachinghospitals.com

Dear Miss Chrissoula Tatsi

**Re: LTHT R&D Approval of DT08/8473: Slow release fluoride glass devices in the prevention of enamel demineralization during fixed appliance orthodontic treatment. A randomised double-blind controlled clinical trial**

I write with reference to the above research study. I can now confirm that this study has R&D approval and the study may proceed at The Leeds Teaching Hospitals NHS Trust (LTHT). This organisational level approval is given based on the information provided in the documents listed below.

As principal investigator you have responsibility for the design, management and reporting of the study. In undertaking this research you must comply with the requirements of the *Research Governance Framework for Health and Social Care* which is mandatory for all NHS employees. This document may be accessed on the Department of Health website at <http://www.dh.gov.uk/research>

R&D approval is therefore given on the understanding that you comply with the requirements of the *Framework* as listed in the attached sheet "Conditions of Approval".

If you have any queries about this approval please do not hesitate to contact the R&D Department on telephone 0113 392 2878.

**Indemnity Arrangements**

The Leeds Teaching Hospitals NHS Trust participates in the NHS risk pooling scheme administered by the NHS Litigation Authority 'Clinical Negligence Scheme for NHS Trusts' for: (i) medical professional and/or medical malpractice liability; and (ii) general liability. NHS Indemnity for negligent harm is extended to researchers with an employment contract (substantive or honorary) with the Trust. The Trust

Chairman Martin Buckley Chief Executive Maggie Boyle

The Leeds Teaching Hospitals incorporating: Chapel Allerton Hospital Cookridge Hospital Leeds Chest Clinic  
Leeds Dental Institute Seacroft Hospital St James's University Hospital The General Infirmary at Leeds  
Wharfedale Hospital

WT2280

only accepts liability for research activity that has been managerially approved by the R&D Department.

The Trust therefore accepts liability for the above research project and extends indemnity for negligent harm to cover you as principal investigator and the researchers listed on the R&D approval form provided that each member of the research team has an employment contract (substantive or honorary) with the Trust. Should there be any changes to the research team please ensure that you inform the R&D Department and that s/he obtains an employment contract with the Trust if required.

Yours sincerely



Dr D R Norfolk  
Associate Director of R&D

**Approved documents**

The documents reviewed and approved are listed as follows

<i>Document</i>	<i>Version</i>	<i>Date of document</i>
Protocol	1	18/01/08
SSI Form	5.5	04/01/08
CMT Approval		08/05/08
NHS REC Application Form	5.5	04/01/08
MREC Letter confirming favourable opinion		29/04/08

**Conditions of R&D Approval**

- Approval from your local Clinical Management Team must be obtained before starting the study.
- Approval of the appropriate Research Ethics Committee, where necessary, must be obtained before starting the study. Any changes made to the project during ethical review must be reviewed and approved by the R&D Department to maintain R&D Approval status.
- Arrangements must be made to ensure that all members of the research team, where applicable, have employment contracts with the Trust (either full or honorary).
- Agreements must be in place with appropriate support departments regarding the services required to undertake the project and arrangements must be in place to recompense them for the costs of their services.
- Arrangements must be in place for the management of financial and other resources provided for the study, including intellectual property arising from the work.
- Priority should be given at all times to the dignity, rights, safety and well being of participants in the study
- Healthcare staff should be suitably informed about the research their patients are taking part in and information specifically relevant to their care arising from the study should be communicated promptly.
- Each member of the research team must be qualified by education, training and experience to discharge his/her role in the study. Students and new researchers must have adequate supervision, support and training.
- The research must follow the protocol approved by the relevant research ethics committee. Any proposed amendments to or deviations from the protocol must be submitted for approval to the Research Ethics Committee, the research sponsor, regulatory authority and any other appropriate body. The R&D Department should be informed where the amendment has resource implications within the CMT and the CMT research lead/clinical director notified.
- Any adverse events/adverse drug reactions must be reported to the appropriate research ethics committee, research sponsor and any other regulatory authority. Adverse events in clinical trials of investigational medicinal products must be reported in accordance with the Medicines for Human Use (Clinical Trials) Regulations 2004. All serious adverse events as defined in the research protocol,

that occur within LTHT should be reported via the IR1 reporting system to the Risk Management Department, Trust Headquarters, St James's University Hospital, Beckett Street, Leeds LS9 7TF.

- Reports on the progress and outcomes of the work must be produced on time and to an acceptable standard. Please send a copy of the progress report produced for the Research Ethics Committee to the R&D Department for monitoring.
- Procedures should be in place to ensure collection of high quality, accurate data and the integrity and confidentiality of data during processing and storage.
- Arrangements must be made for the appropriate archiving of data when the research has finished. Records must normally be kept for 15 years.
- All data and documentation associated with the study must be available for audit at the request of the appropriate auditing authority. Currently 10% of REC approved projects are randomly selected for audit inspection by the R&D Department each year. You will be informed by letter if your study is selected.
- Findings from the study should be disseminated promptly and fed back as appropriate to research participants.
- Findings from the study should be exposed to critical review through accepted scientific and professional channels.

#### **Commercially Sponsored Trials**

If the study is commercially sponsored approval is given subject to provision of the following documents.

- Clinical Trials Agreement - agreed and signed off by the R&D Department (on behalf of the Leeds Teaching Hospitals NHS Trust) and the Sponsor.
- Indemnity agreement, if not included in the Clinical Trials Agreement- (standard ABPI no fault arrangements apply) signed by the R&D Department and the Sponsor

It is essential that all the responsibilities set out in the Research Governance Framework and outlined above are fulfilled. The Trust reserves the right to withdraw R&D approval for a study, and therefore the provision of indemnity cover (for negligent harm) for its employees, where it is found that the above criteria have not been met. The Trust will not accept liability for any activity that has not been fully approved.

### 12.3 Declaration of the end of the study

**NRES Committee Yorkshire & The Humber - Leeds Central**

Yorkshire and Humber REC Office  
 First Floor, Millside  
 Mill Pond Lane  
 Meanwood  
 Leeds  
 LS6 4RA

Tel: 0113 30 50166  
 Fax: 0113 85 56191

01 May 2012

Miss Chrysoula Tatsi  
 PhD Student  
 Leeds Dental Institute  
 Clarendon Way  
 Leeds  
 West Yorkshire  
 LS2 9LU

Dear Miss Tatsi

**Study title:** **Slow Release Fluoride Glass Devices in the prevention of enamel demineralization during fixed appliance orthodontic treatment. A randomised double-blind controlled clinical trial.**

**REC reference:** 08/H1313/6

**Protocol number:** n/a

Thank you for sending the declaration of end of study form, notifying the Research Ethics Committee that the above study concluded on 15 February 2012. I will arrange for the Committee to be notified.

A summary of the final research report should be provided to the Committee within 12 months of the conclusion of the study. This should report on whether the study achieved its objectives, summarise the main findings, and confirm arrangements for publication or dissemination of the research including any feedback to participants.

<b>08/H1313/6:</b>	<b>Please quote this number on all correspondence</b>
--------------------	---

Yours sincerely

**Mr Marc Neal**  
 Assistant Committee Co-ordinator

E-mail: marc.neal@nhs.net

Copy to: *University of Leeds*



## 12.4 Invitation Letter for Participation in a Research Study

Version 1, 18/01/2008



### Invitation letter for participation in a research study

Dear Parent/Guardian of

Your child is invited to participate in a research study entitled "Slow Release Fluoride Glass Devices in the prevention of enamel demineralization during fixed appliance orthodontic treatment. A randomized double-blind controlled clinical trial".

We are hoping to recruit volunteers who are about to start orthodontic treatment to straighten their teeth with fixed brackets/braces and follow them throughout the course of their treatment.

The aim of our study is to test a method to prevent white spots (early caries) developing around the brackets. We will compare a placebo (dummy) glass-bead to an identical glass-bead that releases fluoride in the mouth; fluoride is found in toothpastes and mouth-rinses to prevent dental decay. The glass-beads will be placed in a plastic holder threaded onto the orthodontic wire on both sides in the upper arch.

To try to make sure the two groups with the devices (placebo or fluoride) are same to start with, each volunteer is placed into a group by chance (randomly); the chance to have a placebo or fluoride device will be exactly the same (50-50) for all volunteers. The study is double-blind meaning that the volunteers and the dentist will not know what device they have.

Our study has been reviewed and given favourable opinion by Leeds Central Research Ethics Committee. (Ref No: 08/H1313/6).

Please find enclosed the "Child Information Sheet Aged 6-10 years / Young People Information Sheet Aged 11-15 years" and "Parental Information Sheet about the Research" which fully explain our study and hopefully will answer your questions. Please do not hesitate to contact us if you do have any questions or concerns. We will be more than happy to answer them.

Participation in the study is entirely voluntarily; you can withdraw permission for your child to participate in the study at any time without giving a reason. A decision to withdraw at any time or a decision not to take part will not affect the standard of care your child will receive in any way.

If you are interested for your child to participate in our study please do inform reception / dental nurse / member of staff in the Orthodontic or Children's Department at the Leeds Dental Institute, Level 5 when you arrive for your next scheduled appointment.

Yours sincerely

Dr Chrysoula Tatsi  
PhD student, Principal Investigator

## 12.5 Parental Information Sheet About The Research

Version 2, 10/06/2008



### Parental Information Sheet About The Research. Part 1.....

#### Study title:

**Slow release fluoride glass devices in the prevention of enamel demineralization during fixed appliance orthodontic treatment.  
A randomized double-blind controlled clinical trial.**

Your child is being invited to take part in a research study. Before you decide whether you are happy for your child to be involved, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like any further information. Take time to decide whether or not you wish your child to take part. Thank you for reading this.

#### What is the purpose of the study?

The purpose of this study is to investigate a method to reduce the number and the severity of white spots that sometimes appear in children's teeth that have braces.

#### Why has my child been invited?

All children who are starting orthodontic treatment to straighten their teeth with fixed brackets/braces are being asked to participate in this study.

#### Does my child have to take part?

It is up to you and your child to decide whether or not to take part. If you do decide to let your child take part you will be given this information sheet and be asked to sign a consent form to show you agree for your child to take part in the study. You will keep a copy of the consent form and of the information sheet. We will ask for your child to sign an assent form to show that he/she agrees to take part in the study. Even if you decide to let your child take part you are still free to withdraw your permission at any time and without giving a reason. A decision to withdraw at any time or a decision not to take part in the study will not affect the standard of care your child will receive in any way.

#### What will happen to my child if he/she takes part?

For our study we want to test a new method; a small glass bead that constantly and slowly releases fluoride in the mouth. We will have two groups both will have identical devices; one will have the fluoride device and the other will have a placebo. A placebo is a 'dummy treatment', which looks like the genuine device but contains no active ingredient. To try to make sure the groups are the same to start with, each patient is put into a group by chance (randomly). The chance for your child to receive the placebo or the device is exactly the same. Our study is a "double blind trial" meaning that neither you nor your dentist will know in which group your child will be (although, if your dentist needs to find out he/she can do so). Regardless of the group your child will be in the orthodontic treatment and review appointments will not be affected in any way. All children in both groups will have the full preventive treatment and advice as given to all children who have orthodontic treatment. The glass bead will be an additional measure to this preventive treatment and advice.

Version 2, 10/06/2008

**What will my child have to do?**

- Your child will have to have digital photographs taken of their six (6) upper front teeth before and after he/she has completed the orthodontic treatment.
- Your child will have to give a saliva sample eight (8) times throughout the study; he/she will be given a plastic tube to spit in for 5min while seating in the dental chair. We will ask your child not to use the fluoride mouth-rinse and to use a fluoride-free tooth-paste the night before and the morning of the day scheduled to give a saliva sample.
- The plastic holders with the beads should not be removed by your or your child.

**Expenses and payments**

No payments will be provided and travel expenses will not be covered unless appointments are scheduled at different date/time to the normal review appointment.

**What is the device that is been tested?**

The device that is being tested is a glass bead secured in a plastic holder that will be threaded onto the orthodontic wire in between two teeth at the back on both sides in the upper dental arch. The glass bead contains fluoride that is constantly and slowly released in the mouth therefore it does not rely on the child/parent compliance to use fluoride toothpaste/mouth-rinse to prevent white spots/caries.

**What are the possible disadvantages and risks of taking part?**

The bead may feel uncomfortable for the first days since it is something new in the mouth but volunteers from other studies that used the bead said they got used to it within 1-2 days.

**What are the possible benefits of taking part?**

We hope that the information obtained from this study will provide us with evidence to show that the glass bead can effectively reduce the number and/or the severity of white spot lesion that develop around the orthodontic brackets by slowly and constantly releasing fluoride. We hope that this information will provide us with a new tool to improve prevention of white spots in children who have orthodontic treatment in future.

**Will taking part in this study alter my child's treatment in any way?**

Absolutely not. All children in both groups will be asked to follow the standard advice given to all patients that have braces in order to prevent white spots i.e. to brush their teeth twice daily with adult fluoride toothpaste and to rinse once a day with a fluoride mouth-rinse. Review appointments will be scheduled as normal.

**What if something goes wrong?**

Whilst it is extremely unlikely, any complaint about the way your child has been dealt with during the study or any possible harm he/she might suffer will be addressed. The detailed information on this is given in Part 2.

**Will my child's taking part in this study be kept confidential?**

Yes, we will follow ethical and legal practice and all information about your child during the research will be handled in confidence. The details are included in part 2.

**If the information in Part 1 has interested you and you are considering participation for your child, please read the additional information in Part 2 before making any decision.**



Version 2, 10/06/2008



## Parental Information Sheet About The Research. ....Part 2

### **What if relevant new information becomes available?**

If any new information becomes available during the study, we will let you know as soon as possible and discuss and explain the changes to you. You will have the chance to ask us any questions before deciding whether you would like for your child to continue with the study. If the changes are significant and you decide for your child to continue in the study we may ask you to sign an updated consent form. Please let us stress that, should this occur, you will be under no obligation whatsoever to continue with the study and you may still withdraw your permission for participation at any point without giving a reason.

### **What will happen if I don't want for my child to carry on with the study?**

If you withdraw permission for your child to participate in the study, we will destroy all his/her samples that can still be identified, but we will need to use the data collected up to withdrawal.

### **What if there is a problem?**

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (contact Dr Chrysoula Tatsi 0113 3436195). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the Leeds Dental Institute.

In the event that something does go wrong and your child is harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against the University of Leeds but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

### **Will my child's taking part in this study be kept confidential?**

All information which is collected about your child during the course of the research will be kept strictly confidential and any information about your child which leaves the Leeds Dental Institute will have his/her name and address removed so that he/she cannot be recognised. Anything you say and/or your child says will be treated in confidence, no names will be mentioned in any reports of the study and care will be taken so that individuals cannot be identified from details in reports of the study.

### **Notification of the General Practitioner/Family Doctor (GP) and General Dental Practitioner/Family Dentist (GDP)**

We will notify by letter of your child's participation in our study both the Family Doctor and the Family Dentist.

### **What will happen to any samples my child gives?**

Saliva samples will be collected in sterile plastic tubes from participants. The samples will be anonymous and given a unique identifying number. Collection, storage and use of the samples will be permitted only to the research student and the academic supervisors. The samples will be stored throughout the course of the study. The digital photographs and the data of the study will be stored in a computer for 15 years for any possible intended use in the future for research that cannot yet be specified.

Version 2, 10/06/2008

**What will happen to the results of the research study?**

We will report on the results of this study as a research thesis for a higher degree. We hope to publish the results in an international journal and present our findings at both national and international meetings. Your child will not be identified by name in any reports we write.

**Who is the organising and funding the research?**

The study is carried out by Dr Chrysoula Tatsi, a PhD student funded by a Scholarship from the University of Leeds. The study is supervised by the following members of staff:

- Prof. M.S. Duggal (Professor in Paediatric Dentistry, Consultant in Paediatric Dentistry and Head of Paediatric Dentistry Department at Leeds Dental Institute)
- Prof K.J. Toumba (Professor in Paediatric and Preventive Dentistry, Consultant in Paediatric Dentistry and Director of Taught Postgraduate Studies, Leeds Dental Institute).
- Dr F. Luther (Senior Lecturer, Honorary Consultant in Orthodontics, Academic Head of Orthodontics and Postgraduate Course Leader, Deputy Head of Clinical Orthodontics, Leeds Dental Institute).

**Who has reviewed the study?**

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by Leeds Central Research Ethics Committee. (Ref No 08/H1313/6).

**Further information**

- General information about research:
  - <http://www.nres.npsa.nhs.uk/public/index.htm>  
Public area of the National Research Ethics Service, National Patient Safety Agency, NHS.
  - <http://www.leedsteachinghospitals.com/>.  
The website of the Leeds Teaching Hospitals NHS Trust.
- Specific information about this research project: Contact any of the people listed below.
- Advice as to whether your child should participate: Any member of staff at the Leeds Dental Institute, the Family Dentist or the Family Doctor.
- Who you should approach if unhappy with the study: Dr Chrysoula Tatsi, contact details listed below.

**Contact details**

Dr Chrysoula Tatsi (0113 343 6195) or e-mail: [den3ct@leeds.ac.uk](mailto:den3ct@leeds.ac.uk)

Prof Jack Toumba (0113 343 6141) or e-mail [k.i.toumba@leeds.ac.uk](mailto:k.i.toumba@leeds.ac.uk)

Prof. Monty Duggal (0113 343 6138) or e-mail: [m.s.duggal@leeds.ac.uk](mailto:m.s.duggal@leeds.ac.uk)

Dr Friedy Luther (0113 343 6180) or e-mail: [f.luther@leeds.ac.uk](mailto:f.luther@leeds.ac.uk)

1 for parent/legal guardian; 1 for researcher site file; 1 (original) to be kept in medical notes

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Thank you for reading this information sheet.

## 12.6 Child Information Sheet aged 6-10 years old

Version 1, 18/01/2008



### Child Information Sheet Aged 6-10 years



A clear bead placed on your brace to check  
if you will get any white spots on your front teeth

- Research is a way we try to find out the answers to questions. We want to see if our clear beads can stop white spots appearing on your teeth when you have braces to make your teeth straight.
- We are asking you to take part in our research because you will soon start to have your teeth straightened with braces.
- Before any research is allowed to happen, it has to be checked by a group of people called a Research Ethics Committee. They make sure that the research is fair. Your research has been checked by the Leeds Central Research Ethics Committee.
- You do not have to do this if you do not want to. If you decide to take part and then change your mind that is fine too. Just tell your parents, dentist or nurse. They will not be cross with you. Your parents know about this research and we will ask them to sign a piece of paper called consent if you are all happy to take part.
- We will hide your name so nobody will know you are doing this unless it is necessary.
- We cannot promise the research will help you but the information we get might help other children in the future.



Version 1, 18/01/2008

- The beads may feel funny in your mouth for 1-2 days the same as when you wear a new pair of shoes, but quickly you get used to them. You will be able to eat, drink, talk, sing and play the same as before.
- Ⓢ If something goes wrong just tell your parents, dentist or nurse and we will make sure we will do everything we can to make it better.
- Ⓢ If you change your mind and you want to stop the research then we will remove the beads and you will continue with your braces same as before just without the beads.
- Ⓢ If we learn that something else is better from the bead then we will let you and your parents know immediately.

### What is going to happen???

When you come to have your braces we will:  
Take photographs from your upper front teeth only.  
Ask you to spit saliva in a small plastic tube for 5min.  
Thread the plastic holders with the beads between your teeth on both sides of your upper jaw.

Sometimes when you come to see the person responsible for your braces we will come to see you as well and ask you to spit saliva.

When we take away the braces and the beads we will take again photographs from your upper front teeth only.

1 for you; 1 for us; 1 to be kept in your notes

**Thank you very much for reading this**



## 12.7 Young People Information Sheet aged 11-15 years old

Version 2, 10/06/2008



### Young People Information Sheet Aged 11-15 years Part 1...



A transparent bead placed on your brace to check if you will get any white spots on your front teeth

- We are asking if you would take part in a research project to find the answer to the question if we can reduce white spots appearing on your teeth when you have braces. Before you decide if you want to join in it's important to understand why the research is being done and what it will involve for you. So please consider this leaflet carefully. Talk about it with your family, friends, doctor, dentist or nurse if you want to.
- We are doing this study because we want to test a new way to reduce white spots that appear sometimes on your teeth around the brackets. These white spots are the first sign of decay/caries. Fluoride protects your teeth from decay/caries. This is why orthodontists will ask everyone who has braces to use toothpaste and mouth-rinse with fluoride every day. For our study you will still need to follow that advice from the orthodontist as every body who has braces but additionally to that we will test the glass-bead.
- To test the glass-bead we need to compare it to a false one; this is why we need to have two groups; one group will have a "dummy or false" bead and the other group will have a fluoride bead. Both beads look exactly the same and neither you nor your dentist will know which bead you will have. We will clip the bead in a plastic holder and we will thread it onto the wire between two teeth at the back of your upper jaw on both sides.
- To try to make the two groups similar to start with we will put the people in the groups by chance (randomly). It will be like when you toss a coin and the chance to have a dummy or a fluoride device will be the same.
- You have been invited to join our study because you will soon start to have your teeth straightened with brackets and braces. For our study we need to examine up to 100 children. Half of them will have the fluoride bead and half of them the dummy bead.
- Do I have to take part? No. It is up to you. If you do, your dentist will ask you to sign a form giving your consent or assent. You will be given a copy of this information sheet and your signed form to keep. You are free to stop taking part at any time during the research without giving a

Version 2, 10/06/2008

reason. If you decide to stop, this will not affect the care you receive. We will remove the plastic holders with the beads and you will continue with your braces same as before just without the beads.

What will happen?
<p>When you have your braces placed we will:</p> <ul style="list-style-type: none"> <li>● take photos of your (6) upper front teeth only</li> <li>● thread the plastic holders with the beads onto the orthodontic wire</li> <li>● ask for a saliva sample before and after we place the bead</li> </ul> <p>All these should take about 30min extra to your scheduled appointment.</p>
<p>When you come to see your orthodontist for review appointments we will:</p> <ul style="list-style-type: none"> <li>● ask for a saliva sample after 1, 3, 6 months, 1 year, 18 months</li> </ul> <p>This should take 5min extra to your scheduled review appointment.</p>
<p>When you have your braces taken out we will:</p> <ul style="list-style-type: none"> <li>● remove the plastic holders with the beads as well</li> <li>● take photos of your (6) upper front teeth only and</li> <li>● ask for a saliva sample</li> </ul> <p>These should take about 30min extra to your scheduled appointment.</p>

- What will I be asked to do? If you decide to take part in our study you will have to have the photos taken, to give us the saliva sample and keep the beads in your mouth. We will give you a tooth-paste without fluoride and ask you not to use your mouth-rinse the night before and the morning of the day that you will give the saliva sample; this way we will check only the effect of the beads.
- The beads may feel like something new in your mouth for 1-2 days, just as the braces will. You will be able to eat, drink and talk the same as before.
- We cannot promise the research will help you but the information we get might help other young people having braces in the future.
- If you want to contact us please do not hesitate to ask for:
  - Dr Chrysoula Tasi on 0113 3436195 or e-mail: [den3ct@leeds.ac.uk](mailto:den3ct@leeds.ac.uk).
  - Dr Friedy Luther on 0113 3436180 or e-mail : [f.luther@leeds.ac.uk](mailto:f.luther@leeds.ac.uk)
  - Prof Jack Toumba on 0113 3436141 or e-mail: [k.j.toumba@leeds.ac.uk](mailto:k.j.toumba@leeds.ac.uk).

**Thank you for reading so far-if you are still interested, please go to Part 2:**

Version 2, 10/06/2008



**Young People Information Sheet**  
**Aged 11-15 years**  
 .....Part 2



**A transparent bead placed on your brace to check  
 if you will get any white spots on your front teeth**

- We will keep your information in confidence. This means we will only tell those who have a need or right to know for example we will let your doctor and dentist know that you are taking part in our study. Wherever possible, we will only send out information that has your name and address removed.
- What will happen to any samples I give? The saliva samples will be stored in a freezer and will be analysed to see how much fluoride they have using special equipment. The digital photos will be analysed by a computer and checked by a dentist to see if there are any white spots.
- Who is organising and funding the study? The study is organised by the Department of Paediatric Dentistry and Orthodontics at the Leeds Dental Institute. The student who is doing the study is funded by a Scholarship from the University of Leeds.
- What if something goes wrong? If something goes wrong just tell your parents, dentist or nurse and we will make sure we will do everything we can to make it better.
- Before any research is allowed to happen, it has to be checked by a group of people called a Research Ethics Committee. They make sure that the research is fair. Your research has been checked by the Leeds Central Research Ethics Committee.

**1 for participant; 1 for researcher site file; 1 to be kept in medical notes**

**Thank you for reading this - please ask any questions if you need to.**

## 12.8 Letter to Doctor/Dentist

Version 1, 18/01/2008



Name and address of Doctor/Dentist: \_\_\_\_\_

Date / /

Re: \_\_\_\_\_

We understand that the above named subject is currently under your care. He/she has volunteered and his/her parent(s)/legal guardian(s) have given written consent for participation in an oral healthcare study entitled "Slow Release Fluoride Glass Devices in the prevention of enamel demineralization during fixed appliance orthodontic treatment. A randomized double blind controlled clinical trial".

The subjects, all of whom will be healthy volunteers, will be visiting the Leeds Dental Institute for their orthodontic treatment. For the purpose of our study the following interventions/procedures will take place:

- Saliva sample collection. Participants will be asked to collect saliva in a sterile tube at the review visits for their orthodontic treatment on eight (8) different time points.
- Attachment of a placebo or fluoride slow-release glass device, threaded onto the orthodontic wire in between posterior teeth on both sides of the upper arch.
- Digital photographs of six (6) upper anterior teeth before and after completion of orthodontic treatment with fixed appliances.

During the study period the volunteers will follow standard procedure of using fluoride toothpaste twice daily and fluoride mouth-rinse once daily but will need to refrain from any additional fluoride.

The study has been reviewed and approved by the Leeds Central Research Ethics Committee (Ref...08/H1313/6).

If you have any questions regarding the study please do not hesitate to contact me. Unless we hear from you to the contrary we will assume that you have no objection. All information will be treated in the strictest confidence. I can be contacted at the Leeds Dental Institute on (0113 3436195) or via e-mail [den3ct@leeds.ac.uk](mailto:den3ct@leeds.ac.uk).

Yours sincerely

**Dr Chrysoula Tatsi**  
PhD student, Principle Investigator

**Prof. M. S. Duggal**  
Lead Academic Supervisor



## 12.9 Consent Form

Version 1, 18/01/2008



UNIVERSITY OF LEEDS

Centre Number:  
Study Number:  
Patient Identification Number for this trial:

### CONSENT FORM

Title of Project: **Slow Release Fluoride Glass Devices in the prevention of enamel demineralization during fixed appliance orthodontic treatment. A randomised double blind controlled clinical trial.**

Name of Researcher: Dr Chrysoula Tatsi

Please initial box

1. I confirm that I have read and understand the information sheet dated .....  
(version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
2. I understand that my child's participation is voluntary and that I am free to withdraw at any time, without giving any reason, without his/hers medical care or legal rights being affected.
3. I understand that relevant sections of any of my child's medical notes and data collected during the study may be looked at by clinicians involved in the study, from regulatory authorities or from the NHS Trust where it is relevant to his/her taking part in research. I give permission for these individuals to have access to my child's records.
4. I agree for my child's General Practitioner and General Dental Practitioner to be informed of his/her participation in the study.
5. I agree for my child to take part in the above study.

\_\_\_\_\_  
Name of Parent/Legal Guardian      Date      Signature

\_\_\_\_\_  
Name of Person taking consent      Date      Signature

When completed, 1 for parent/legal guardian; 1 for researcher site file; 1 (original) to be kept in medical notes.  
Version 1, 18/01/2008; Research Ethics Committee Reference Number 08/H1313/6

**12.10 Case Record Form (CRF)**

**Leeds Dental Institute**  
The Centre for Oral Health Sciences



University of Leeds School of Dentistry with the  
Dental Hospital at Leeds  
Division of Child Dental Health  
A Centre for Children with Special Needs

Subject code:	Randomisation no.:	Screening no.:
---------------	--------------------	----------------

**Case Record Form**

**Slow Release Fluoride Glass Devices in the prevention of enamel  
demineralization during fixed appliance orthodontic treatment.  
A randomised double-blind controlled clinical trial.**

**Ethics Committee Ref No: 08/H1313/6**

**Principal Investigator**

**Dr Chrysoula Tatsi**

Division of Child Dental Health

**Academic Supervisors**

**Professor K. J. Toumba  
Professor M. S. Duggal  
Dr F Luther**

Division of Child Dental Health

**Volunteer Personal Sheet**

First name:		Family name:	
Date of birth: __/__/____		Gender: Male <input type="checkbox"/> Female <input type="checkbox"/>	
Contact address:			
		Post code:	
<b>Emergency contact</b>			
Name			
Relationship			
Address:			
		Post code:	
Tel:	Mobile:	e-mail:	

Principal Investigator's signature:..... Date \_\_/\_\_/\_\_\_\_

Subject code:	Randomisation no.:	Screening no.:
---------------	--------------------	----------------

**MEDICAL STATUS**

Any medical conditions to report? <input type="checkbox"/> YES <input type="checkbox"/> NO Name/address of GP (Family Doctor) :..... ..... .....
---

**Any medication**

Drug's name	Cause of medication	Dosage	Date started	Date stopped (if applicable)

Principal Investigator's signature:..... Date \_/ \_/ \_

Subject code:	Randomisation no.:	Screening no.:
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**DENTAL STATUS**

Name and address of GDP (Family Dentist): .....

.....

.....

**Oral Cavity Examination**

**Dental Tissues:** *Note:* Subject must have all first permanent molars and upper anteriors.

<i>Right</i>				<i>upper</i>										<i>Left</i>			
18	17	16	5	4	13	12	11	21	22	23	4	5	26	27	28		
48	47	46	5	4	3	2	1	1	2	3	4	5	36	37	38		

DMFT:	DMFS:	dmft:	dmfs:
-------	-------	-------	-------

**Soft Tissues:**

Normal  Abnormal  Describe abnormality

**Salivary Flow Rate:**

- Unstimulated: \_\_. \_\_ ml/min. (Must be at least  $\geq 0.2$  ml/min.)

Principal Investigator's signature:..... Date \_\_/\_\_/\_\_\_\_

<b>Subject code:</b>	<b>Randomisation no.:</b>	<b>Screening no.:</b>
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**Inclusion Criteria Check List\***

	<b>Yes</b>	<b>No</b>
<b>Age:</b> Aged between 6-18 years at the start of the study.	<input type="checkbox"/>	<input type="checkbox"/>
<b>General Health:</b> <ul style="list-style-type: none"> <li>• No relevant medical history and under no medication that is known to affect the oral cavity, the oral flora status and salivary flow rate.</li> <li>• Non-pregnant female volunteers</li> </ul>	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
<b>Dental Examination:</b> <ul style="list-style-type: none"> <li>• Presence of first permanent molars and upper anterior teeth.</li> <li>• Free from visual signs of untreated caries or periodontal disease.</li> <li>• Not exposed to water fluoride levels &gt;0.1ppmF.</li> <li>• Unstimulated Salivary flow rate <math>\geq</math> 0.2 ml/min and</li> <li>• Refrain from using any additional fluoride products during the study period other than 1,000-1,450ppmF tooth-paste and 225ppmF mouth-rinse</li> </ul>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
<b>Compliance:</b> Understand and is willing, able and likely to comply with all study procedures and restrictions.	<input type="checkbox"/>	<input type="checkbox"/>
<b>Parental Consent:</b> Parent(s)/legal guardian(s) demonstrates understanding of the study and willingness for the volunteer to participate as evidenced by voluntary written informed consent.	<input type="checkbox"/>	<input type="checkbox"/>

\***Note:** If any of the above questions are answered "No", the subject should be discontinued from the study as a "Screen failure" on the study conclusion page.

Principal Investigator's signature:..... Date / /

Subject code:	Randomisation no.:	Screening no.:
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**Exclusion Criteria Checklist\***

Age:	Yes	No
Aged above 18 years old at the start of the study.	<input type="checkbox"/>	<input type="checkbox"/>
<b>General Health</b>		
<ul style="list-style-type: none"> <li>• Current or previous history of serious, severe or unstable physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to fully complete the study.</li> </ul>	<input type="checkbox"/>	<input type="checkbox"/>
<ul style="list-style-type: none"> <li>• Pregnant female volunteer.</li> </ul>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Medication:</b>		
Medication that is known to affect the oral cavity, oral flora status and salivary flow rate.	<input type="checkbox"/>	<input type="checkbox"/>
<b>Dental Details:</b>		
<ul style="list-style-type: none"> <li>• Dental disease that require immediate treatment.</li> </ul>	<input type="checkbox"/>	<input type="checkbox"/>
<ul style="list-style-type: none"> <li>• Absence of all four first permanent molars and upper anterior teeth.</li> </ul>	<input type="checkbox"/>	<input type="checkbox"/>
<ul style="list-style-type: none"> <li>• Exposure to water fluoride levels of &gt;0.1ppmF.</li> </ul>	<input type="checkbox"/>	<input type="checkbox"/>
<ul style="list-style-type: none"> <li>• Use of fluoride products other than 1,000-1,450 ppmF tooth-paste and 225ppmF mouth-rinse.</li> </ul>	<input type="checkbox"/>	<input type="checkbox"/>
<ul style="list-style-type: none"> <li>• Unstimulated Salivary flow rate &lt; 0.2 ml/min and</li> </ul>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Clinical Trials:</b>		
Participation in another clinical study or receipt of an investigational drug within 30 days of the screening visit at the start of the study.	<input type="checkbox"/>	<input type="checkbox"/>
<b>Parental Consent:</b>		
Parent(s)/legal guardian(s) are not willing for the volunteer to participate	<input type="checkbox"/>	<input type="checkbox"/>

*\*Note:* If any of the above questions are answered "Yes", the subject should be discontinued from the study as a "Screen failure" on the study conclusion page.

Principal Investigator's signature:..... Date / /

**Leeds Dental Institute**  
The Centre for Oral Health Sciences



University of Leeds School of Dentistry with the  
Dental Hospital at Leeds  
Division of Child Dental Health  
A Centre for Children with Special Needs

<b>Subject code:</b>	<b>Randomisation no.:</b>	<b>Screening no.:</b>
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**Fitness and Eligibility to Participate in the Study**

In the investigator's opinion, on the basis of the screening assessments and Inclusion and Exclusion criteria, is the subject eligible to participate in the study?

Yes

No

Principal Investigator's signature: ..... Date \_/ \_/ \_



<b>Subject code:</b>	<b>Randomisation no.:</b>	<b>Screening no.:</b>
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**Screening Visit Check List**

Personal data sheet completed	Yes	No
Medical history checked	Yes	No
Dental Examination completed	Yes	No
Inclusion criteria sheet completed	Yes	No
Exclusion criteria sheet completed	Yes	No
Eligibility sheet completed	Yes	No
Participant' s Information Sheet	Yes	No
Participant's Assent Form (optional)	Yes	No
Parental Information Sheet	Yes	No
Parental Consent Form Signed	Yes	No
Letter to GP	Yes	No
Letter to GDP	Yes	No
Consent to have orthodontic treatment	Yes	No
Fluoride-free toothpaste and instructions?	Yes	No
Study baseline appointment arranged?	Yes	No

Investigator's signature:..... Date \_/ \_/ \_

<b>Subject code:</b>	<b>Randomisation no.:</b>	<b>Screening no.:</b>
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**Study baseline appointment Date** \_\_ / \_\_ / \_\_

Use of F-free toothpaste the night before?	<b>Yes</b>	<b>No</b>
...the morning of appointment?	<b>Yes</b>	<b>No</b>
Saliva before placement of device*	<b>Yes</b>	<b>No</b>
Löe Plaque Index for subject	<b>Yes</b>	<b>No</b>
Löe Gingival Index for subject	<b>Yes</b>	<b>No</b>
Dental prophylaxis (F-free paste)	<b>Yes</b>	<b>No</b>
QLF	<b>Yes</b>	<b>No</b>
Any problems?	<b>Yes</b>	<b>No</b>
If yes please give details		
Digital photographs	<b>Yes</b>	<b>No</b>
Any problems?	<b>Yes</b>	<b>No</b>
If yes please give details		

Subject code:	Randomisation no.:	Screening no.:
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**Study baseline appointment Date** \_\_\_ / \_\_\_ / \_\_\_\_\_  
**(continuation)**

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Randomisation	<b>Yes</b>	<b>No</b>
Device between 16-15	<b>Yes</b>	<b>No</b>
Any problems?	<b>Yes</b>	<b>No</b>
If yes please give details		
Device between 26-25	<b>Yes</b>	<b>No</b>
Any problems?	<b>Yes</b>	<b>No</b>
If yes please give details		
Saliva after placement of device*	<b>Yes</b>	<b>No</b>
Appointment arranged after 1 month?	<b>Yes</b>	<b>No</b>
Reminder to use F-free toothpaste?	<b>Yes</b>	<b>No</b>

**\*Saliva sample (whole unstimulated saliva pooled over FSRGD for 2min-need to have at least 2ml)**

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<b>Subject code:</b>	<b>Randomisation no.:</b>	<b>Screening no.:</b>
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**Pre-orthodontic treatment**

<b>Plaque Index</b>	<b>16</b>	<b>12</b>	<b>11</b>	<b>24</b>	<b>26</b>	<b>36</b>	<b>32</b>	<b>31</b>	<b>44</b>	<b>46</b>
Buccal										
Mesial										
Lingual										
Distal										
Mean PI tooth										

PI (light, drying of teeth and gingivae, mirror and pocket probe)  
 0=No plaque.  
 1=Plaque recognized only by running a probe across the tooth surface.  
 2=Plaque visible with naked eye.  
 3=Abundance of soft matter.

<b>Gingival Index</b>	<b>16</b>	<b>12</b>	<b>11</b>	<b>24</b>	<b>26</b>	<b>36</b>	<b>32</b>	<b>31</b>	<b>44</b>	<b>46</b>
Buccal										
Mesial										
Lingual										
Distal										
Mean GI tooth										

GI (light, drying of teeth and gingivae, mirror and pocket probe)  
 0=Normal gingivae; absence of inflammation  
 1= No bleeding on pressure; mild inflammation; slight change in colour and texture  
 2=Bleeding on pressure; moderate inflammation; glazing, redness, oedema and hypertrophy.  
 3=Tendency to spontaneous bleeding; marked redness and hypertrophy, ulceration.

You can examine the following 6 teeth. 16, 12, 24, 36, 32, 44.  
 Teeth and gums need to be dried with a blast of air.  
 You can examine one proximal site (either mesial or distal) but you need to double the score to calculate the final score.  
 PI / GI for tooth = add score for buccal, lingual, mesial, distal site / 4 sites.  
 PI / GI for group of teeth = add PI / GI for all incisors / number of incisors.  
 PI / GI for subject = add PI / GI for group of teeth / 6 groups of teeth.

Subject code:	Randomisation no.:	Screening no.:
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**Pre-Orthodontic white spot lesions with QLF**

Tooth	13	12	11	21	22	23	Details
Presence							
Absence							
Location							

Examiner: .....

Examination: 1 / 2

Additional Comments

Subject code:	Randomisation no.:	Screening no.:
---------------	--------------------	----------------

**Follow-up appointment (1 month) Date** \_ \_ / \_ \_ / \_ \_ \_ \_

Use of F-free toothpaste the night before?	<b>Yes</b>	<b>No</b>
...the morning of appointment?	<b>Yes</b>	<b>No</b>
Saliva sample*	<b>Yes</b>	<b>No</b>
Any problems?	<b>Yes</b>	<b>No</b>
If yes please give details		
Change of fluoride device?	<b>Yes</b>	<b>No</b>
Loss of fluoride device?	<b>Yes</b>	<b>No</b>
Breakage of fluoride holder?	<b>Yes</b>	<b>No</b>
Appointment arranged after 2 months?	<b>Yes</b>	<b>No</b>
Reminder to use F-free toothpaste?	<b>Yes</b>	<b>No</b>

Additional Comments i.e. change of orthodontic wire

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**\*Saliva sample (whole unstimulated saliva pooled over FSRGD for 2min-need to have at least 2ml)**

Subject code:	Randomisation no.:	Screening no.:
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**Follow-up appointment (3 months) Date** \_ \_ / \_ \_ / \_ \_ \_ \_

Use of F-free toothpaste the night before?	<b>Yes</b>	<b>No</b>
...the morning of appointment?	<b>Yes</b>	<b>No</b>
Saliva sample*	<b>Yes</b>	<b>No</b>
Any problems?	<b>Yes</b>	<b>No</b>
If yes please give details		
Change of fluoride device?	<b>Yes</b>	<b>No</b>
Loss of fluoride device?	<b>Yes</b>	<b>No</b>
Breakage of fluoride holder?	<b>Yes</b>	<b>No</b>
Appointment arranged after 3 months?	<b>Yes</b>	<b>No</b>
Reminder to use F-free toothpaste?	<b>Yes</b>	<b>No</b>

Additional Comments i.e. change of orthodontic wire

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**\*Saliva sample (whole unstimulated saliva pooled over FSRGD for 2min-need to have at least 2ml)**

Subject code:	Randomisation no.:	Screening no.:
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**Follow-up appointment (6 months) Date** \_\_ / \_\_ / \_\_\_\_

Use of F-free toothpaste the night before?	<b>Yes</b>	<b>No</b>
...the morning of appointment?	<b>Yes</b>	<b>No</b>
Saliva sample*	<b>Yes</b>	<b>No</b>
Any problems?	<b>Yes</b>	<b>No</b>
If yes please give details		
Change of fluoride device?	<b>Yes</b>	<b>No</b>
Loss of fluoride device?	<b>Yes</b>	<b>No</b>
Breakage of fluoride holder?	<b>Yes</b>	<b>No</b>
Appointment arranged after 6 months?	<b>Yes</b>	<b>No</b>
Reminder to use F-free toothpaste?	<b>Yes</b>	<b>No</b>

Additional Comments i.e. change of orthodontic wire

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**\*Saliva sample (whole unstimulated saliva pooled over FSRGD for 2min-need to have at least 2ml)**



Subject code:	Randomisation no.:	Screening no.:
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**Follow-up appointment (12 months) Date** \_ / \_ / \_ \_ \_ \_

Use of F-free toothpaste the night before?	<b>Yes</b>	<b>No</b>
...the morning of appointment?	<b>Yes</b>	<b>No</b>
Saliva sample*	<b>Yes</b>	<b>No</b>
Any problems?	<b>Yes</b>	<b>No</b>
If yes please give details		
Change of fluoride device?	<b>Yes</b>	<b>No</b>
Loss of fluoride device?	<b>Yes</b>	<b>No</b>
Breakage of fluoride holder?	<b>Yes</b>	<b>No</b>
Appointment arranged after 6 months?	<b>Yes</b>	<b>No</b>
Reminder to use F-free toothpaste?	<b>Yes</b>	<b>No</b>

Additional Comments i.e. change of orthodontic wire

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**\*Saliva sample (whole unstimulated saliva pooled over FSRGD for 2min-need to have at least 2ml)**

Subject code:	Randomisation no.:	Screening no.:
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**Follow-up appointment (18 months) Date** \_\_ / \_\_ / \_\_\_\_

Use of F-free toothpaste the night before?	<b>Yes</b>	<b>No</b>
...the morning of appointment?	<b>Yes</b>	<b>No</b>
Saliva sample*	<b>Yes</b>	<b>No</b>
Any problems?	<b>Yes</b>	<b>No</b>
If yes please give details		
Change of fluoride device?	<b>Yes</b>	<b>No</b>
Loss of fluoride device?	<b>Yes</b>	<b>No</b>
Breakage of fluoride holder?	<b>Yes</b>	<b>No</b>
Appointment arranged after 6 months or debond?	<b>Yes</b>	<b>No</b>
Reminder to use F-free toothpaste?	<b>Yes</b>	<b>No</b>

Additional Comments

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**\*Saliva sample (whole unstimulated saliva pooled over FSRGD for 2min-need to have at least 2ml)**

Subject code:	Randomisation no.:	Screening no.:
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**De-bonding appointment**                      **Date** \_ \_ / \_ \_ / \_ \_ \_ \_

Use of F-free toothpaste the night before?	<b>Yes</b>	<b>No</b>
...the morning of appointment?	<b>Yes</b>	<b>No</b>
Saliva sample*	<b>Yes</b>	<b>No</b>
Löe Plaque Index for subject	<b>Yes</b>	<b>No</b>
Löe Gingival Index for subject	<b>Yes</b>	<b>No</b>
Removal of fixed appliances/devices	<b>Yes</b>	<b>No</b>
Any problems?	<b>Yes</b>	<b>No</b>
If yes please give details		
Dental prophylaxis (F-free paste)	<b>Yes</b>	<b>No</b>
Dental examination	<b>Yes</b>	<b>No</b>
QLF	<b>Yes</b>	<b>No</b>
Any problems?	<b>Yes</b>	<b>No</b>
If yes please give details		
Questionnaire to participant	<b>Yes</b>	<b>No</b>
Questionnaire to orthodontist	<b>Yes</b>	<b>No</b>
Digital photographs	<b>Yes</b>	<b>No</b>

**\*Saliva sample (whole unstimulated saliva pooled over FSRGD for 2min-need to have at least 2ml)**

<b>Subject code:</b>	<b>Randomisation no.:</b>	<b>Screening no.:</b>
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**Post-orthodontic treatment**

<b>Plaque Index</b>	<b>16</b>	<b>12</b>	<b>11</b>	<b>24</b>	<b>26</b>	<b>36</b>	<b>32</b>	<b>31</b>	<b>44</b>	<b>46</b>
Buccal										
Mesial										
Lingual										
Distal										
Mean PI tooth										

PI (light, drying of teeth and gingivae, mirror and pocket probe)  
 0=No plaque.  
 1=Plaque recognized only by running a probe across the tooth surface.  
 2=Plaque visible with naked eye.  
 3=Abundance of soft matter.

<b>Gingival Index</b>	<b>16</b>	<b>12</b>	<b>11</b>	<b>24</b>	<b>26</b>	<b>36</b>	<b>32</b>	<b>31</b>	<b>44</b>	<b>46</b>
Buccal										
Mesial										
Lingual										
Distal										
Mean GI tooth										

GI (light, drying of teeth and gingivae, mirror and pocket probe)  
 0=Normal gingivae; absence of inflammation  
 1= No bleeding on pressure; mild inflammation; slight change in colour and texture  
 2=Bleeding on pressure; moderate inflammation; glazing, redness, oedema and hypertrophy.  
 3=Tendency to spontaneous bleeding; marked redness and hypertrophy, ulceration.

You can examine the following 6 teeth. 16, 12, 24, 36, 32, 44.  
 Teeth and gums need to be dried with a blast of air.  
 You can examine one proximal site (either mesial or distal) but you need to double the score to calculate the final score.  
 PI / GI for tooth = add score for buccal, lingual, mesial, distal site / 4 sites.  
 PI / GI for group of teeth = add PI / GI for all incisors / number of incisors.  
 PI / GI for subject = add PI / GI for group of teeth / 6 groups of teeth.

Subject code:	Randomisation no.:	Screening no.:
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**Post-Orthodontic orthodontic white spot lesions QLF**

Tooth	13	12	11	21	22	23	Details
Presence							
Absence							
Location							

Examiner:.....

Examination:      1    /    2

Additional Comments

## Leeds Dental Institute

The Centre for Oral Health Sciences



Subject code:	Randomisation no.:	Screening no.:
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University of Leeds School of Dentistry with the  
Dental Hospital at Leeds  
Division of Child Dental Health  
A Centre for Children with Special Needs

### Adverse Events

Adverse Event	Onset Date	End Date	Duration	Outcome	Pattern	Intensity	Relation to study	Action taken	Serious*
	___/___/___	___/___/___							
	___/___/___	___/___/___							
	___/___/___	___/___/___							
	___/___/___	___/___/___							
	___/___/___	___/___/___							
	___/___/___	___/___/___							
	___/___/___	___/___/___							
<b>Duration(Units)</b> 1. S-Seconds 2. M-Minutes 3. H-Hours 4. D-Days	<b>Outcome</b> 1. Resolved 2. Ongoing	<b>Pattern</b> 1. Continuous 2. Intermittent	<b>Intensity</b> 1. Mild 2. Moderate 3. Severe	<b>Relationship to study</b> 1. Not relate 2. Unlikely 3. Possible 4. Highly possible	<b>Action Taken (regarding the study)</b> 1. None 2. Interrupted 3. Discontinued	<b>Serious</b> 1. No 2. Yes*			

\* All serious adverse events must be reported to the study monitor within 24 hours and require special action

Subject code:	Randomisation no.:	Screening no.:
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**DIARY**

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Date of first contact in orthodontic department.....  
Date of orthodontic treatment plan & consent.....  
Date of study screening & study consent form.....  
Date of bonding of fixed appliances.....  
1 month review.....  
3 months review.....  
6 months review.....  
12 months review.....  
18 months review.....  
Date of de-bonding of fixed appliances.....  
Review/orthodontic retainer appointment.....

Additional Comments

Subject code:	Randomisation no.:	Screening no.:
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**STUDY CONCLUSION**

Did the subject complete the entire study?                      Yes       No\*

*If "No" is checked, please complete the following (please check as an appropriate):*

Screen Failure                     

Adverse Event                     

Lost of Follow-up                     

Protocol Deviation                       \_\_\_\_\_

Withdrawal of Volunteer                       \_\_\_\_\_

Other                       \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**Investigator's Signature**

I confirm that I have reviewed all the data collected in this Case Report Form and take responsibility that the information is accurate and complete.

**Study Investigator's Name**.....

**Study Investigator's Signature**.....

**Date** \_\_\_/\_\_\_/\_\_\_\_\_



## 12.11 Satisfaction Questionnaire for Orthodontists

Version 1, 18/01/2008



### Satisfaction Questionnaire for Orthodontists

Please circle all you agree with:

1. It was easy to work with the bead in the patient's mouth.  
Strongly agree / Agree / Disagree / Strongly disagree
2. It was difficult to work with the bead in the patient's mouth.  
Strongly agree / Agree / Disagree / Strongly disagree
3. It was straightforward to place/remove the plastic holder with the bead.  
Strongly agree / Agree / Disagree / Strongly disagree
4. It was time consuming to place/remove the plastic holder with the bead.  
Strongly agree / Agree / Disagree / Strongly disagree
5. Did the bead interfere with the orthodontic fixed appliance? Yes / No
6. If yes in what way? .....
7. Anything you want to tell us about the bead? .....
8. Anything you would like us to change to the bead? .....

## 12.12 Satisfaction Questionnaire for Participants

Version 2, 4/23/2008



Centre Number:  
Study Number:  
Patient Identification Number for this trial:

### Satisfaction Questionnaire for Participants (Children aged 6-10 years and Young People aged 11-15 years)

Please circle all you agree with:

1. Has it been easy for you to have the bead in the mouth? Yes / No

2. Has it been difficult to have the bead in the mouth? Yes / No

3. Did the bead feel uncomfortable/strange at all? Yes / No

4. Would it feel different without the bead in your mouth? Yes / No

5. Anything you want to tell us about the bead? .....

6. Anything you would like us to change to the bead? .....

**12.13 Randomisation codes**

Females		Males	
ID	Group	ID	Group
1	A	1	A
2	A	2	A
3	A	3	B
4	A	4	B
5	B	5	A
6	A	6	A
7	A	7	A
8	A	8	B
9	B	9	A
10	A	10	B
11	A	11	B
12	A	12	A
13	B	13	B
14	B	14	B
15	A	15	B
16	A	16	B
17	A	17	A
18	B	18	B
19	B	19	A
20	B	20	B
21	A	21	A
22	A	22	A
23	B	23	B
24	B	24	B
25	B	25	A
26	B	26	A
27	B	27	B
28	B	28	B
29	B	29	A
30	B	30	A
31	B	31	B
32	B	32	B
33	A	33	B
34	B	34	A
35	B	35	A
36	A	36	A
37	A	37	B
38	B	38	B
39	B	39	B
40	B	40	A
41	A	41	A
42	B	42	A
43	A	43	B
44	A	44	B
45	A	45	A
46	B	46	A
47	A	47	B
48	A	48	A
49	B	49	A
50	A	50	B
51	B	51	B
52	A	52	B
53	A	53	B
54	B	54	B
55	A	55	A
56	B	56	A
57	B	57	A
58	B	58	B
59	A	59	A
60	A	60	A

12.14 Questionnaire on WSLs emailed to members of British Orthodontic Society

**Questionnaire regarding the effect of white spot lesions (WSLs) on fixed orthodontic treatment (FAOT). We need your current clinical opinion. We do not expect you to look up references, books, etc. for this "correct" answer!**

Male / Female Age : \_\_\_\_\_ years Orthodontic qualification (year and where obtained) \_\_\_\_\_

In what dental settings do you work? (Please indicate proportion of time e.g. 50% Hospital ; 50% University; 100% Community )

Specialist \_\_\_\_\_ Community \_\_\_\_\_ GDP \_\_\_\_\_ NHS Specialist Practice \_\_\_\_\_ University \_\_\_\_\_

In your opinion, what is the problem with WSLs for patients under 18 years of age who have FAOT?

X next to all that apply

delay completion of orthodontic treatment

Need for restorative care

Other (please specify) \_\_\_\_\_

For each patient asked you to quantify the risk of developing WSLs, what percentage would you give them? \_\_\_\_\_

What is the protocol you follow to prevent WSLs additional to adult (>1,100ppm F) toothpaste?

X next to all that apply

fluoride mouth-rinse (225 ppmF)

fluoride mouth-rinse (900 ppmF)

fluoride concentration toothpaste (>1,450 ppm F)

fluoride ionomer based bonding materials for bonding brackets

Professional dental plaque removal

Professional dental plaque removal with F applicator

Other (please specify) \_\_\_\_\_

In your opinion which are the most important risk factors for developing WSLs?

Most important  
Second most important  
Third most important

In your opinion are the following risk factors (as identified in the literature) important in developing WSLs?  
 (Delete as appropriate)

a. Pre-existing WSLs	Yes / No	d. DMFT	Yes / No	g. Oral hygiene	Yes / No
b. Socio-Economic-Status	Yes / No	e. Age of patient	Yes / No		
c. Duration of orthodontic treatment	Yes / No	f. Gender of patient	Yes / No		

How do you diagnose the presence of WSLs during orthodontic treatment? (Type X next to all that apply)

Visual clinical examination	<u>Use of specialised equipment (please name)</u>
Photographs	<u>Other (please specify)</u>

How do you assess the severity of WSLs during orthodontic treatment? (Type X next to all that apply)

Visual clinical examination	<u>Use of scoring system (please name)</u>
Photographs	<u>Use of specialised equipment (please name)</u>
<u>Other (please specify)</u>	

If WSLs are found during and/or after completion of treatment what is your protocol? (Type X next to all that apply)

Fluoride application - in surgery	Termination of orthodontic treatment	Microabrasion
Fluoride application - at home	Diet advice	Restorative care
Oral Hygiene Instructions	Discussion with patient/parent(s)	
<u>Other (please specify)</u>		

Would you consider using a simple glass bead threaded onto an orthodontic wire that continuously released fluoride, if it was proven to effectively prevent WSLs?

(See attached photograph) Yes / No

12.15 Abstract for International Association of Dental Research (IADR)  
Meeting in Barcelona, Spain July 2010

**Socio-economic status – might it affect clinical trial recruitment?**

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Leeds Dental Institute, UK.

**Objectives:** To investigate socio-economic characteristics of eligible participants who were invited to participate in a clinical trial as these may influence clinical trial recruitment.

**Methods:** Following ethical approval,<sup>(1)</sup> n=60 participants had to be recruited in a randomized clinical trial involving orthodontic patients at the Leeds Dental Institute. The aim was to test the effectiveness of the fluoride slow release glass devices for the prevention of white spot lesions on the anterior maxillary teeth during the course of fixed appliance orthodontic treatment. All relevant information was posted to eligible volunteers to allow them time to consider participation. Their postcodes were used to identify their Multiple Deprivation Index, constructed by the Social Disadvantage Research Centre at the University of Oxford, at Lower Layer Super Output Area (LSOA) level,<sup>(2)</sup> published in 2007 by the Department of Communities and Local Government. Each LSOA in England is given an overall MDI score; the lower the score the lower the deprivation of the area.<sup>(3)</sup>

**Results:** After 10 months 63 participants volunteered to participate in the trial whereas 28 refused to do so. The range of their MDI scores was 3.14-59.05 (mean=21.24, median=17.37). Descriptive statistics and crosstabs showed significant differences across the quartiles of the MDI scores between the groups. Amongst those who refused participation 43% (12/28) were living in the least deprived areas and 14% (4/28) in the most deprived areas. Within the group who volunteered to participate, the majority 32% (20/63) belonged to the third quartile (MDI 17.37-31.26) whereas the minority 16% (10/63) belonged to the least deprived group.

**Conclusions:** In this sample the majority of participants live in a more deprived area whereas the majority of those who refused participation live in the least deprived areas. This difference may be a source of bias that may need to be addressed in future clinical trials.

References:

(1) Ethical approval from Leeds Central Research Ethics Committee (08/H1313/6) and Research & Development office (DT08/8473).

(2) <http://www.spsw.ox.ac.uk/fileadmin/statio/sdrc/>

(3) <http://www.communities.gov.uk/communities/neighbourhoodrenewal/deprivation/deprivation07/>

## 12.16 Poster presentation for International Association of Dental Research (IADR) Meeting in Barcelona, Spain July 2010

**Leeds Dental Institute**  
FACULTY OF MEDICINE AND HEALTH

  
UNIVERSITY OF LEEDS

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**Socio-economic status – might it affect clinical trial recruitment?**

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Department of Paediatric Dentistry and Orthodontics, Leeds Dental Institute, UK

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**Objectives:** To investigate whether socio-economic characteristics of orthodontic patients invited to participate in a clinical trial may influence recruitment.

**Methods:** Following a favourable opinion from the Leeds Central Research Ethics Committee (Ref 08/H1313/6) and Research & Development Office (DT08/8473), relevant information was posted to eligible volunteers to allow them time to decide whether to participate in a double blind, randomised clinical trial. The aim of the trial was to test the effectiveness of fluoride slow release glass devices for the prevention of white spot lesions on the anterior maxillary teeth during the course of fixed appliance orthodontic treatment. There was no active involvement of the patients and digital photographs were taken at the start and the end of their treatment. Following a sample size calculation, recruitment of n=60 orthodontic patients at the Leeds Dental Institute began in November 2008. The postcodes of eligible volunteers were used to identify their overall Multiple Deprivation Index, constructed by the Social Disadvantage Research Centre at the University of Oxford UK, at Lower Layer Super Output Area (LSOA) level.<sup>(1)</sup> The Output Area is a geographical unit, built from clusters of adjacent postcodes, designed to have similar population sizes and to be as socially homogenous as possible. Each LSOA in England is given an overall MDI score (range 0.37–85.46); the higher the score the higher the deprivation of the area.<sup>(2)</sup> The latest figures were published in 2007 by the Department of Communities and Local Government.

**Results:**



Multiple Deprivation Index score	YES=agreed to participate	NO=declined to participate
Q1 (3.14-8.74)	10	12
Q2 (8.74-17.37)	15	9
Q3 (17.37-31.26)	20	3
Q4 (31.26-59.05)	18	4

After 10 months, a total of 91 eligible participants had been informed about the study; 63/91 volunteered to participate in the clinical trial whereas 28/91 declined.

Amongst those who refused to participate, the majority (12/28 or 43%) were living in the least deprived areas and the minority (4/28 or 14%) in the most deprived areas.

For those who agreed to participate, the results were almost the opposite. The majority (20/63 or 32%) belonged to the third quartile across the spectrum (MDI 17.37-31.26) whereas the minority (10/63 or 16%) belonged to the least deprived group.

In this sample Fischer's Exact test showed a statistically significant association ( $p < 0.002$ ) between MDI score (quartile group) and participation in the study.

**Conclusions:** In this random sample, there was similar representation from both sides of the spectrum with n=45 subjects living in the most deprived areas and n=46 subjects in the least deprived areas. Results though showed that the difference in MDI score amongst those who volunteered to participate and those who declined was statistically significant. This difference may be a source of bias that may need to be addressed in future clinical trials. The outcome of recruitment for clinical trials is based on voluntary participation hence it is a variable that cannot be controlled and ideally should result in a study sample that is homogenous and representative of the population or the setting that it refers to. It should not be unbalanced with regards to major confounding factors and socio-economic strata may warrant further investigation. It may also make recruitment to orthodontic trials more difficult since there is greater uptake of orthodontic services from children from higher socio-economic strata. <sup>(3,4)</sup>

**References:** (1) <http://www.spsw.ox.ac.uk/fileadmin/static/sdrc/>  
(2) <http://www.communities.gov.uk/communities/neighbourhoodrenewal/deprivation/deprivation07/>  
(3) Jenkins PM et al 1984. The effect of social factors on referrals for orthodontic advice and treatment. British Journal of Orthodontics 11(1): 24-6  
(4) Drugan CS et al 2007. Inequality in uptake of orthodontic services. British Dental Journal 24, 202(6): E15

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