Consequences of salivary contamination and its remedies on bond quality; comparing the modern adhesive concepts

Dissertation From Pooja Nair

2020

From Department of Operative Dentistry, University Hospital of Ludwig Maximilians University, Munich
Director: Prof. Dr. med. dent. Reinhard Hickel

Consequences of salivary contamination and its remedies on bond quality; comparing the modern adhesive concepts

Dissertation
to acquire the degree of Doctor of Philosophy (Ph.D.) of the Medical faculty of Ludwig Maximilians University, Munich

Submitted by
Pooja Nair

From
Bangalore

Year
2020

With permission from the medical faculty of Ludwig-Maximilians-Universität München

Supervisor:	Prof. Dr. Nicoleta Ilie
Second evaluator:	Prof. Dr. Dipl. Ing. Bogna Stawarcyzk
Dean:	Prof. Dr. med. dent. Reinhard Hickel
Date of oral defence :	05 June 2020

To my family and friends who have always supported me to achieve my dreams.
To my son for inspiring me to take on new challenges.
To my professor who has been a source of knowledge and inspiration

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Dean's Office
Medical Faculty

MMRS MUNICH MEDICAL RESEARCH SCHOOL

Affidavit

Nair, Pooja

Surname, first name

Street

Zip code, town

Country

I hereby declare, that the submitted thesis entitled

Consequences of salivary contamination and its remedies on bond quality; comparing the modern adhesive concepts

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

Binningen, 14.07.2020

Place, date

Pooja Nair

Signature doctoral candidate

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Dean's Office
Medical Faculty

MMRS MUNICH MEDICAL RESEARCH SCHOOL

Confirmation of congruency between printed and electronic version of the doctoral thesis

Nair, Pooja

Surname, first name

Street

Zip code, town

Country

I hereby declare that the electronic version of the submitted thesis, entitled
Consequences of salivary contamination and its remedies on bond quality; comparing the modern adhesive concepts

is congruent with the printed version both in content and format.

Binningen, 14.07.2020

Place, date

Pooja Nair

Signature doctoral candidate

List of Abbreviations

ANOVA	Analysis of Variance
BBR	Beautifil Bulk Restorative
BF-RC	Bulk-fill Resin composite
Bis-GMA	Bisphenol-A-Glycidyl Dimethacrylate
Bis-MPEPP	Bisphenol A Polyethoxy Methacrylate
C1	Contamination after Surface preparation
C2	Contamination after Primer application
C3	Contamination after Curing
CI	Confidence Interval
CX	Ceram-X
DC1	Decontamination after Surface preparation
DC2	Decontamination after Primer application
DC3	Decontamination after Curing
EBADMA	Ethoxylated Bisphenol-A-Dimethacrylate,
FBF	Filtek Bulk-fill
HEMA	2- Hydroxyethyl Methacrylate
NC	No contamination
Ormocers	Organically Modified Ceramics
RC	Resin Composite
SBS	Shear Bond Strength
SD	Standard Deviation
SDR	Surefil SDR
SE	Self Etch
SF2	Sonicfil 2
TEC	TetricEvo Ceram
TEF	TetricEvo Flow
TEGDMA	Triethyleneglycol Dimethacrylate
U	Universal
UDMA	Urethane Dimethacrylate
UV	Ultra Violet
VBF	Venus Bulk-Fill

List of Publications

Ph.D Project

The topic of my Ph.D.thesis was "Consequences of salivary contamination and its remedies on bond quality; comparing the modern adhesive concepts"

To fulfill my requirements to achieve Ph.D, I worked on the following projects:

Project 1: In-vitro evaluation of low and high viscosity bulk-fill restoratives vs conventional resin composite in terms of their shear bond strength

Project 2: The long term consequence of salivary contamination at various stages of adhesive application and clinically feasible remedies to decontaminate

Publications and Presentations

1. Nair P and Ilie N (2016) Are novel universal adhesives more resilient to salivary contamination? Dental Materials 32:e46-e47.

2. Nair P, Hickel R and Ilie N (2017) Adverse effects of salivary contamination for adhesives in restorative dentistry. A literature review. American journal of dentistry 30:156-164.

3. Nair P and Ilie N (2020) The long-term consequence of salivary contamination at various stages of adhesive application and clinically feasible remedies to decontaminate. Clinical oral investigations. (Online ahead of print)

List of Figures

List of Tables

Contents

Introduction

In standard dental operative treatment procedures, a rubber dam isolation is recommended [1]. However, there are situations where rubber dam isolation is not possible or isolation protocol is violated, which leads to the operative site to be exposed to contamination. These contaminants may or may not have a detrimental effect on improved and advanced dental adhesive materials. The most common and probable contaminant a clinician has to confront is the saliva.

Saliva contamination

It is recognized that saliva comprises of several hydrolytic enzymes that are competent of reacting with the tooth and the material through different biochemical processes which could modify the surface of the tooth structure and thus compromise bond strength [2]. It has been documented that saliva contact should be avoided since a surface that is acid-etched, attracts salivary proteins, decreases the surface energy and makes it unreliable for adhesion [3].

The modern self-etching adhesives comprise of monomers like bisphenol A diglycidyl ether dimethacrylate (bis-GMA) that bonds to the enamel marvellously. However, the dentin is moist and bis-GMA is hydrophobic, which will make it difficult to infiltrate into dentin. Hence, more hydrophilic monomers like 2-hydroxylethyl methacrylate (HEMA) are used to enhance the monomer penetration into the tubules. Additionally, monomers also contain hydrophilic groups such as carboxylic acid, hydroxyl, ester and amine to boost the water solubility of adhesives. These additives make them more prone to hydrolysis in

the mouth[4] because of water absorption occuring at the adhesive level and act as a semi-permeable membrane [5]. When a universal adhesive is used on dentin, the precise balance between the hydrophilic and hydrophobic components plays a significant role. The monomers must primarily be hydrophilic enough to moisten, penetrate, and interact with the dentin substrate, nevertheless after they are polymerized, if they remain hydrophilic, they can escalate the water sorption, which could lead to hydrolysis and degenerate the adhesive interface over time. After placing a universal adhesive, a highly cross-linked hydrophobic polymer matrix is anticipated, which is attached to the dentin on one end and restorative materials on the other end [6].

Several in-vitro studies are performed for understanding the possible influence of salivary contamination on adhesives. A clinical study demonstrated lower bond strength when subjected to contamination and suggested rubber-dam placement before cavity preparation resulted in significantly higher bond quality. SEM images of samples contaminated with saliva disclosed the presence of structural imperfections at the interface and showed a shorter resin tags on pull-out [7].

A recent in vitro study investigated the consequence of relative humidity and saliva contamination on the bond strength in dentin after one year and observed that the two self-etching adhesives showed a stable bond strength over time [8]. The concept of universal adhesives was introduced a decade ago and innovative universal adhesives with distinctive features are endlessly produced by different manufacturers. It is recognized that moisture trapped within the adhesive during polymerization may cause an inferior polymerization of the adhesive monomers [9]. It is essential to identify the consequences of salivary contamination on these new adhesives when the ingredients are sensitive to moisture. A little or no evidence is available on the long term bonding performance of universal adhesives to saliva contaminated dentin and on the comparative distinctions of its properties in comparison with the well established self-etch systems.

Aim of the Study

The objective of this doctoral thesis was to contribute to a better understanding of the influence of salivary contamination on the quality of bonding of contemporary dental adhesives and its consequences on the bonding effectiveness to dentin in long term. Also, if the effect of contamination is found to be substantial, the study also aims to understand which stage in the adhesive application is more vulnerable to contamination. Furthermore, to understand if clinically feasible decontamination procedures can regain the original bond quality.

The null hypothesis verified were that:

a. Salivary contamination does not affect the bond quality of modern dental adhesives

b. Decontamination methods employed at various stages of the restorative process does not affect the bond quality of modern dental adhesives.

c. The stage of salivary contamination or decontamination is not critical to the bond quality

d. The type of salivary decontamination is not imperative to the bond quality

e. Aging of the substrates does not alter their bond quality

f. The type of adhesive does not differ in their ability of bonding

g. The type of composite restorative material does not influence the bond strength.

Organization of Thesis

This thesis is presented in a three-article dissertation construct.

- Chapter one of this thesis is dedicated to the literature review done on the adverse effects of salivary contamination on the dental adhesives and is published in the American Journal of dentistry.

- Chapter two describes a small pilot study that was done to test the methodology by evaluating various commercially available bulk-fill RC in two different viscosities.

- Chapter three explains the major experiment, the long-term consequences of salivary contamination on various stages of self-etching and universal adhesive application and the clinically feasible remedies to decontaminate during the treatment and also to identify the most vulnerable situation in case of salivary contamination clinically.

References

1. Batchelder KF, Richter RS, Vaidyanathan TK. Clinical factors affecting the strength of composite resin to enamel bonds. The Journal of the American Dental Association 1987; 114: 203-205.

2. Chauncey HH, Lionetti F, Winer RA, Lisanti VF. Enzymes of Human Saliva:I. the Determination, Distribution, and Origin of whole Saliva Enzymes. Journal of Dental Research 1954; 33: 321-334.

3. Buonocore MG. Caries prevention in pits and fissures sealed with an adhesive resin polymerized by ultraviolet light: a two-year study of a single adhesive application. J Am Dent Assoc 1971; 82: 1090-1093.

4. Aboushelib MN. Clinical performance of self-etching adhesives with saliva contamination. J Adhes Dent 2011; 13: 489-493.

5. Ferracane JL. Hygroscopic and hydrolytic effects in dental polymer networks. Dental Materials 2006; 22: 211-222. 6. Tay FR, Frankenberger R, Krejci I, Bouillaguet S, Pashley DH, Carvalho RM, Lai CNS. Single-bottle adhesives behave as permeable membranes after polymerization. I. In vivo evidence. Journal of Dentistry 2004; 32: 611-621.

7. Van Landuyt KL, Snauwaert J, Peumans M, De Munck J, Lambrechts P, Van Meerbeek B. The role of HEMA in one-step self-etch adhesives. Dent Mater 2008; 24: 1412-1419.

8. Amsler F, Peutzfeldt A, Lussi A, Flury S. Long-Term Bond Strength of Self-Etch Adhesives to Normal and Artificially Eroded Dentin: Effect of Relative Humidity and Saliva Contamination. J Adhes Dent 2017.

9. Cadenaro M, Maravic T, Comba A, Mazzoni A, Fanfoni L, Hilton T, Ferracane J, Breschi L. The role of polymerization in adhesive dentistry. Dental Materials 2019; 35: e1-e22.

Chapter 1

Adverse effects of salivary contamination of adhesives in restorative dentistry-a literature review

1.1 Abstract

Purpose: The study aims to review the literature concerning the influence of salivary contamination on the bond quality of adhesives used in restorative materials by comparing and contrasting the different adhesive materials and critically analyzing them.

Method: A detailed search on PUBMED, Cochrane Library, Google scholar and Web of Science was carried out to identify publications on salivary contamination and dental adhesive materials, from 1990-2017 (March) which resulted in a total of 6202 web-identified publications. After screening titles/abstracts and de-duplicating, 54 publications were selected, that matched the requirements for this review. The condition for selection was English literature concerning the effect of salivary contamination on the adhesives used in restorative dentistry. The obtained articles were systematically evaluated

Results: Salivary contamination of adhesives during restorative procedures statistically (64.6%) shows an adverse effect on adhesives, occurring either at one or many stages of restoration. Methodological dissimilarities impeded the direct comparison of the selected studies. Nevertheless, it was observed that, 2-step etch and rinse adhesive were relatively less vulnerable to salivary contamination than the others. Sixty five percent of the evaluated studies for decontamination, achieved improved bonding when the contaminated surface was subjected decontamination procedure of some kind. However, the duration and other specificities are not standard in all the evaluations and needs further research to assess the course of action. It is necessary to do long term studies to evaluate the effectiveness of contaminated adhesive over time.

Significance: Salivary contamination is a potential cause for a poor bond quality of adhesive systems during restorative process and to provide a successful treatment proper care must be taken to ensure the operating area is free from contamination. Understanding the properties of the materials and its constituents as well as considering measures to manage the potential vulnerabilities due to salivary contamination in the area of bonding might help a clinician to produce better results.

1.2 Introduction

Over the past decades, dental adhesives have progressed with changes in chemistry, application and technique. The evolved adhesive materials have led to intense reconsiderations in the practice of restorative dentistry. The foremost objective of a dental adhesive in restorative dentistry is offering retention to composite fillings. In adjunct to enduring shrinkage stress and mechanical forces from the overlaying RC material, an ideal adhesive ought to be able to inhibit leakage at the margins of the restoration [1].

The ability of modern formulations of adhesives is based on dual function. On one end, the adhesive attaches to the composite by co-polymerization of residual double bonds (-C=C-) and on the other end, it holds on to the tooth substrate which is principally based on micromechanical adhesion [2].This is achieved by substituting the inorganic tooth material with resin monomers which form tags that gets intermingled in dentin on polymerization [3].

The usual treatment procedures are often known to expose these materials to various factors in and around the oral cavity which may result in contamination and cause difficulty in their infiltration to provide the necessary mechanical bonding and eventually deteriorate quality. Saliva is the most common component present in oral cavity and has a high probability to influence an operative field. It constitutes of 99.4% water and 0.6% solids. They are mainly aggregates of molecules such as glycoproteins, sugar, proteins and amylase and inorganic components like sodium, chloride, calcium [4]. An acid conditioned tooth surface readily absorbs salivary constituents and decreases the surface energy, leaving the surface unfavorable for bonding [5]. A prerequisite for a durable adhesive bonds is clean restorative surface and maintain a high energy state. Presence of water, organic debris, and/or biofilms in a clinical condition might interfere with the wetting and spreading [6, 7].

This review provides a gist of the published articles, concerning the influence of salivary contamination on the quality of bonding of different generation of adhesives in restora-

tive dentistry, and also critically analyses the approaches and protocols used by the researchers.

1.3 Materials and Methods

For this literature review, 54 references [8-61] were selected. An extensive search on PUBMED, Cochrane Library, Google scholar and Web of Science imparted a total of 6202 published articles. The search terminologies used for searching on the online database were (saliva) AND (contamination) AND (adhesive) AND (dental). The search was restricted for the years 1990-2017 (March). The web search was also supplemented by a manual search of reference list from the identified papers. After screening titles and de-duplicating, 54 papers were shortlisted that matched the conditions entirely.

The criteria for selection of articles for this review were English literature pertaining to salivary contamination of adhesives in restorative dentistry. Studies were included if the investigators evaluated the influence of salivary contamination of enamel, dentin or both on the bond quality of adhesive systems in restorative dentistry. The obtained papers were meticulously evaluated under various categories; Year of publishing, type of adhesive, type of contaminant, type of test, parameters of the test, results, surface preparation, method of contamination, quantity and details of contaminant, stages of contamination, decontamination procedure, time between contamination and testing, type of aging, size of bonding area, type of substrate and number of specimens.

1.4 Results

1.4.1 Dental Adhesives

Dental professionals use various adhesive systems in their day to day clinical practice. Depending on the adhesive system used, bonding RC to tooth structure involves multiple steps and the operating surface might get contaminated during any of these steps. Dental

adhesives are broadly categorized into two groups, i.e. Etch and Rinse and Self-etch adhesives (Fig.1.1) [62]. 64.6% of the evaluated adhesives were prone to have a deleterious impact due to salivary contamination.

1.4.1.1 Etch and rinse adhesives

Etching with phosphoric acid dissolves the apatite crystals in hydroxyapatite rich enamel surface to create microporosities, which increases the surface area as well as the surface energy but does not modify the chemical alignment of the surface. In dentin, acid treatment removes the smear layer and demineralises the surface of intertubular dentin to reveal the underlying collagen matrix [63]. Subsequently, either a distinct primer along with an adhesive resin is applied in a 3-step process or a mixture of primer and adhesive resin combined together is applied in a shortened 2-step process [63]. The 3 and 2-step etch-and-rinse adhesives depend on a adhesion mechanism that is similar. The intention is to micro-mechanically interlock and polymerize the monomers that penetrates into the etched enamel and the dentin tubules. It is implicit that etch and rinse adhesive involve multiple steps in their application. Increased number of steps increases the vulnerability of the restorative surface for salivary contamination.

• 3-step-etch and rinse

The seven reviewed articles tested nine 3-step etch and rinse adhesive for consequences of salivary contamination (Table 1.1) and almost 77% of adhesives depicted a negative impact when there was salivary contamination (Fig.1.2). It was observed that, it always had an adverse effect when enamel was contaminated and 62.5% showed negative influence on dentin. According to Xie et al.,[57] the contamination after etching reduced the bond strength in enamel and dentin by 40% and rinsing the contaminated surface with water, air drying and re-etching followed by application of the adhesive, the proteins could be rinsed away improving the bond strength. Patil et al.,[39] reported that just rinsing the contaminated surface after curing the adhesive in 3-step etch and rinse adhesive, could

not reverse the harmful effect.

• 2-step-etch and rinse

A total of 30 articles investigated the influence of salivary contamination on 48 two-step etch and rinse adhesive and 46% found to have a deleterious outcome on the bond quality. The rest suggested that effect of salivary contamination was non-significant. 80% of the contamination tested in enamel had an adverse impact however, 47.2% suffered negatively in dentin. el Kalla et al.,[17] believed that saliva contamination did not prevent hybrid layer formation in 2 step etch and rinse adhesives or the resin penetration into the dentin tubules, while Park et al.,[38] suggested that following the salivary contamination of etched surface, blotting and applying the primer could recover the bond strength.

1.4.1.2 Self-etch adhesives

Self-etching adhesives contain non-rinse acidic monomers that condition and prime dentin alongside. Self-etching process dissolves and modifies the smear layer, however, it fails to eliminate the dissolved calcium phosphates, as it is not rinsed. This method reduces the clinical time as well as technique-sensitivity [3]. Self-etch adhesives are available as 'two-step' and 'one-step' adhesives, depending on if they are available in 2 bottles of self-etching primer and adhesive resin or if they are combined into one single solution (Fig 1.1). The self-etch primers and self-etch adhesive systems contain a mixture of acidic functional monomers, whose pH is slightly higher than phosphoric acid based etchant [64]. Most self-etching adhesives comprises of functional monomers that defines the performance. These monomers etch and improve infiltration into the tooth substrates and also creates a chemical contact between the adhesive and the dental substrates [65].

• 2-step self-etch adhesives

Around 20 articles investigated 24 different 2-step self-etch. 81.5% suggested that salivary contamination adversely influenced their bond quality (Fig.1.2). It was also interesting

to note that many of the articles suggested that the contamination occurring after the application of primer drastically affected the bond quality [10, 13, 31, 36, 38, 56]. 84.2% of the investigation conducted on dentin and 85.7% on enamel reported an unfavourable impact. Vieira et al., [56] suggested that salivary contamination in 2 step self-etching adhesive was deleterious in enamel as well as dentin at all the steps and decontamination methods like rinsing with water, air drying or reapplication of primer couldn't restore the bond quality. Cobanoglu et al.,[13] reported that when the salivary contamination took place after curing the adhesive, repeating the bonding procedure regained the bond strength. However, when the salivary contamination occurred before or after application of the primer, it negatively affected their bond strength. Townsend et al.,[52] observed that saliva contamination of the 2-step self-etching adhesive did not affect the dentin shear bond strength but, it had a negative effect on enamel bond strength.

- **1-step self-etch**

1-step self-etching adhesives are considered all in one adhesives. They are a mixture of an etchant, primer and bonding agent, hence contain hydrophobic and hydrophilic monomers, acidic functional monomers, organic solvents and water in one single formulation. These one-step adhesives are also called "Universal or Multi-Mode Adhesives", which is applied either on etched or un-etched enamel or dentin [62]. A total of 20 papers investigated 30 one step self-etch adhesives (Table 1.1). 73.3% of the adhesives were found to have deleterious effect when contaminated with saliva (Fig.1.2). The negative effect was more pronounced when the contamination occurred either after adhesive application or after polymerizing the adhesive. It was always negative when tested on enamel and 66.6% tested negative on dentin. Bhatia et al.,[12] observed that the salivary contamination significantly affected the bond strength of both 1-step self-etching adhesives evaluated. However, the reapplication of the adhesive system after the salivary contamination improved the bond strength values. Santschi et al.,[45] stated that saliva contamination reduced the bond quality of 1-step self-etching adhesive and it was

prominent when the contamination happened before polymerization than after. In both conditions, decontamination by reapplying the adhesive reinstated the bond strength.

Figure 1.1: Stages of possible salivary contamination on different classes of adhesives (classified as per to Van Meerbeek et al.,[3])

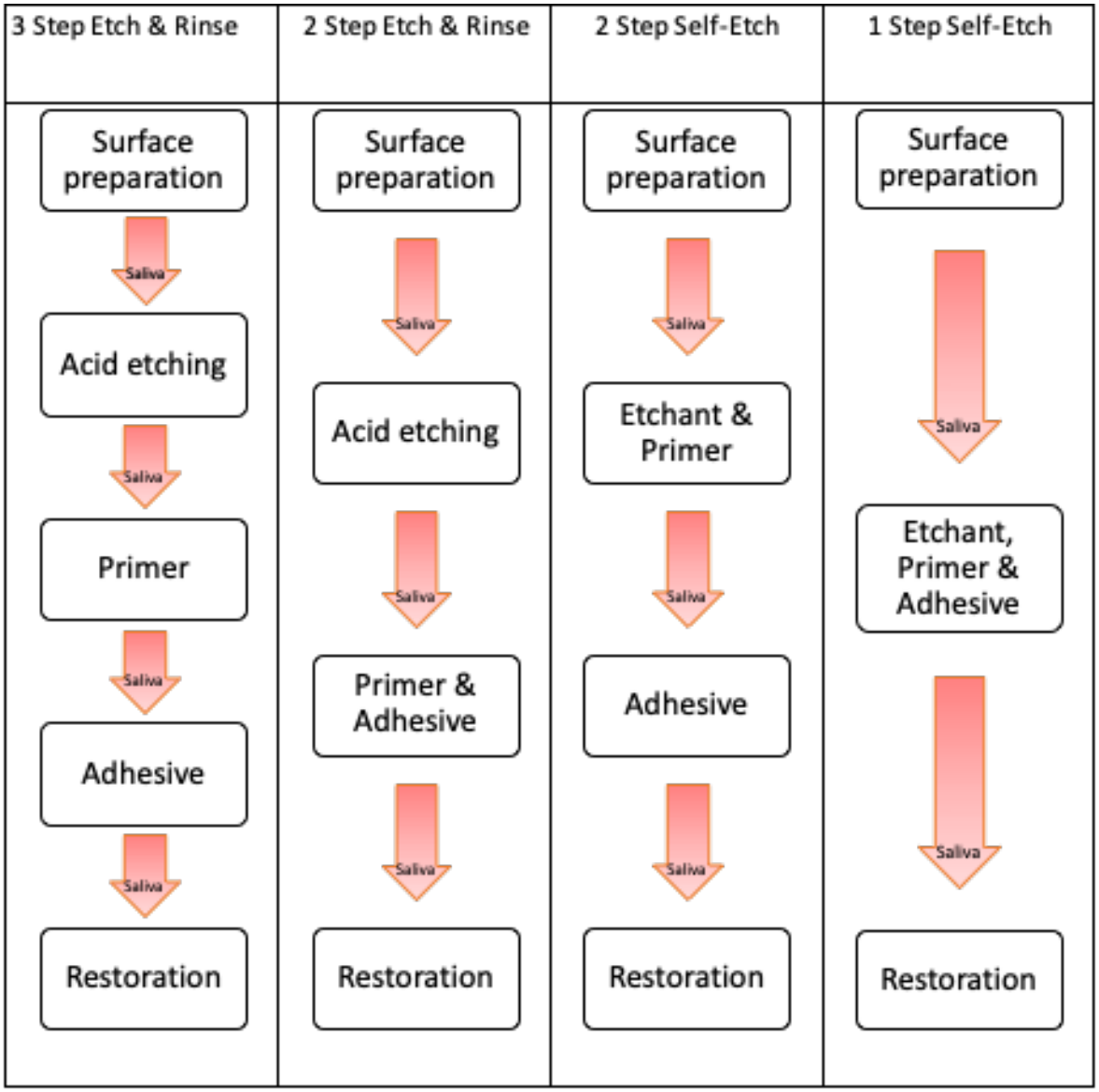


Table 1.1: Articles included in the review describing the influence of salivary contamination on various adhesive

Sl.No	Author	Year	Brand Name	Type of Adhesive	Substrate	Test	Contamination Result
1	Abdalla	1998	Scotchbond 1	2-step etch and rinse	Dentin	Shear Bond Strength	Not significant
			One step	2-step etch and rinse	Dentin		Not significant
			Prime & Bond 2.1	2-step etch and rinse	Dentin		Not significant
			Syntac SC	2-step etch and rinse	Dentin		Negative
			Scotch Bond Multi Purpose Plus	3-step etch and rinse	Dentin		Not significant
2	Aboushelib	2011	Clearfil SE Bond	2-step self etch	Dentin	Micro-Tensile Bond Strength	Negative
3	Ari Hale	2008	Clearfil SE Bond	2-step self etch	Dentin	Micro-Tensile Bond Strength	Negative
4	Benderli	1999	Scotch Bond Multi Purpose	3-step etch and rinse	Enamel	Shear Bond Strength	Negative
5	Bhatia	2015	Adper Easy One	1-step self etch	Dentin	Shear Bond Strength	Negative
			Xeno V	1-step self etch	Dentin		Negative
6	Cobanoglu	2013	Clearfil SE Bond	2-step self etch	Dentin	Shear Bond Strength	Negative
			Optibond Solo Plus SE	2-step self etch	Dentin		Negative
7	Darabi	2012	Single Bond	2-step etch and rinse	Dentin	Shear Bond Strength	Negative
					Enamel		Negative
8	Dietrich	2000	Scotchbond 1	2-step etch and rinse	Dentin	Microleakage	Not significant
9	Duarte	2005	Single Bond	2-step etch and rinse	Dentin	Microscopic Analysis	Negative
			Single Bond	2-step etch and rinse	Enamel		Negative
10	el-Kalla	1997	Prime & Bond 2.1	2-step etch and rinse	Dentin	Shear Bond Strength	Not significant
					Enamel		Not significant
			One step	2-step etch and rinse	Dentin		Not significant
					Enamel		Not significant
			Tenure Quik	2-step etch and rinse	Dentin		Not significant
					Enamel		Not significant
			Syntac SC	2-step etch and rinse	Dentin		Not significant
					Enamel		Negative

Sl.No	Author	Year	Brand Name	Type of Adhesive	Substrate	Test	Contamination Result
11	el-Kalla	1999	Prime & Bond 2.1	2-step etch and rinse	Dentin	Micromorphological assesment	Not significant
			One step	2-step etch and rinse	Dentin		Not significant
			Tenure Quik	2-step etch and rinse	Dentin		Not significant
			Syntac SC	2-step etch and rinse	Dentin		Not significant
12	el-Kalla	1997	Prime & Bond 2.1	2-step etch and rinse	Enamel	Micromorphological assesment	Not significant
			One step	2-step etch and rinse	Enamel		Not significant
			Tenure Quik	2-step etch and rinse	Enamel		Not significant
			Syntac SC	2-step etch and rinse	Enamel		Negative
13	Elkassas	2016	Single Bond	2-step etch and rinse	Dentin	Micro-Shear Bond Strength	Negative
14	Fakhri	2009	Clearfil SE Bond	2-step self etch	Both	Microleakage	Not significant
15	Farmer	2014	Optibond Solo Plus	2-step etch and rinse	Both	Microleakage	Negative
16	Fritz	1998	ARX(experimental adhesive)	2-step etch and rinse	Enamel	Shear Bond Strength	Negative
					Dentin		Negative
17	Guerriero	2009	Single Bond 2	2-step etch and rinse	Dentin	Shear Bond Strength	Negative
18	Gupta	2015	Single Bond	2-step etch and rinse	Dentin	Micro-Tensile Bond Strength	Negative
			Adper SE Plus	2-step self etch	Dentin		Negative
			Single Bond Universal	1-step self etch	Dentin		Negative
19	Hegde	2008	Xeno III	1-step self etch	Dentin	Shear Bond Strength	Negative
			Clearfil SE Bond	2-step self etch	Dentin		Negative
20	Hiraishi	2003	Clearfil SE Bond	2-step self etch	Dentin	Micro-Shear Bond Strength	Negative
21	Hitmi	1999	Syntac Sprint	2-step etch and rinse	Dentin	Shear Bond Strength	Negative
			One step	2-step etch and rinse	Dentin		Negative
			Clearfil liner bond 2	2-step self etch	Dentin		Negative
22	Jiang	2010	Clearfil SE Bond	2-step self etch	Enamel	Micro-Tensile Bond Strength	Negative
			Xeno III	1-step self etch	Enamel		Negative
			Frog	2-step self etch	Enamel		Negative
			FL Bond H	2-step self etch	Enamel		Negative

Sl.No	Author	Year	Brand Name	Type of Adhesive	Substrate	Test	Contamination Result
23	Johnson	1994	All-Bond 2	3-step etch and rinse	Dentin	Shear Bond Strength	Not significant
			Scotch Bond Multi Purpose	3-step etch and rinse	Dentin		Not significant
24	Justin	2012	Single Bond	2-step etch and rinse	Dentin	Shear Bond Strength	Negative
			UniFil bond	2-step self etch	Dentin		Negative
25	Kermanshah	2010	Scotch Bond Multi Purpose Plus	3-step etch and rinse	Dentin	Shear Bond Strength	Negative
			Single Bond	2-step etch and rinse	Dentin		Negative
			Adper Prompt L-Pop	1-step self etch	Dentin		Not significant
26	Khoroushi	2008	i-Bond	1-step self etch	Enamel	Shear Bond Strength	Negative
27	Koppolu	2012	Xeno III	1-step self etch	Enamel	Shear Bond Strength	Negative
					Dentin		Negative
28	Kumar	2012	Single Bond	2-step etch and rinse	Both	Microleakage	Not significant
			i-Bond	1-step self etch	Both		Negative
29	Munaga	2014	Filtek P90	2-step self etch	Dentin	Shear Bond Strength	Negative
30	Neelagiri	2010	AdheSE	2-step self etch	Dentin	Shear Bond Strength	Negative
			Adper Prompt L-Pop	1-step self etch	Dentin		Negative
31	Park	2004	One step	2-step etch and rinse	Dentin	Shear Bond Strength	Negative
			Clearfil SE Bond	2-step self etch	Dentin		Negative
32	Patil	2014	Scotch Bond Multi Purpose	3-step etch and rinse	Enamel	Shear Bond Strength	Negative
					Dentin		Negative
			Single Bond	2-step etch and rinse	Enamel		Negative
					Dentin		Negative
33	Pinzon	2010	Prime and Bond NT	2-step etch and rinse	Dentin	Micro-Tensile Bond Strength	Not significant
			Single bond plus	2-step etch and rinse	Dentin		Negative
			Clearfil SE Bond	2-step self etch	Dentin		Negative
			Clearfil S3 Bond	1-step self etch	Dentin		Negative
34	Pinzon	2011	One up bond F Plus	1-step self etch	Dentin	Shear Bond Strength	Not significant
			Adper Prompt L-Pop	1-step self etch	Dentin		Not significant
35	Powers	1995	Gluma 2000	3-step etch and rinse	Enamel	Shear Bond Strength	Negative
					Dentin		Negative

Sl.No	Author	Year	Brand Name	Type of Adhesive	Substrate	Test	Contamination Result
36	Ramires-Romito	2004	OptiBond Solo	2-step self etch	Enamel	Micro-Tensile Bond Strength	Not significant
			Prime and Bond NT	2-step etch and rinse	Enamel		Not significant
37	Saayman	2005	Prime and Bond NT	2-step etch and rinse	Dentin	Microleakage	Not significant
					Enamel		Negative
38	Santschi	2015	Xeno V+	1-step self etch	Dentin	Shear Bond Strength	Negative
			Scotchbond Universal	1-step self etch	Dentin		Not significant
39	Sattabanasuk	2006	One up bond F Plus	1-step self etch	Dentin	Micro-Tensile Bond Strength	Negative
			Adper Prompt L-Pop	1-step self etch	Dentin		Negative
40	Sheikh	2010	Adper Prompt L-Pop	1-step self etch	Dentin	Micro-Tensile Bond Strength	Not significant
			Adper Easy bond	1-step self etch	Dentin		Not significant
			Clearfil SE Bond	2-step self etch	Dentin		Not significant
41	Shimazu	2014	Clearfil S3 Bond	1-step self etch	Enamel	Microleakage and Shear Bond Strength	Negative
					Dentin		Negative
			OptiBond Solo Plus	2-step etch and rinse	Enamel		Not significant
					Dentin		Negative
42	Suresh	2010	Single Bond	2-step etch and rinse	Dentin	Shear Bond Strength	Negative
43	Suryakumari	2011	Single Bond	2-step etch and rinse	Dentin	Shear Bond Strength	Negative
44	Taskonak	2002	Prime and Bond NT	2-step etch and rinse	Dentin	Shear Bond Strength	Not significant
			Gluma one bond	2-step etch and rinse	Dentin		Not significant
			Syntac SC	2-step etch and rinse	Dentin		Not significant
45	Townsend	2004	na	2-step self etch	Enamel	Shear Bond Strength	Negative
				2-step self etch	Dentin		Not significant
46	Tuncer	2014	One step Plus	2-step etch and rinse	Dentin	Shear Bond Strength, Microleakage	Negative
			G- Bond	1-step self etch	Dentin		Negative
47	Ulusoy	2012	Prime and Bond NT	2-step etch and rinse	Dentin	Micro-Tensile Bond Strength	Negative
			Clearfil Protect Bond	2-step self etch	Dentin		Negative

Sl.No	Author	Year	Brand Name	Type of Adhesive	Substrate	Test	Contamination Result
48	van Schalkwyk	2003	Scotchbond 1	2-step etch and rinse	Dentin	Shear Bond Strength	Not significant
			Prime and Bond NT	2-step etch and rinse	Dentin		Not significant
49	Vieira	2010	Clearfil SE Bond (24hrs)	2-step self etch	Enamel	Micro-Tensile Bond Strength	Negative
					Dentin		Negative
			Clearfil SE Bond (6 months)		Enamel		Negative
					Dentin		Negative
50	Xie	1993	All-Bond 2	3-step etch and rinse	Enamel	Tensile Bond Strength	Negative
					Dentin		Negative
			Scotch Bond Multi Purpose	3-step etch and rinse	Enamel		Negative
					Dentin		Negative
51	Yalcin	2013	Clearfil SE Bond	2-step self etch	Dentin	Micro-Tensile Bond Strength	Not significant
			Clearfil S3 Bond	1-step self etch	Dentin		Not significant
52	Yazici	2007	Single Bond	2-step etch and rinse	Both	Microleakage	Not significant
			Futura Bond NR	1-step self etch	Both		Not significant
53	Yoo(saliva)	2006	One up bond F Plus	1-step self etch	Dentin	Micro-Shear Bond Strength	Negative
			Xeno III	1-step self etch	Dentin		Negative
			Adper Prompt L-Pop	1-step self etch	Dentin		Negative
54	Yu	2014	Adper Easy One	1-step self etch	Dentin	Micro-Tensile Bond Strength	Negative
			Clearfil S3 Bond	1-step self etch	Dentin		Negative

1.4.2 Experimental Procedure

- **Contamination**

The foremost objective of evaluating contamination-based study is to simulate the possible oral condition and effectively create a situation that takes place in a clinical practice. Most of the authors have described the procedure by mentioning, “contaminating the specimen” or “applying saliva on the substrate”. Only 18.5% papers had specified quantities of contaminants and 48.1% mentioned the duration of contamination. The saliva used for testing were mostly natural (85.2%), and they were either freshly collected from

one or many donors just prior to the experiment, or collected in advance, frozen at –80°C and thawed just before use [45, 46, 56]. Few investigators also used artificial saliva (14.8%) for their experiments.

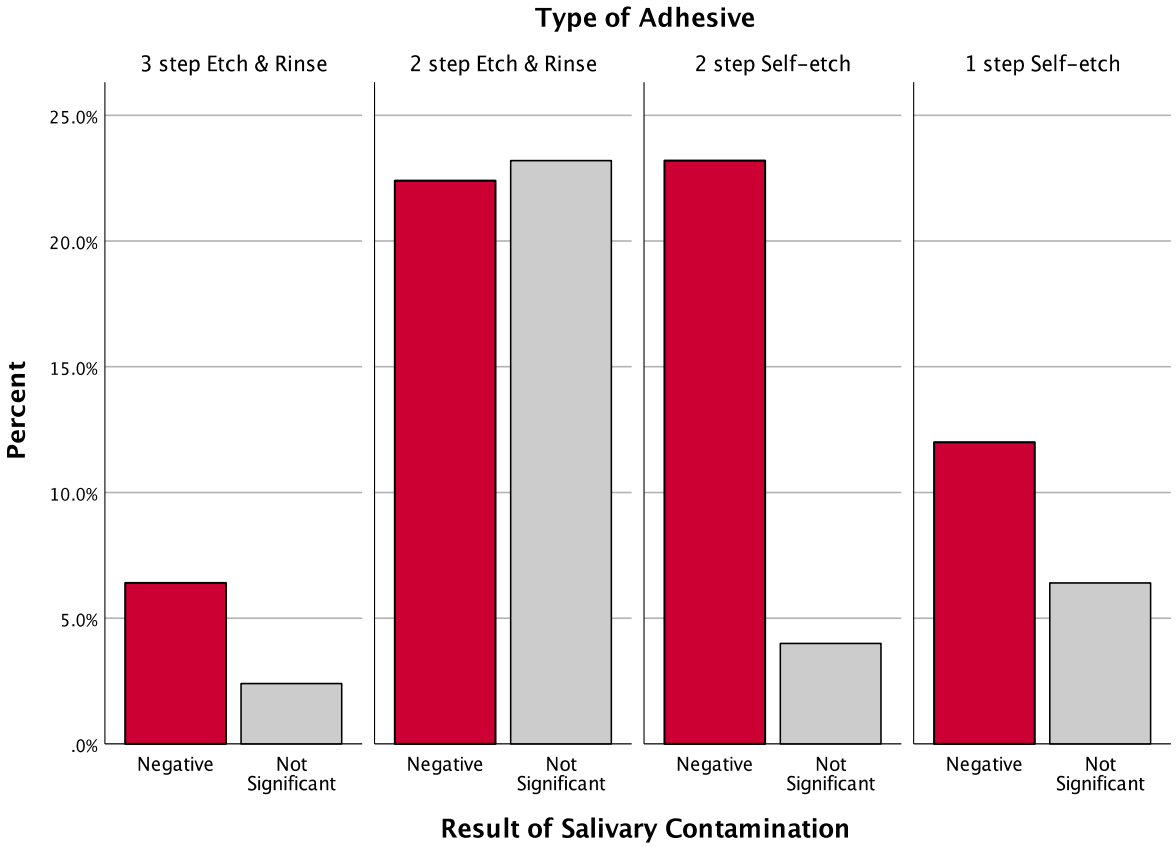
- **Decontamination**

70% of the articles indicated that some form of decontamination procedure might restore the values to control levels and have adopted a variety of approaches. While 33.3% of them tried to blow-dry the contaminant, 53.7% chose to rinse and dry, 20.4% re-etched the contaminated surface, 11.1% re-primed and 25.9% reapplied the adhesive in order to decontaminate.

Sheikh et al.,[47] proposed cleaning with agents like sodium hypochlorite, ethanol, acetone and chlorhexidine to improve the quality but found saliva and the cleansing solutions had no influence on the bond strengths in both one and two step self-etch adhesive systems. When the priming stage was contaminated in 2 step self -etching adhesive, re-priming improved the bond strengths considerably [21, 27, 36-38]. 65% have claimed to have improved or restored the bond strength whereas 35% failed to restore the values or found no significant difference after decontamination.

.

Figure 1.2: Influence of contamination on different adhesives



- **Type of test**

Generally, the quality of bonding via experiments on contamination is determined in-vitro, except for one study done in-vivo [9]. Although, intentionally contaminating a tooth for the purpose of experiment in an in-vivo tests may be considered unreasonable. Aboushelib et al., [9] carried out the study on teeth intending to be extracted for orthodontic purpose and the teeth were extracted 3 years after restoration. More than half (54.7%) of the reviewed articles used shear bond strength test to evaluate the bonding, followed by 22.6 % that used micro-tensile bond strength test and 11.3% that used microleakage for assessment. The other testing procedures used were micro-shear bond strength (3.8%), tensile bond strength (1.9%) and microscopic analysis (5.7%). All the tests are almost always accompanied by a microscopic evaluation of the specimens by

a stereomicroscope or a scanning electron microscope. The irregularities of each testing method are represented (Table.1.2).

• **Surface Preparation**

Preparation of the surface varied in different test protocols and is often modified by individual researchers. The adaptation of different materials and substrates to different surface conditions could not be contrasted for an evaluation. The variability in the surface preparation procedure in different test procedures used is illustrated (Table.1.2). It is observed from the literatures that, grinding the surface with 600 grit silicon carbide (SiC) paper is the most widely used method of surface preparation (38.9%) for bond strength test, followed by serial grinding (27.8%) with 2 or more different grit size or roughness of SiC.

In bond strength testing, while conducting a test following the ISO/TS 11405 (2003), the most often overlooked specification is that "a limitation of the bonding area is important" [66]. It is moreover essential to consider, if the precise bonding area is maintained from the stage of etching. This step however, is not very clear from all of the literature. In the methodology explained, even most of the newer studies in bond strength have not specified whether the whole area or the defined area is subjected to the contamination, etching or bonding. This may lead to discrepancy in the data.

• **Substrate**

Almost all (87%) of the investigations were done using human teeth as their substrates and 7.5% were conducted on extracted primary teeth. 5% were investigated on enamel, 61% were on dentin and 29% were conducted on both enamel and dentin. However, 5.6% studies were done on extracted bovine teeth.

- **Aging**

The aging process can be simulated either by thermocycling or by storing for a stipulated amount of time in water or different solutions. 27.7 % opted to perform thermocycling and it was done between 5 and 55°C and at various frequencies of 500, 1000, 2000, 2500 and 5000 cycles. Majority (62.9%) of the researchers stored the specimen for 24 hours at 37°C (Table.1.2) in either distilled water or dye. Few studies combined different duration of aging, in order to compare the variation. In-vivo/clinical study had an advantage of leaving the test specimens in the natural oral environment, which ensured an authentic condition for aging [9]. There was only one in-vitro study which examined the adhesive efficacy for a longer term, after a 6-month interval [56].

Table 1.2: Comparision of test parameters to demonstrate variability among studies

		TYPE OF TEST						
PARAMETERS	**VALUES**	**Shear bond**	**Tensile bond**	**Micro-Shear**	**Micro-Tensile**	**Microleakage**	**Others**	**Total**
SURFACE PREPERATION	600 grit SIC	9	1	3	7	0	1	**21**
	<600 grit SIC	1	0	0	0	0	0	01
	Serial grinding	13	0	0	2	0	0	15
	Cavity preparation	1	0	0	2	6	2	11
	Flattened with Bur/disc	5	0	0	0	0	0	05
	Not available	0	0	0	1	0	0	01
SAMPLE SIZE/GROUP(n)	1-5	3	1	2	7	0	1	14
	6-10	18	0	0	4	4	1	**27**
	11-15	5	0	1	1	1	0	08
	16-20	3	0	0	0	1	1	05
THERMOCYCLING	Yes	8	0	0	0	6	1	15
	No	21	1	3	12	0	2	**39**
STORAGE TIME	24 hours	19	1	2	10	3	1	**36**
	48 hours	6	0	0	0	0	1	7
	1 week	0	0	1	0	1	0	2
	3 weeks	1	0	0	0	1	0	2
	6 months	0	0	0	1	0	0	1
	3 years	0	0	0	1	0	0	1
	Not available	3	0	0	0	1	1	5
DURATION OF CONTAMINATION	0-5 seconds	2	0	0	2	1	0	05
	6-10 seconds	2	0	0	2	1	1	06
	11-15 seconds	5	0	0	0	0	0	05
	16-20 seconds	4	0	1	0	0	0	05
	21-30 seconds	2	0	0	0	0	0	02
	1 minute	0	0	1	1	0	0	02
	Not available	14	1	1	7	4	2	**29**

1.5 Discussion

Dental adhesives are complex blends of components. Insightful knowledge of these ingredients is vital to recognise the performance of adhesives while using in clinic conditions. Better understanding of the components provides awareness in the correct clinical use of adhesives [62].

The idea of the possible interactions of adhesives with saliva are understood to be that, when the surface gets contaminated with saliva after etching (in etch and rinse) or surface preparation, the presence of water and glycoproteins of saliva on the surface may hamper with the proper infiltration of adhesives and subsequently hamper the micromechanical adhesion.

When the surfaces are contaminated with saliva after application of adhesive but before polymerising, saliva may affect the degree of conversion because molecules with their hydrophilic nature may hold moisture within the adhesive layer and get dispersed in water, thus they become unable to participate in chain growth during polymerization and eventually alter the bond strength.

When surfaces are contaminated after polymerization process, absorption of salivary proteins to the polymerized surface may cause reduction of bond strength. These glycoproteins may prevent complete infiltration of the subsequent resin layer and prevent copolymerization [32].

Hydrophilic monomers are incorporated in water, ethanol, or acetone to be used as primers and form a hybrid layer. After applying the primer, air drying evaporates the carrier solvent which deposits the resin material in the collagen. The bonding agent co-polymerizes with the primer, wetting the dentin surface and facilitating further penetration of the monomers [67].

Preferably, before light-curing, the applied adhesive must be devoid of all the solvents and water. Hence, a bit of time for the sake of evaporation is provided between application and curing of the adhesive resin. Nevertheless, the ratio of water to monomer decreases

as water evaporates from the adhesive, lowering the vapor pressure of water and further reduces the capacity of water and other solvents to completely evaporate from the adhesive. In case of contamination, it is likely that residual moisture from saliva along with the solvent will be confined inside the adhesive. This may degrade the bonding and hamper the strength of the adhesive [67].

This review discovered that 2-step etch and rinse adhesive tolerated better when there is salivary contamination. However, there were mixed thoughts observed. This variation in behavior between the tested materials could be assigned to the difference in the chemical configuration. Some of the materials tested include acetone as their solvent. Acetone is a "water chaser" and assists to replace the water with primer on the dentin surface. When acetone based primers come in contact with moistened surface, the boiling point of water decreases and that of acetone increases and they evaporate leaving behind the resin [8, 68]. However, when water based solvents are used, the moisture in saliva tends to dilute the adhesive, reducing its efficacy. The favorable response to salivary contamination in dentin could be reasoned that, saliva increased the hydration of dentin surface producing a favorable performance to acetone based primers [8].

In-vivo clinical performance of any adhesive cannot be represented entirely based on the in-vitro results[69]. This does not however suggest, that proper technique and moisture control should not be followed while applying these adhesives.

Although, there is a likelihood of salivary contamination during restorative process, 1-step self-etching adhesives are simpler as well as faster than etch-and-rinse adhesives making it less technique sensitive. The simplification of the bonding process will certainly have clinical advantages, but these adhesives contain very hydrophilic monomers and they aid in absorbing moisture from dentinal tubules through osmosis. Monomers that are not polymerised tend to leach out through water sorption which leads to expansion of the polymer. Usually, an increase in water sorption is coexistent with increase in solubility, which leads to hydrolytic degradation and nanoleakage, resulting in a reduced bond quality over time [65, 70].

Adhesive systems commonly contain Hydroxyethyl-methacrylate (HEMA) monomer. These monomers are included to offer strength to the cross-linking formed from monomeric matrix. HEMA-containing adhesives are vulnerable to moisture in saliva, as their presence in the uncured adhesive encourages absorption of water and will end up diluting the monomers to a degree that polymerization process is hinderd [62].

In a good scientific research, specificities of contaminants like the quantity and duration are crucial to compare the results and also to validate exactly how much contaminant is adversely affecting the material examined. The haphazardness of tests protocols makes it difficult not only to compare the test specimens within the study but also from one study to another. Thus, making the findings non-reproducible.

Few studies used artificial saliva for experiments. Various types of artificial saliva have been formulated for the studies in dentistry. Although, these formulations try to have a composition as similar as that of natural saliva, their use for contamination studies is questionable. Saliva is known to be very inconsistent [71] and it comprises of several hydrolytic enzymes competent of reacting with the tooth structure through different biochemical processes, which could modify the surface of the tooth structure and also compromise the material bond strength [72]. Hence, the studies excluding these organic constituents might not entirely simulate the clinical contamination. One study however, incorporated mucin alone in the artificial saliva but could not elicit dramatic ill effects on bond strength [41]. Further investigations could be done to evaluate the effect of other salivary proteins at different protein concentrations as well as the influence of other salivary constituents in the adhesion to tooth structures in order to have a better understanding on the exact consequence.

There is still an apparent unpredictability in the decontamination procedures in all the investigations. The duration and other precise details of the decontamination process mentioned are not consistent and thus making it unsuitable for a comparative analysis. But then again, findings indicate towards the fact that, if contamination is discovered, the material strength could be salvaged if the remedial measures are taken.

Unavailability of extracted human teeth and the need of a large sample group have compelled researchers to find unconventional ways to conduct experiments. At present, bovine teeth are widely used in experiments. Nakimichi et al.[73] found no statistical difference in bond strength in human teeth and bovine teeth when enamel and the superficial layer of dentin were used for experiment. Usually, an early or a 24-hour bond strength is verified but, it is necessary to test bonding efficiency of adhesives in a clinically appropriate situations and after aging for longer durations. Undeniably, many commercially existing adhesives have shown a good short-term bond strength, while the clinical results have not been comparable [66].Therefore, more resilience testing of adhesion is required, than only determining the intermediate bond strength.

Thermocycling and storing in water and are the most common methods to age the substrate artificially [74].The co-relation of bond-strength tests and clinical results was explored and was concluded that, aging the specimens will encourage the results to be more clinically relevant [66]. Also, long-term durability of dentin bonding adhesives depends on the bonding capability of the functional monomer [75].

It is clear from the literature that, in most of the adhesives tested so far, saliva has the potential to impair the immediate bond quality. These altered circumstances need to be tested in a long-term study to understand if it deteriorates with time.

It is not an unfamiliar idea in dentistry that, contamination may harm the materials and it will never become an old subject for research. There is constant research in developing novel and improved adhesive materials. These newer materials ought to be verified under simulated conditions of oral cavity. However, the test protocols need to be more standardized as well as the explanation of the test procedure need to be more transparent in order for the tests to be reproducible and to get a fair comparison between the materials.

1.6 References

1. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P, Van Meerbeek B. Systematic review of the chemical composition of contemporary dental adhesives. Biomater 2007; 28: 26: 3757-3785.

2. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P, Vanherle G. Adhesives and cements to promote preservation dentistry. Oper Dent 2001; 26: 119-144.

3. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. Buonocore memorial lecture. Adhesion to enamel and dentin: Current status and future challenges. Oper Dent 2003; 28: 3: 215-235.

4. Eiriksson SO, Pereira PN, Swift EJ, Jr., Heymann HO, Sigurdsson A. Effects of saliva contamination on resin-resin bond strength. Dent Mater 2004; 20: 1: 37-44.

5. Buonocore MG. Caries prevention in pits and fissures sealed with an adhesive resin polymerized by ultraviolet light: A two-year study of a single adhesive application. J Am Dent Assoc 1971; 82: 5: 1090-1093.

6. Baier RE. Principles of adhesion. Oper Dent 1992; Suppl 5: 1-9.

7. Marshall SJ, Bayne SC, Baier R, Tomsia AP, Marshall GW. A review of adhesion science. Dent Mater 2010; 26: 2: 11-16.

8. Abdalla AI, Davidson CL. Bonding efficiency and interfacial morphology of one-bottle adhesives to contaminated dentin surfaces. Am J Dent 1998; 11: 6: 281-285.

9. Aboushelib MN. Clinical performance of self-etching adhesives with saliva contamination. J Adhes Dent 2011; 13: 5: 489-493.

10. Ari H, Donmez N, Belli S. Effect of artificial saliva contamination on bond strength to pulp chamber dentin. Eur J Dent 2008; 2: 2: 86-90.

11. Benderli Y, Gokce K, Buyukgokcesu S. In vitro shear bond strength of adhesive to normal and fluoridated enamel under various contaminated conditions. Quint Int 1999; 30: 8: 570-575.

12. Bhatia TK, Asrani H, Banga H, Jain A, Rawlani SS. Influence of salivary contamination on the dentin bond strength of two different seventh generation adhesive systems: In vitro study. J Conserv Dent 2015; 18: 6: 467-470.

13. Cobanoglu N, Unlu N, Ozer FF, Blatz MB. Bond strength of self-etch adhesives after saliva contamination at different application steps. Oper Dent 2013; 38: 5: 505-511.

14. Darabi F, Tavangar M, Davalloo R. Effect of different decontamination procedures from a saliva-contaminated cured bonding system (single bond). Dent Res J (Isfahan) 2012; 9: 4: 399-403.

15. Dietrich T, Kraemer M, Losche GM, Wernecke KD, Roulet JF. Influence of dentin conditioning and contamination on the marginal integrity of sandwich class ii restorations. Oper Dent 2000; 25: 5: 401-410.

16. Duarte SJ, Lolato AL, de Freitas CR, Dinelli W. Sem analysis of internal adaptation of adhesive restorations after contamination with saliva. J Adhes Dent 2005; 7: 1: 51-56.

17. el-Kalla IH. Saliva contamination and resin micromorphological adaptation to cavity walls using single-bottle adhesives. Am J Dent 1999; 12: 4: 172-176.

18. el-Kalla IH, Garcia-Godoy F. Saliva contamination and bond strength of single-bottle adhesives to enamel and dentin. Am J Dent 1997; 10: 2: 83-87.

19. el-Kalla IH, Garcia-Godoy F. Effect of saliva contamination on micromorphological adaptation of single-bottle adhesives to etched enamel. J Clin Pediatr Dent 1999; 24: 1: 69-74.

20. Elkassas D, Arafa A. Assessment of post-contamination treatments affecting different bonding stages to dentin. Eur J Dent 2016; 10: 3: 327-332.

21. Fakhri M, Seraj B, Shahrabi M, Motahhary P, Hooshmand T. Effect of salivary contamination on microleakage of resin composites placed with a self-etch adhesive in primary teeth: An in vitro study. Pediatr Dent 2009; 31: 4: 334-339.

22. Farmer SN, Ludlow SW, Donaldson ME, Tantbirojn D, Versluis A. Microleakage of composite and two types of glass ionomer restorations with saliva contamination at different steps. Pediatr Dent 2014; 36: 1: 14-17.

23. Fritz UB, Finger WJ, Stean H. Salivary contamination during bonding procedures with a one-bottle adhesive system. Quint Int 1998; 29: 9: 567-572.

24. Guerriero LN, Vieira SN, Scaramucci T, Kawaguchi FA, Sobral MAP, Matos AB. Effect of saliva contamination on the bond strength of an etch-and-rinse adhesive system to dentin. Rev. odonto ciênc 2009; 24: 4: 410-413.

25. Gupta N, Tripathi AM, Saha S, Dhinsa K, Garg A. Effect of saliva on the tensile bond strength of different generation adhesive systems: An in-vitro study. J Clin Diagn Res 2015; 9: 7: Zc91-94.

26. Hegde MN, Hegde P, Shetty SK. The influence of salivary contamination on the

shear bond strength of two newer generation dentin bonding agents - an in vitro study. J Conserv Dent 2008; 11: 3: 127-130.

27. Hiraishi N, Kitasako Y, Nikaido T, Nomura S, Burrow MF, Tagami J. Effect of artificial saliva contamination on ph value change and dentin bond strength. Dent Mater 2003; 19: 5: 429-434.

28. Hitmi L, Attal JP, Degrange M. Influence of the time-point of salivary contamination on dentin shear bond strength of 3 dentin adhesive systems. J Adhes Dent 1999; 1: 3: 219-232.

29. Jiang Q, Pan H, Liang B, Fu B, Hannig M. Effect of saliva contamination and decontamination on bovine enamel bond strength of four self-etching adhesives. Oper Dent 2010; 35: 2: 194-202.

30. Johnson ME, Burgess JO, Hermesch CB, Buikema DJ. Saliva contamination of dentin bonding agents. Oper Dent 1994; 19: 6: 205-210.

31. Justin RM, Paranthaman H, Rajesh AG, Varghese RP, Ranganath LM. Effect of salivary contamination on the bond strength of total-etch and self-etch adhesive systems: An in vitro study. J Contemp Dent Pract 2012; 13: 5: 655-660.

32. Kermanshah H, Ghabraei S, Bitaraf T. Effect of salivary contamination during different bonding stages on shear dentin bond strength of one-step self-etch and total etch adhesive. J Dent (Tehran) 2010; 7: 3: 132-138.

33. Khoroushi M, Karimi B. Saliva contaminated and re-etched all-in-one adhesive: Influence on bond strength. Dent Res J 2006; 3: 1: 10-14

34. Koppolu M, Gogala D, Mathew VB, Thangala V, Deepthi M, Sasidhar N. Effect of saliva and blood contamination on the bond strength of self-etching adhesive system-an in vitro study. J Conserv Dent 2012; 15: 3: 270-273.

35. Kumar P, Shenoy A, Joshi S. The effect of various surface contaminants on the microleakage of two different generation bonding agents: A stereomicroscopic study. J Conserv Dent 2012; 15: 3: 265.

36. Munaga S, Chitumalla R, Kubigiri SK, Rawtiya M, Khan S, Sajjan P. Effect of saliva contamination on the shear bond strength of a new self-etch adhesive system to dentin. J Conserv Dent 2014; 17: 1: 31-34.

37. Neelagiri K, Kundabala M, Shashi RA, Thomas MS, Parolia A. Effects of saliva contamination and decontamination procedures on shear bond strength of self-etch dentine bonding systems: An in vitro study. J Conserv Dent 2010; 13: 2: 71-75.

38. Park JW, Lee KC. The influence of salivary contamination on shear bond strength

of dentin adhesive systems. Oper Dent 2004; 29: 4: 437-442.

39. Patil SB, Shivakumar AT, Shah S. Effect of salivary contamination on shear bond strength of two adhesives: An in vitro study. Dental Hypoth 2014; 5: 3: 115.

40. Pinzon LM, Oguri M, O'Keefe K, Dusevish V, Spencer P, Powers JM, Marshall GW. Bond strength of adhesives to dentin contaminated with smoker's saliva. Odontology 2010; 98: 1: 37-43.

41. Pinzon LM, Powers JM, O'Keefe KL, Dusevish V, Spencer P, Marshall GW. Effect of mucoprotein on the bond strength of resin composite to human dentin. Odontology 2011; 99: 2: 119-128.

42. Powers JM, Finger WJ, Xie J. Bonding of resin composite to contaminated human enamel and dentin. J Prosthodont 1995; 4: 1: 28-32.

43. Ramires-Romito AC, Reis A, Loguercio AD, de Goes MF, Grande RH. Micro-tensile bond strength of adhesive systems applied on occlusal primary enamel. J Clin Pediatr Dent 2004; 28: 4: 333-338.

44. Saayman CM, Grobler SR, Rossouw RJ, Oberholzer TG. Effect of saliva contamination on microleakage of a bonding system. Sadj 2005; 60: 3: 109, 111-102.

45. Santschi K, Peutzfeldt A, Lussi A, Flury S. Effect of salivary contamination and decontamination on bond strength of two one-step self-etching adhesives to dentin of primary and permanent teeth. J Adhes Dent 2015; 17: 1: 51-57.

46. Sattabanasuk V, Shimada Y, Tagami J. Effects of saliva contamination on dentin bond strength using all-in-one adhesives. J Adhes Dent 2006; 8: 5: 311-318.

47. Sheikh H, Heymann HO, Swift EJ, Jr., Ziemiecki TL, Ritter AV. Effect of saliva contamination and cleansing solutions on the bond strengths of self-etch adhesives to dentin. J Esthet Restor Dent 2010; 22: 6: 402-410.

48. Shimazu K, Karibe H, Ogata K. Effect of artificial saliva contamination on adhesion of dental restorative materials. Dent Mater 2014; 33: 4: 545-550.

49. Suresh B, Pushpa R. Effect of saliva contamination and different decontamination modes on dentin bond strength during bonding with single bottle adhesive. Annals and Essences of Dent. 2010; 2: 3: 11-16.

50. Suryakumari NB, Reddy PS, Surender LR, Kiran R. In vitro evaluation of influence of salivary contamination on the dentin bond strength of one-bottle adhesive systems. Contemp Clin Dent 2011; 2: 3: 160-164.

51. Taskonak B, Sertgoz A. Shear bond strengths of saliva contaminated 'one-bottle'

adhesives. J Oral Rehabil 2002; 29: 6: 559-564.

52. Townsend RD, Dunn WJ. The effect of saliva contamination on enamel and dentin using a self-etching adhesive. J Am Dent Assoc 2004; 135: 7: 895-901

53. Tuncer S, Demirci M, Tekçe N, İşler SC, Uysal Ö. Effect of saliva contamination on shear bond strength and microleakage of one-bottle etch-and-rinse and self-etch adhesives: Scanning electron and confocal laser microscopic analyses. J of Adh Sci and Tech 2014; 28: 6: 525-545.

54. Ulusoy AT, Olmez S. Effect of saliva contamination on the bond strenght of dentin adhesives to central and peripheral primary dentin in vitro. Eur J Dent and Med 2012; 4: 2: 26-33.

55. van Schalkwyk JH, Botha FS, van der Vyver PJ, de Wet FA, Botha SJ. Effect of biological contamination on dentine bond strength of adhesive resins. Sadj 2003; 58: 4: 143-147.

56. Vieira SN, Kawaguchi FA, Botta SB, Matos AB. Longitudinal evaluation of the effect of saliva contamination during the bonding protocol with a self-etch adhesive system. Braz. J. Oral Sci 2010; 9: 2: 98-103

57. Xie J, Powers JM, McGuckin RS. In vitro bond strength of two adhesives to enamel and dentin under normal and contaminated conditions. Dent Mater 1993; 9: 5: 295-299.

58. Yalçin M, Simsek N, Keles A, Ahmetoglu F, Dündar A, Umar I. Effect of salivary contamination on micro-tensile bond strength of self-etch adhesives systems after bonding procedure. J of Rest Dent 2013; 1: 2: 55.

59. Yazici AR, Tuncer D, Dayangac B, Ozgunaltay G, Onen A. The effect of saliva contamination on microleakage of an etch-and-rinse and a self-etching adhesive. J Adhes Dent 2007; 9: 3: 305-309.

60. Yoo HM, Oh TS, Pereira PN. Effect of saliva contamination on the microshear bond strength of one-step self-etching adhesive systems to dentin. Oper Dent 2006; 31: 1: 127-134.

61. Yu M, Wu Z, Pan H, Li M, Wang C, Zhang Z, Fu B, Hannig M. Effects of saliva contamination on bonding performance of self-etching adhesives. J of Adh Sci and Tech 2014; 28: 20: 2032-2045.

62. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P, Van Meerbeek B. Systematic review of the chemical composition of contemporary dental adhesives. Biomat 2007; 28: 26: 3757-3785.

63. Perdigão J. New developments in dental adhesion. Dental Clinics of North America

2007; 51: 2: 333-357.

64. Giannini M, Makishi P, Ayres APA, Vermelho PM, Fronza BM, Nikaido T, Tagami J. Self-etch adhesive systems: A literature review. Braz dentl J 2015; 26: 1: 3-10.

65. Milia E, Cumbo E, Cardoso JA, Gallina G. Current dental adhesives systems. A narrative review. Curr pharm des 2012; 18: 34: 5542-5552.

66. Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, De Munck J. Relationship between bond-strength tests and clinical outcomes. Dent Mater 2010; 26: 2: e100-121.

67. Yiu CK, Pashley EL, Hiraishi N, King NM, Goracci C, Ferrari M, Carvalho RM, Pashley DH, Tay FR. Solvent and water retention in dental adhesive blends after evaporation. Biomater 2005; 26: 34: 6863-6872.

68. Kanca J, 3rd. Effect of resin primer solvents and surface wetness on resin composite bond strength to dentin. Am J Dent 1992; 5: 4: 213-215.

69. Green DJ, Banerjee A. Contemporary adhesive bonding: Bridging the gap between research and clinical practice. Dent Update 2011; 38: 7: 439-450.

70. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, Rueggeberg FA, Foulger S, Saito T, Nishitani Y, Yoshiyama M, Tay FR, Pashley DH. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomater 2005; 26: 33: 6449-6459.

71. Mandel ID. Relation of saliva and plaque to caries. J Dent Res 1974; 53: 2: 246-266.

72. Finer Y, Santerre J. Salivary esterase activity and its association with the biodegradation of dental composites. J Dent Res 2004; 83: 1: 22-26.

73. Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible substitutes in the adhesion test. J Dent Res 1983; 62: 10: 1076-1081.

74. Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, De Munck J. Relationship between bond-strength tests and clinical outcomes. Dental Mater2010; 26: 2: 100-121.

75. Inoue S, Koshiro K, Yoshida Y, Munck JD, Nagakane K, Suzuki K, Sano H, Meerbeek BV. Hydrolytic stability of self-etch adhesives bonded to dentin. J Dent Res 2005; 84: 12: 1160-1164.

Chapter 2

In-vitro Evaluation of Low and High Viscosity Bulk-fill Restoratives vs Conventional Resin Composite in terms of their Shear Bond Strength

2.1 Abstract

Purpose To evaluate the variance in high and low viscosity bulk-fill restorative material and conventional resin composite in terms of their shear-bond strength to dentin.

Materials and Methods: Human third molars were sectioned mid-coronally, embedded in cold-cure acrylic and wet ground with 600 grit silicon carbide paper in order to attain flat dentinal surface and then randomly allocated into ten groups (n=20). The region to be bonded was delimited and treated with self-etch adhesive (Clearfil SE 2). Four high viscosity bulk-fill restorative materials (BF-RC); Sonic fill-2 (SF2), Tetric Evo-Ceram (TEC), Admira Fusion x-tra (AFX) and Beautifil-bulk restorative (BBR) and five low viscosity BF-RC; Tetric EvoFlow (TEF), Surefill SDR (SDR), Venus Bulk-fill (VBF), Beautifil-bulk flowable (BBF) and Filtek Bulk-fill (FBF) were dispensed in one 4 mm increment and polymerized for 20 seconds. One conventional resin composite (RC); Ceram-x (CX) was dispensed in two 2 mm increments. Shear bond strength (SBS) was determined at a crosshead speed of 0.5 mm/min after seven days of storing submerged in distilled water at 37°C. The data was statistically analyzed using one-way ANOVA with Tukey HSD post-hoc ($\alpha = 0.05$) and Weibull statistical analysis.

Results: The study could not identify a statistically significant difference in SBS between the ten restorative materials tested. Weibull statistic ranked the materials in the order BBR<CX<FBF<TEC<AFX<BBF<VBF<SDR<SF2<TEF

Clinical Significance: The BF-RC functions comparable to conventional RC in terms of SBS and the type of material had no significant influence.

2.2 Introduction

Improvements through the years in chemical configuration, filler permutations and adhesive approaches, has steered the development of many new and modified categories of resin composite (RC) materials. The entry of bulk-fill (BF) RCs into the market has been very promising for the clinicians [1]. The BF-RC originated to address the limitation of incremental material placement of conventional RC, as they exhibit an inadequate depth of cure and increased polymerization shrinkage [2]. Moreover, the incremental placement procedure has several drawbacks like the inclusion of voids or chances of contamination between layers and increased effort to deposit in posterior cavities with inadequate access, all of which makes it clinically time consuming. BF-RC allows a single 4-5 mm increment to be polymerized efficiently and are reported to have improved curing and controlled shrinkage [3], which effectively brings about reduction in the chair time [4, 5].

The BF-RCs that are commercially available differ in their rheological properties, chemical composition and filler ratio and hence differ in their mechanical properties [6-8]. At the moment, they are available in high viscosity or restorative/sculptable form and low viscosity or flowable form. The low viscosity BF-RC are apt for procedures in narrow cavities that are 4-5 mm deep, owing to its better flowability in the lesser accessible posterior cavity configurations, where a higher adaptation is required [9]. They have proven to be effectively used clinically in large cavities using a capping layer [10]. The high viscosity BF-RC is used in high stress-bearing areas and in situations that necessitate producing functional cuspal configurations [6, 9].

The junction of a RC and the tooth is often subjected to an assortment of stresses which can theoretically lead to impairment of the restoration [11]. Much prior to the restored tooth being exposed to functional burden and thermal stresses, it is subjected to an interfacial stress that occurs between the RC and the tooth during the polymerization [11]. It stems from the complex interaction between the volumetric contraction, reaction kinesis, as well as the viscoelastic behaviors of the RC [12]. Thus, the primary concern

of curing large increments is increased polymerization shrinkage stress at the interface. When the stress value exceeds the adhesive resistance, it can lead to the formation of gaps [13]. Therefore, an ideal restorative should develop low shrinkage stress to ensure a better seal [14]. The adhesion of bonded RC to dental tissues is a primary concern for clinical success and durability of restoration, especially when the innate property of the materials demonstrate the potential to shrink when polymerized [11]. An inferior adaptation tends to increase the risk of microleakage and result in debonding, secondary caries and postoperative sensitivity [15].

With the intention of regulating the reaction kinetics and minimize stress formation in RC, manufactures included advanced high-molecular-weight base monomers, pre-polymer stress relievers, and stress-relaxant polymerization modulators in their bulk-fill materials [7,16]. There has been a great deal of investigations on BF RCs in-vitro, which has ascertained that they can be cured and used in increments up to 4 mm thickness successfully [17]. BF-RC generated more controlled shrinkage stress when compared to conventional RC, particularly when bigger increments were evaluated [2,14,16,18,19]. However, this assumption is distinctively material dependent [20]. When the effect of viscosity on the stress-reducing potential of BF-RC was investigated, the high viscosity BF-RC showed similar stress values as a high viscosity conventional RC; whereas the low viscosity BF-RC produced less stress than the conventional equivalent [2, 21]. Contrarily, in another study, the low viscosity BF-RC showed more shrinkage compared with high viscosity BF-RC [22]. Overall, BF-RC with high filler amount exhibited most satisfactory shrinkage force properties [16].

Clinical performance so far has shown an assuring prospect for the BF-RC in the direct restorations of posterior teeth which were similar to the conventional RC, within an interval of12 to 72 months [10, 23]. There has been much debate regarding the bond strength of BF-RCs. In one research, there was no significant difference between the micro-SBS of low viscosity BF-RC, conventional or high viscosity BF-RC in terms of restoring the occlusal layer [18], although in another study, low viscosity BF-RC showed more satis-

factory micro tensile bond strength than conventional RC and different C-factors did not affect the bond strength [24]. In few studies, bond strength of BF-RCs was found to be product dependent [25, 26]; which was thought to be due to the differences in mechanical properties and consistency than to differences in induced shrinkage stress.

A reduction in the degree of conversion, as well as an increase in increment thickness, have been shown to negatively affect the bond strength of conventional RC to dentin [27, 28] but, SBS remained constant with increasing increment thickness for BF-RC [25, 28]. If the differences in material properties distinguished by their viscosities affect the adhesion to dentin and bond quality is still quite ambiguous. Therefore, this study aimed to understand the influence materials of different viscosities on adhesion by comparing the SBS of commercially available modern BF restoratives in both high viscosity and low viscosity with a conventional nano-hybrid RC as reference.

The null hypotheses to be verified are;

a. The BF-RCs do not significantly differ from that of a conventional RC in terms of their SBS.

b. The low viscosity and the high viscosity BF-RC do not demonstrate a significant difference in terms of their SBS.

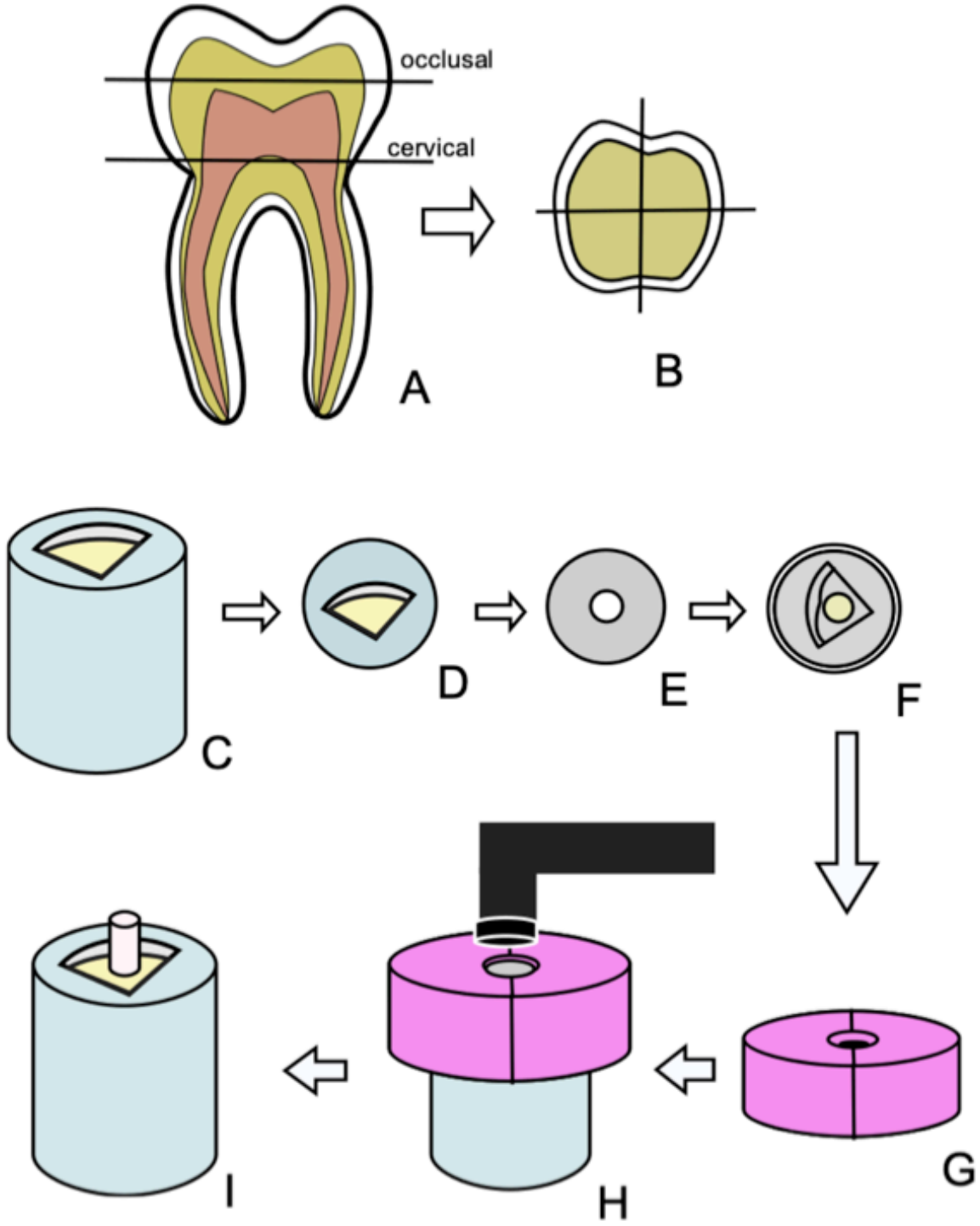
2.3 Materials and Methods

Extracted carious free third molars were stored in sodium azide solution at 4°C. They were thoroughly cleaned and then sectioned mid-coronally parallel to the occlusal line (Fig.2.1A), using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) with water cooling to obtain two halves, “occlusal” and “cervical” sections. Each divided portion was further sectioned into 2 or 4 parts depending on the size of the tooth (Fig.2.1B). The obtained 200 dentin surfaces were embedded in methacrylate resin (Technovit 4004, Heraeus Kulzer; Hanau, Germany) with the help of stainless-steel cylinders (Fig.2.1C). The embedded dentin surfaces were ground with 600 grit silicon carbide grinding paper

(Leco, St. Joseph; USA) on a grinding system (Exakt 400 cs, Norderstedt, Germany) and ensured to have a flat surface (Fig.2.1D). They were then randomly allocated into ten groups (n=20) (Fig.2.1). A thin adhesive sheet (Fig.2.1E) with a circular hole (3.2 mm diameter) was placed on the surface, delimiting the region to be bonded (Fig.2.1F). The exposed surfaces of all substrates were then treated with a 2-step self-etching dental adhesive Clearfil SE Bond 2 (Kuraray Noritake; Osaka, Japan, Lot:000031). The primer was applied with a micro brush and left undisturbed for 20s. It was then blow-dried with mild air for 5s so that the primer does not move anymore. Then the bond liquid was applied with a micro brush and dried with mild air to ensure even and a thin layer of application. The adhesive was then cured for 10s (Bluephase; Ivoclar-Vivadent; Schaan, Lichtenstein). Custom made vinyl polysiloxane split mold (Regisil PB; Dentsply Caulk, Milford, Delaware, USA) (Fig.2.1G) with a central cylindrical cavity (3.2 mm in diameter and 4 mm in height) was positioned on the specimen.

.

Figure 2.1: Diagrammatic representation of the overview of specimen preparation



Ten restoratives were used (Fig.2.1), of which, four were high viscosity BF-RC; Sonic fill 2 (SF2), Tetric EvoCeram (TEC), Admira Fusion x-tra (AFX) and Beautifil-bulk restorative (BBR), five were low viscosity BF-RC; Tetric EvoFlow (TEF), Surefill SDR (SDR), Venus Bulk-fill (VBF), Beautifil-bulk flowable (BBF), Filtek Bulk-fill (FBF) and one conventional nano-hybrid RC; Ceram-x (CX). The RC was then placed inside the cavity and condensed for better adaptation. It was deposited in one increment of 4-mm for all the BF-RC and cured for 20s with Bluephase curing lamp (Ivoclar-Vivadent; Schaan, Lichtenstein) with an irradiance of 1316 ± 51 mW/cm2 as measured with MARC simulator (BlueLight Analytics, Halifax, Canada) (Fig.2.1H). Two consecutive increments of 2 mm thickness were polymerized for the conventional RC (CX).

The prepared samples (Fig.2.1I) were stored vertically for seven d at 37 °C in distilled water and were subjected to SBS test with a broad chisel head in a universal testing machine (MCE 2000ST, Quicktest Prüfpartner; Langenfeld, Germany) at a crosshead speed of 0.5 mm/min until fracture. The loaded force at fracture was recorded. The diameter of the fractured fragments was measured at two perpendicular points (to calculate an average), and then the bonded area was determined. The SBS was calculated by dividing the loaded force by the bonded area.

The fractured bits were examined closely with a 10x magnification. The fracture progression was categorized to be in three distinct patterns. If the fracture followed straight line between dentin and resin, it was identified as adhesive. A fracture that occurred partly in tooth/RC to advance through one or both of the substrates (tooth/RC) defined a mixed fracture. The occurrence of a fracture which did not follow the bonding surface but ran exclusively through RC or dentin, such a pattern was designated as cohesive.

Table 2.1: Material Description

Product (ACRONYM) Manufacturer (LotNo)	**Type Of RC**	**Matrix**	**Filler**	**Filler % Wt /Vol**
Ceram-X (CX) Dentsply Sirona, York, PA, USA (1508000010)	Conventional RC	Methacrylate modified polysiloxane. Dimethacrylate resin	Ba-Al-F-B-Si Glass, SiO_2 nano filler	77/55
Sonic fill 2 (SF2) Kerr,Orange CA, USA (5767358)	High Viscosity BF-RC	Bis-GMA. TEGDMA. EBADMA	SiO_2, zirconium oxide, Glass oxide and YbF_3	83.5/66
Tetric EvoCeram (TEC) Ivoclar vivadent, Schaan, Liechtenstein (V08737)	High Viscosity BF-RC	Bis-GMA. UDMA	Barium glass, YbF_3. mixed oxide and prepolymer.	77/54
Admira Fusion x-tra (AFX) Voco, Cuxhaven Germany (1537600)	High Viscosity BF-RC	Ormocer	SiO_2	84/69
Beautifil-bulk restorative (BBR) Shofu, Kyoto Japan (091301)	High Viscosity BF-RC	Bis-GMA. UDMA. Bis-MPEPP. TEGDMA	S-PRG based on fluoro-boro-alumino-silicate glass.	87/74.5
Venus Bulk-fill (VBF) Heraeus Kulzer, Hanau, Germany (010108)	Low Viscosity BF-RC	UDMA. EBADMA	Ba-Al-F silicate glass. YbF_3. and SiO_2	65/38
Tetric EvoFlow (TEF) Ivoclar vivadent,Schaan, Liechtenstein (U12113)	Low Viscosity BF-RC	Bis-GMA. UDMA	Barium glass, YbF_3 and copolymers	68.2/46.4
Surefill SDR (SDR) Dentsply Sirona, York, PA, USA (1508000518)	Low Viscosity BF-RC	Modified UDMA. TEGDMA. EBADMA	Ba-Al-F-B-Si glass and St-Al-F-Si glass	68/44
Beautifil-bulk flowable (BBF) Shofu, Kyoto Japan (121301)	Low Viscosity BF-RC	Bis-GMA. UDMA. Bis-MPEPP. TEGDMA	S-PRG based on fluoro-boro-alumino-silicate glass	72.5/51
Filtek bulk-fill (FBF) 3MESPE, Seefeld Germany (N692537)	Low Viscosity BF-RC	Bis-GMA. EBADMA. UDMA	Zirconia, SiO_2	64.5/42.5

Bis-GMA: Bisphenol-A-Glycidyl Dimethacrylate, UDMA: Urethane Dimethacrylate,
Bis-MPEPP: Bisphenol A Polyethoxy Methacrylate, EBADMA: Ethoxylated Bisphenol-A-Dimethacrylate,
TEGDMA: Triethyleneglycol Dimethacrylate,
wt: weight percentage, vol: volume percentage.

2.4 Statistical Analysis

The SBS results were statistically analyzed (Version 25.0; IBM SPSS Statistics. USA) for normality and homogeneity of variance using the Kolmogorov-Smirnov test and Levene's test, respectively. The SBS results were then compared using a one-way analysis of variance (ANOVA) with the Tukey HSD post-hoc test ($\alpha = 0.05$) and the univariate analysis (general linear model with partial eta squared (η_p^2))($\alpha = 0.05$) verified the influence of type of RC, part of the tooth and experimental groups on the bond strength. Statistical power analysis and post-hoc power analysis was performed to verify if the sample size was adequate.

Weibull is a representation for the cumulative probability of failure (P_f) at applied stress: $P_f(\sigma_c) = 1 - exp\left[-\left(\frac{\sigma_c}{\sigma_0}\right)^m\right]$, m is the Weibull modulus, σ_c is the measured strength and σ_0 is the characteristic strength. It is described as the stress at which the probability of failure is 0.63. The double logarithm of this expression gives: $\left(\frac{1}{1-F}\right) = mln(\sigma) - mln(\sigma_0)$. By mapping lnln $\left(\frac{1}{1-F}\right)$ versus ln(σ), a linear upward gradient m and its intersection with the x-axis gives the logarithm of the characteristic strength (σ_0). The scatter in the computed Weibull parameters as well as the bias are analysed and compared to results by using the $P_f = \frac{(1-0.5)}{n}$ estimator [29]

A Pearson's correlation test was done to estimate if there was any correlation between the filler weight % data (as per manufacturers) and the bond strength as well as the obtained Weibull's modulus.

2.5 Results

Kolmogorov-Smirnov test ascertained that the data were normally distributed. One-way ANOVA with Tukey HSD post hoc test ($\alpha = 0.05$) failed to identify significant differences among the different BF-RC (p = 0.232). The mean SBS value varied from 22.42 (2.43) MPa for TEF and 19.37 (3.25) MPa for VBF (Table.2.2) (Fig.2.2) The univariate analysis (general linear model with partial eta squared (η_p^2) ($\alpha = 0.05$) demonstrated no significant influence seen by the type of RC (p = 0.905), part of the tooth (p=0.078) and different experimental groups (p = 0.232) on the SBS. A statistical power analysis suggested that at least 14 samples were required for an adequate evaluation of bond strength. Therefore, this experiment was performed with sample sizes of 20 and the posthoc power tests indicated that the sample size was adequate.

The Weibull analysis revealed that TEF was the most reliable material, and BBR was found to be the least reliable out of the tested materials (Table.2.2). The plot of the statistics is presented in Fig.2.3.

A very weak inverse linear correlation ($r = -.297, p < 0.001$) was found between the filler weight percentage and the Weibull's modulus and no other significant correlations was found between filler weight and SBS.

The overall fracture pattern was predominantly adhesive (58.5%) and mixed (35.5%), with few cohesive (6%) (Fig.2.5), and no pre-failures were registered. In high viscosity BF-RC, the fracture pattern was 53.75% adhesive, 36.25% mixed, and 10% cohesive. In low viscosity BF-RC, 64% adhesive, 32% mixed and 4% cohesive. The conventional composite showed a 50% adhesive and 50% mixed fractures and no cohesive fractures. High viscosity BF-RC had more fractures involving the dentin (22%), and low viscosity BF-RC had more fractures involving composite (25%). It was also observed that cohesive failures are related to high bond strength with a mean of 23.59 (3.28) MPa.

.

Table 2.2: Shear bond strength of all materials (mean with SD) and Weibull Statistics

Type Of RC	**Resin Composite**	**Shear Bond Strength (MPa)**	**Weibull Parameters** (at *95% confidence bounds*)	
		Mean (SD)	m (CI)	σ_0 (MPa)
Conventional RC	Ceram-X **(CX)**	21.31 (4.3)	5.04 (0.15)	23.27
High Viscosity BF-RC	Sonic Fill 2 **(SF2)**	20.08 (2.4)	9.81 (0.12)	21.12
	Tetric Evoceram **(TEC)**	22.21 (4.5)	5.96 (0.16)	23.95
	Admira Fusion X-Tra **(AFX)**	20.90 (3.9)	6.24 (0.11)	22.48
	Beautifil Bulk Restorative **(BBR)**	21.77 (5.9)	3.77 (0.14)	24.21
Low Viscosity BF-RC	Venus Bulk-Fill **(VBF)**	19.37 (3.2)	7.16 (0.18)	20.68
	Tetric Evoflow **(TEF)**	22.42 (2.4)	11.09 (0.11)	23.46
	Surefil SDR **(SDR)**	21.51 (3.0)	8.36 (0.13)	22.79
	Beautifil Bulk Flowable **(BBF)**	22.18 (3.7)	6.65 (0.13)	23.78
	Filtek Bulkfill **(FBF**)	22.01 (4.2)	5.59 (0.10)	23.84

SD=Standard deviation, m=Weibull's modulus, CI-Confidence interval, σ_0 =Characteristic strength, MPa=Megapascal

Figure 2.2: Shear bond strength of all restorative materials

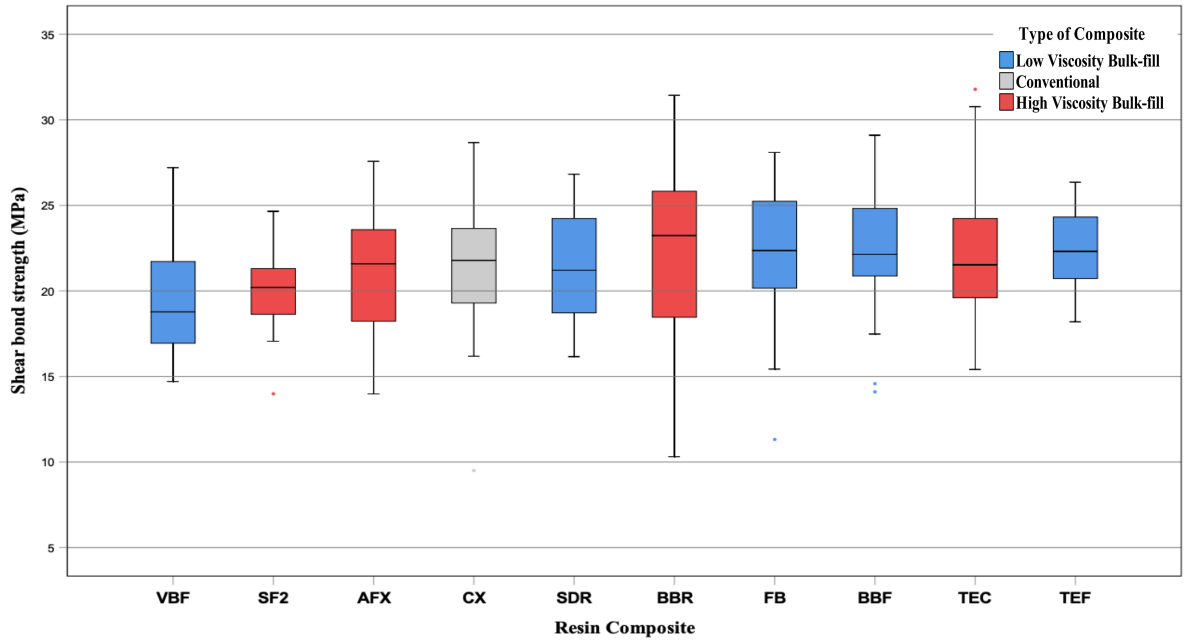
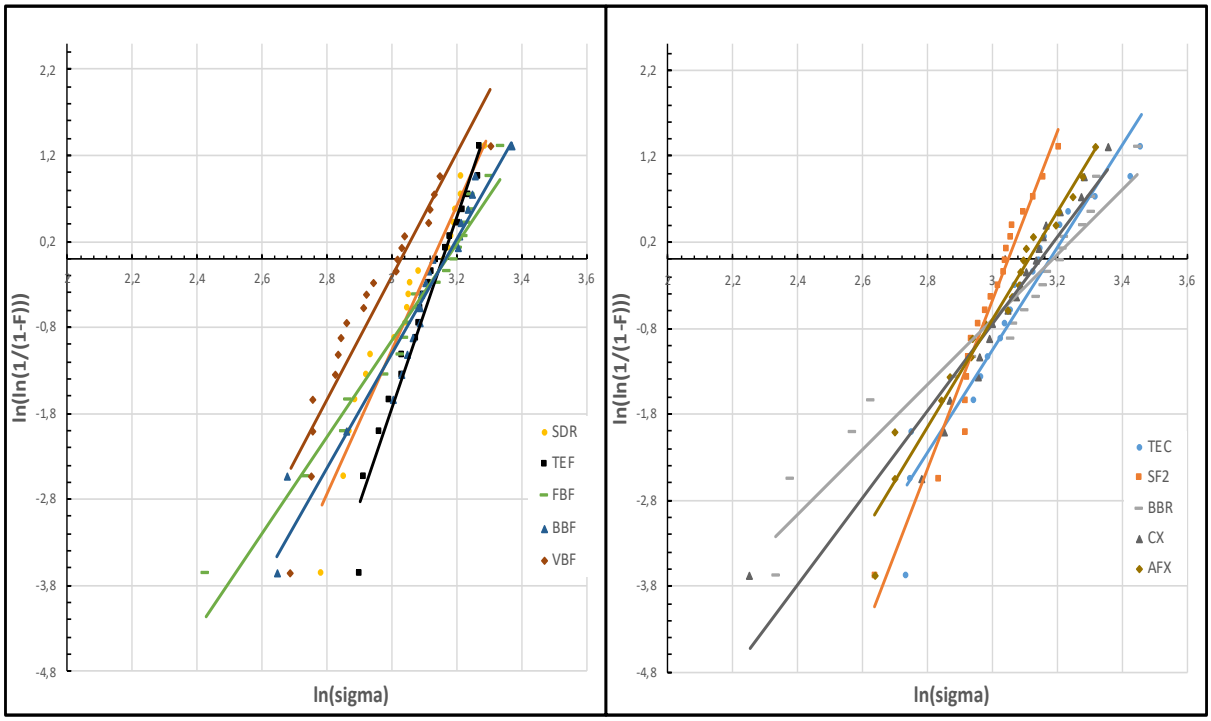


Figure 2.3: Weibull graph for all restorative materials



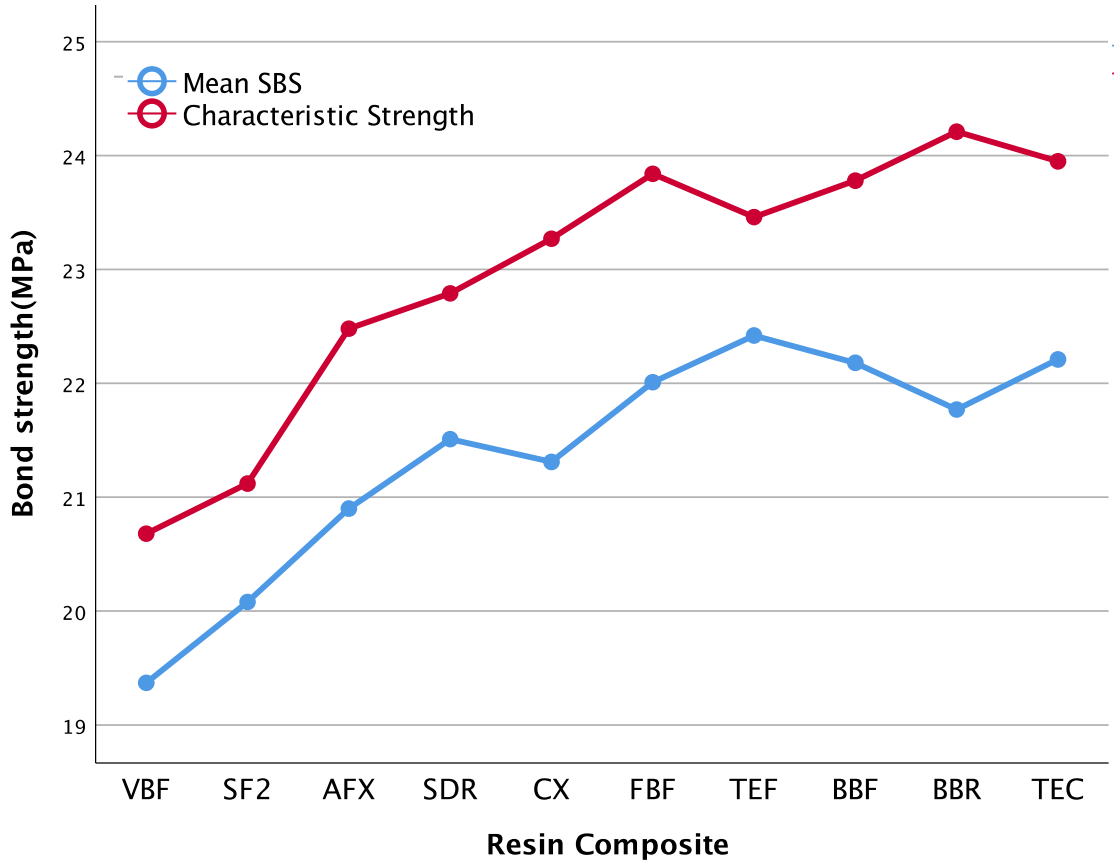
2.6 Discussion

In this study, the BF-RC showed similar values for SBS as conventional RC, regardless of their respective viscosities. Thus, both the null hypothesizes, that there was no statistically significant difference in mean SBS observed among the different viscosities of BF-RC or with conventional RC was accepted.

The bond strength test results with just mean SBS with standard deviation does not convey the complete information about the performance of the materials. In the majority of cases, the 95% CI (confidence interval) is used to calculate variances in datasets, as it provides a precise estimation. It is sometimes challenging to identify significant differences among the mean values. Nonetheless, it is broadly exercised and establishes a statistically significant difference between groups of data. Furthermore, this method of measurement can not specify the intrinsic strength as it depends mainly on the test methodology, the surface exposed to stress and the size of the test. Besides, the use of low sample size may also increase the error from the true mean of the population and true standard deviation [30]. We observed that the characteristic strength of BBR is highest compared to the other tested materials, whereas the mean strength was 21.77 (5.9) MPa (Table.2.2) (Fig.2.4). The characteristic strength value depicts the bond strength distribution in a group of samples, instead of estimating the arithmetic mean bond strength value. A change in σ_0 shifts the whole strength distribution of the data set. Thus, this value could be assumed as a statistical guide for estimating the strength of the material [31].

.

Figure 2.4: Comparison of mean shear bond strength and characteristic strength



Though the SBS results obtained in this study could not identify significant differences with respect to mean SBS, Weibull statistical analysis could assess the materials as per their reliability (Table.2.2) Weibull distribution is used to describe variability in measured material strength of brittle materials. If the obtained SBS values show high disparity among themselves, the computed Weibull modulus would be small. This reveals that flaws are arranged inconsistently, and the measured strength will be unpredictable [29]. However, in commercially available products, the evaluation is not as straightforward. There are various confounding features that result from dissimilarities in the preparation such as the initiator amount, the filler concentration and other additives, which makes fair comparisons of RCs challenging. Furthermore, stress formation depends on the shrinkage,

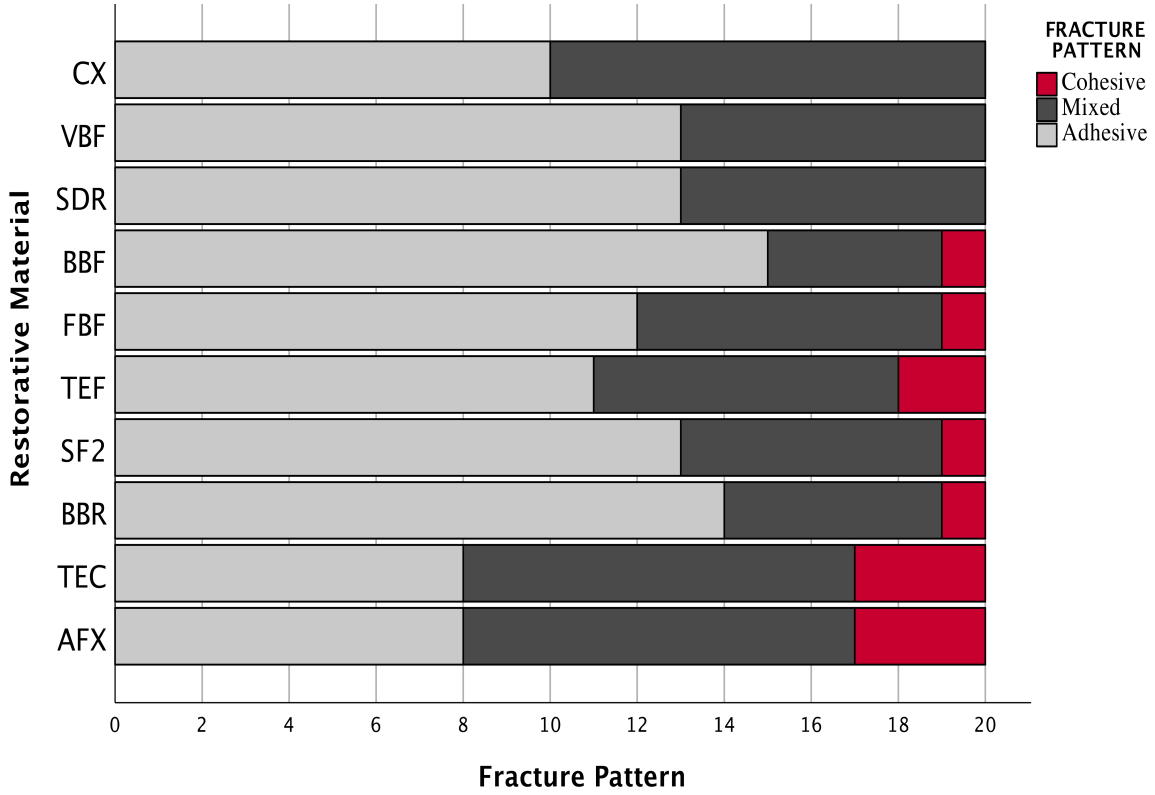
degree of conversion and the elastic modulus of the material, and these properties are affected by modifications in constituents [11, 32]. In bond strength testing, the specimen undergoes an irregular propagation of stresses at the adhesive interface, which is highly influenced by the testing variable. Hence, the absolute bond strength values should never be adjudicated as an intrinsic material property, but rather a measure of material performance under the experimental condition.

The SBS values are assumed to be susceptible to the location of the dentin substrate, as the diameter of the dentinal tubules and the moisture content varies according to the substrate position [33]. However, in order to increase the opportunity of using the available dental tissue, during the substrate preparation the tooth was cut mid coronally to obtain two portions, to obtain an "occlusal" and a "cervical" part. These parts though varied in depth of the dentin only by the thickness of the diamond saw used to cut (0.27mm). The bond strengths obtained in both these parts were compared irrespective of the composite resin used, and there was no statistically significant difference between the occlusal or cervical parts noticed. This indicates that the variation due to the slight disparity in the substrate preparation was inconsequential.

The fractographic analysis revealed that the majority of failure patterns occurred as adhesive, which was an interfacial failure between dentin surfaces and RCs (58%). A mixed fracture involving the dentin were observed to be more in high viscosity than low viscosity BF-RC, which imply that, the material with a higher filler concentration may be more resistant to fracture load and does not become the weakest path for the fracture propagation after initiation.

.

Figure 2.5: Fracture pattern analysis of all restorative materials



Although, the commercially available products broadly fall under the umbrella of BF restoratives, manufactures often bring about variations in their composition and try to encompass innovative and technologically advanced formulations in their RC to facilitate marketing of these products and also make them distinct. With the availability of such diverse materials, it is vital to analyze how the difference in filler ratio and composition affect the different classes of composites as the mechanical properties of BF-RC vary mostly according to the nature of their filler content and composition [34].

The inorganic fillers are the stiffer component in a RC. Thus logically, the higher the amount of filler content, the greater would be the composite elastic modulus ensuing in a higher stress development when tested at low compliance. However, the logic is not that straightforward, as the resin matrix, which has a lower elastic modulus than the inorganic constituents, tends to shrink due to polymerization. Hence, the matrix to filler

ratio has a substantial role in the strain and stress developed. Higher shrinkage, along with a larger elastic modulus would generate added stress within the RC and the bonded interface [35]. In the pre-gel stage, the composite exhibit adequate kinesis to re-arrange and counteract for the shrinkage without generating internal and interfacial stresses [11, 36]. In the post-gel stage, the development of a semi-rigid polymer network hampers the plastic deformation, due to the persistent polymerization shrinkage together with elastic modulus development causing stresses within the material and at the tooth-restoration interface. This state of stress is likely to accelerate gap formation, endangering the bond quality and durability of the restored tooth [36].

The materials considered are different from each other not only in their viscosities but also in their chemical compositions and filler ratio. The study included Ormocers, Giomers, and modified Resin composites. It should be emphasized that evaluating the novel restorative practices can only be achieved by considering different varieties of composites since each procedure has explicit requirements that determine the materials to be used. In the current study, we used the conventional incrementally-placed composite as a control or reference. The nano-hybrid conventional RC (CX) was used as a reference since, a long-term clinical study has proved it to be a reliable material in comparison to a BF-RC after six years [10].

The TEC and TEF both contain a germanium-based light-initiator system (Ivocerin) which unlike the conventional camphorquinone/amine-based photoinitiator systems has the ability to absorb light in the visible region intensively and has a high photoreactivity [37]. The manufacturers claim that they contain a filler that is partly functionalized by silanes which act as a shrinkage stress reliever. Once the RC is cured, the monomers in the fillers along with the silanes initiate a cross-linking process which enables the forces between the individual fillers to come in work and apply stress on the cavity walls. The stress generated is controlled by volumetric shrinkage and the modulus of elasticity. Owing to its low elastic modulus, the shrinkage stress-relieving fillers within TEC and TEF BF-RC functions like a spring among the usual glass fillers which have a greater elastic

modulus. Eventually, the volumetric shrinkage and shrinkage stress in TEF and TEC are reduced during polymerization[38].

BBR and BBF are Giomers which incorporate bioactive fillers like surface pre-reacted glass that is believed to offer the clinical advantages of glass ionomers along with RC [39]. BBR exhibited the least reliability among the tested materials which could be due to its high ratio of filler content. Although a weak, there was an inverse correlation seen between the filler weight % of the RC and the Weibull's modulus. Therefore, probably the high weight % of filler content made the material more rigid and resulted in lower reliability as the fracture became unpredictable.

In contrast to the BBR, BBF showed a higher Weibull modulus. Similarly, TEF exhibited better reliability when compared to the TEC. This implies that the low viscosity equivalent of the material showed higher reliability. Even though, they are produced by the same manufacturers and containing similar composition, a favorable adaptation, better wettability, effortless delivery and void-free restoration produced by the low viscosity BF-RC might be the reason for their higher reliability. Also, essential to consider is the fact that, TEC, TEF, BBR and BBF contain pre-polymerized fillers which makes their filler composition partially organic and thus, the considered filler amount as mentioned by the manufacturer for a correlation might not be entirely inorganic and could cause inadvertences. Pre-polymerized additives decrease the shrinkage of RC by reducing the availability of functional groups to react initially. If the initial volume of a given monomer is similar to the final conversion, a significant volumetric shrinkage is anticipated to transform into higher polymerization stress, and in experimental RCs, a direct correlation has already been established [32].

The bulk-fill nano-ormocer AFX does not contain any conventional methacrylate monomers in the matrix. The organically modified ceramics (Ormocers) features a nanohybrid filler technology with an inorganic filler content of 84% by weight [40]. This improved matrix is believed to reduce the polymerization shrinkage and favor towards having a better bond quality [11]. Previous findings have established that AFX develops lower shrinkage stress

as compared to conventional composites [41]. However, in this study, AFX showed no significant difference in bond strength and exhibited a moderate Weibull modulus.

SF2 is an intriguing material which although is highly-filled high viscosity BF- RC, it incorporates modifiers that react to sonic energy and alter their viscosity [42]. In all the previous in vitro studies testing the mechanical properties which included the Sonic Fill, the predecessor of SF2 has reported highly desirable properties and has been ranked among one of the best in this category of materials [43, 44]. Our study, although could not elicit significant difference in terms of their SBS but the material depicted a relatively high Weibull's modulus.

SDR has modified urethane di-methacrylate (UDMA) monomer, that is responsible for the decrease in polymerization shrinkage and stress. It has been previously seen that SDR improves the dentin bond strength when used in high C-factor cavities in bulk when compared to a hybrid RC and a flowable RC [45]. SDR also showed significantly higher bond strength values than a conventional nano-filler RC in Class II MOD preparations with deep proximal boxes [46]. In this study, it showed comparable values for bond strength as well as fairly high Weibull's modulus.

VBF displayed the lowest SBS value among the tested materials, which also correlates with previous studies showing a lower mechanical property like flexural strength and indentation modulus when compared to other BF RCs [6]. Also, another study elicited that VBF produced higher stress and strain compared to all other bulkfill resins tested [2]. However non-significant, the values on the lower end of the spectrum could be attributed to its lower percentage of its filler content.

A 2 step self-etching adhesive Clearfil SE bond 2 (Kuraray Noritake; Osaka, Japan) was used, which is a simplified, easy to use adhesive and the previous version of this adhesive has been clinically proven to be consistent [47]. In addition, choosing only one type of bonding agent would homogenize the influence of bonding agents as it has been already witnessed that, the actual adhesive used to bond the BF-RC happens to be the most influential factor in bond strength testing [25].

Since the SBS had no significant difference among all of the BF materials analyzed in this study, it can be assumed that under appropriate polymerization situations, a 4-mm increment placed with high viscosity or low viscosity materials in bulk would present comparable outcomes in terms of their bond strength. However, this study was conducted after one week of immersion time, and many of the properties may alter over time. It is also essential to highlight that, differing enhancements adopted by the manufactures can affect the performance of these materials in a longer time frame and therefore results observed cannot be extrapolated for all the tested materials. Further studies are necessary to define the influence of the aging procedures and quantify how much they degrade the adhesion in relation to their composition. The stability of the adhesion should be maintained over time to ensure a successful restoration.

The main strength of this study was that it enables a direct comparison among different types of BF-RC and the specimen preparation was carried out in the same manner by a single operator, consequently diminishing biases related to procedural inaccuracies, environmental influences and other factors that alter the material. Additionally, important to note is that the specimens in this study were prepared in an ideal setting where the curing unit was placed right above the RC material, which is not the case in most of the clinical situation. Thus, when the materials are clinically considered, there may be discrepancies due to this difference. The clinician is in charge of choosing the right material assessing the clinical situation, as according to the results of this study, BF-RC did not show a varied performance as a category of materials than conventional RC and neither did the type of the RC had any influence on the bond strength.

2.7 References

1. van Dijken, J.W. and U. Pallesen, Posterior bulk-filled resin composite restorations: A 5-year randomized controlled clinical study. Journal of Dentistry, 2016. 51: p. 29-35.

2. Al Sunbul, H., N. Silikas, and D.C. Watts, Polymerization shrinkage kinetics and shrinkage-stress in dental resin-composites. Dent Mater, 2016. 32(8): p. 998-1006.

3. Jang, J.H., S.H. Park, and I.N. Hwang, Polymerization shrinkage and depth of cure of bulk-fill resin composites and highly filled flowable resin. Oper Dent, 2015. 40(2): p. 172-80.

4. Chesterman, J., et al., Bulk-fill resin-based composite restorative materials: a review. British Dental Journal, 2017. 222(5): p. 337-344..

5. Al-Ahdal, K., et al., Polymerization kinetics and impact of post polymerization on the Degree of Conversion of bulk-fill resin-composite at clinically relevant depth. Dent Mater, 2015. 31(10): p. 1207-13..

6. Czasch, P. and N. Ilie, In vitro comparison of mechanical properties and degree of cure of bulk fill composites. Clin Oral Investig, 2013. 17(1): p. 227-35.

7. Van Ende, A., et al., Bulk-Fill Composites: A Review of the Current Literature. J Adhes Dent, 2017. 19(2): p. 95-109. 8. Papadogiannis, D., et al., Viscoelastic properties, creep behavior and degree of conversion of bulk fill composite resins. Dent Mater, 2015. 31(12): p. 1533-41..

9. Van Ende, A., et al., Bulk-Fill Composites: A Review of the Current Literature. Journal of Adhesive Dentistry, 2017. 19(2): p. 95-109..

10. van Dijken, J.W.V. and U. Pallesen, Bulk-filled posterior resin restorations based on stress-decreasing resin technology: a randomized, controlled 6-year evaluation. Eur J Oral Sci, 2017. 125(4): p. 303-309..

11. Schneider, L.F.J., L.M. Cavalcante, and N. Silikas, Shrinkage Stresses Generated during Resin-Composite Applications: A Review. Journal of Dental Biomechanics, 2010. 2010: p. 131630..

12. Pfeifer, C.S., et al., Factors affecting photopolymerization stress in dental composites. J Dent Res, 2008. 87(11): p. 1043-7.

13. Peutzfeldt, A., et al., Marginal Gap Formation in Approximal "Bulk Fill" Resin Composite Restorations After Artificial aging. Oper Dent, 2018. 43(2): p. 180-189.

14. Rizzante, F.A.P., et al., Shrinkage stress and elastic modulus assessment of bulk-

fill composites. J Appl Oral Sci, 2019. 27: p. e20180132.

15. Yazici, A.R., et al., Thirty-Six-Month Clinical Comparison of Bulk Fill and Nanofill Composite Restorations. Oper Dent, 2017. 42(5): p. 478-485.

16. Taubock, T.T., F. Jager, and T. Attin, Polymerization shrinkage and shrinkage force kinetics of high- and low-viscosity dimethacrylate- and ormocer-based bulk-fill resin composites. Odontology, 2019. 107(1): p. 103-110.

17. Rizzante, F.A.P., et al., Polymerization shrinkage, microhardness and depth of cure of bulk fill resin composites. Dent Mater J, 2019. 38(3): p. 403-410.

18. Cerda-Rizo, E.R., et al., Bonding Interaction and Shrinkage Stress of Low-Viscosity Bulk Fill Resin Composites With High-Viscosity Bulk Fill or Conventional Resin Composites. Oper Dent, 2019.

19. Meereis, C.T.W., et al., Polymerization shrinkage stress of resin-based dental materials: A systematic review and meta-analyses of composition strategies. Journal of the Mechanical Behavior of Biomedical Materials, 2018. 82: p. 268-281.

20. Rosatto, C., et al., Mechanical properties, shrinkage stress, cuspal strain and fracture resistance of molars restored with bulk-fill composites and incremental filling technique. Journal of dentistry, 2015. 43(12): p. 1519-1528.

21. Kim, R.J., et al., Polymerization shrinkage, modulus, and shrinkage stress related to tooth-restoration interfacial debonding in bulk-fill composites. J Dent, 2015. 43(4): p. 430-9.

22. Ersen, K.A., O. Gurbuz, and M. Ozcan, Evaluation of polymerization shrinkage of bulk-fill resin composites using microcomputed tomography. Clin Oral Investig, 2019.

23. Veloso, S.R.M., et al., Clinical performance of bulk-fill and conventional resin composite restorations in posterior teeth: a systematic review and meta-analysis. Clinical Oral Investigations, 2019. 23(1): p. 221-233.

24. Sagsoz, O., et al., The bond strength of highly filled flowable composites placed in two different configuration factors. J Conserv Dent, 2016. 19(1): p. 21-5.

25. Ilie, N., et al., An in-vitro assessment of the shear bond strength of bulk-fill resin composites to permanent and deciduous teeth. Journal of Dentistry, 2014. 42(7): p. 850-5.

26. Van Ende, A., et al., Effect of Bulk-filling on the Bonding Efficacy in Occlusal Class I Cavities. J Adhes Dent, 2016. 18(2): p. 119-24.

27. Price, R.B., G. Doyle, and D. Murphy, Effects of composite thickness on the shear

bond strength to dentin. Journal of the Canadian Dental Association, 2000. 66(1): p. 35-9.

28. Flury, S., A. Peutzfeldt, and A. Lussi, Influence of increment thickness on microhardness and dentin bond strength of bulk fill resin composites. Dental materials, 2014. 30(10): p. 1104-12.

29. Quinn, J.B. and G.D. Quinn, A practical and systematic review of Weibull statistics for reporting strengths of dental materials. Dent Mater, 2010. 26(2): p. 135-47.

30. Payton, M.E., M.H. Greenstone, and N. Schenker, Overlapping confidence intervals or standard error intervals: What do they mean in terms of statistical significance? Journal of Insect Science, 2003. 3(1).

31. Mehrvar, C., et al., Comparative study of Weibull characteristic strength and mean strength of GPCs to confirm the minimum number of samples needed for confident strength reporting. Journal of the Mechanical Behavior of Biomedical Materials, 2015. 43: p. 53-58.

32. Soares, C.J., et al., Polymerization shrinkage stress of composite resins and resin cements - What do we need to know? Braz Oral Res, 2017. 31(suppl 1): p. e62.

33. Perdigão, J., Dentin bonding—Variables related to the clinical situation and the substrate treatment. Dental Materials, 2010. 26(2): p. e24-e37.

34. Ilie, N., S. Bucuta, and M. Draenert, Bulk-fill resin-based composites: an in vitro assessment of their mechanical performance. Operative Dentistry, 2013. 38(6): p. 618-625.

35. Ferracane, J.L., Buonocore Lecture. Placing dental composites–a stressful experience. Oper Dent, 2008. 33(3): p. 247-57.

36. Boaro, L.C.C., et al., Polymerization stress, shrinkage and elastic modulus of current low-shrinkage restorative composites. Dental Materials, 2010. 26(12): p. 1144-1150.

37. Vivadent, I., Tetric EvoCeram(R) Bulk fill: simplifies composite restoration placement, increases efficiency. Compend Contin Educ Dent, 2014. 35(6): p. 432.

38. Schenck, L., et al., Major breakthrough in the field of direct posterior composite resins-thanks to the combined use of TetricEvoCeram Bulk Fill and Bluephase Style. Die Zahnarzt Woche, 2011. 38(3-15): p. 6.

39. Ilie, N. and G.J. Fleming, In vitro comparison of polymerisation kinetics and the micro-mechanical properties of low and high viscosity giomers and RBC materials. J Dent, 2015. 43(7): p. 814-22.

40. Wolter, H., W. Storch, and H. Ott. New inorganic/organic copolymers (ORMOCER®

S) for dental applications. in MRS Proceedings. 1994. Cambridge Univ Press.

41. Demirel, G., et al., Volumetric Cuspal Deflection of Premolars Restored With Different Paste-like Bulk-fill Resin Composites Evaluated by Microcomputed Tomography. Oper Dent, 2019.

42. Ching, K., Deep and fast: Kerr's SonicFill bulk fill composite. HDA Now, 2012: p. 24-5.

43. Alrahlah, A., N. Silikas, and D.C. Watts, Post-cure depth of cure of bulk fill dental resin-composites. Dental Materials, 2014. 30(2): p. 149-54.

44. Pereira, R., et al., Evaluation of Bond Strength, Nanoleakage, and Marginal Adaptation of Bulk-fill Composites Submitted to Thermomechanical Aging. J Adhes Dent, 2019. 21(3): p. 255-264.

45. Van Ende, A., et al., Bulk-filling of high C-factor posterior cavities: Effect on adhesion to cavity-bottom dentin. Dental Materials, 2013. 29(3): p. 269-277.

46. Kumagai, R.Y., et al., Bond Strength of a Flowable Bulk-fill Resin Composite in Class II MOD Cavities. J Adhes Dent, 2015. 17(5): p. 427-32.

47. Peumans, M., et al., Eight-year clinical evaluation of a 2-step self-etch adhesive with and without selective enamel etching. Dent Mater, 2010. 26(12): p. 1176-84.

Chapter 3

The long term consequence of salivary contamination at various stages of adhesive application and clinically feasible remedies to decontaminate

3.1 Abstract

Purpose: To analyze the long term bond quality to dentin by simulating salivary contamination and decontamination procedures at different stages of restoration.

Method: 1120 human dentin specimens were randomly allocated to 56 groups (14 x 4 intervals) (n=20) to be treated with a self-etching (Clearfil SE Bond 2(SE)) and Universal (Clearfil Universal(U)) adhesive. The experimental procedures were executed after surface preparation, after primer application (for SE) and after adhesive system curing. They were stored (37°C, distilled water) for four intervals (one week, one month, three months and one year) and subjected to shear bond strength (SBS) test at a crosshead speed of 0.5 mm/min.

Results: One-way ANOVA with Tukey's test (α=0.05) revealed significant reduction in SBS in all the groups in U adhesive compared to the control group (no contamination) at one week ($p < 0.0001$) and in SE when the contamination took place after primer application. However, decontamination improved the SBS in SE but not in U adhesive. The Weibull analysis showed reliability of U adhesive reduced over time compared to SE. The univariate analysis confirmed significant influences ($p < 0.0001$) seen by the stage of influence (η_p^2=0.600), experimental groups (η_p^2=0.518), type of adhesive (η_p^2=0.328), aging (η_p^2=0.130) and treatment procedure (η_p^2=0.075).

Conclusion: Saliva contamination is detrimental after primer application in SE but, decontamination regained the SBS and maintained it over time. In U adhesive, SBS deteriorated over time irrespective of the contamination.

Clinical Relevance: The salivary contamination showed significant influence on SBS when restoring with contemporary dental adhesives.

3.2 Introduction

Adhesive systems have momentously transformed dentistry, allowing dental procedures that were considered impossible in the past without fashioning retentive features in cavity preparations and losing healthy tooth structure [1, 2]. Adhesives typically incorporate monomers like bisphenol A diglycidyl ether dimethacrylate (bis-GMA) that functions exceptionally well while bonding to enamel, whereas, the dentin is moist and inherently hydrophilic, and bis-GMA being hydrophobic is incompetent to penetrate into the tubules completely. Therefore, more hydrophilic monomers like 2-hydroxylethyl methacrylate (HEMA) are used to enhance wetting. Additionally, monomers also contain hydrophilic groups such as carboxylic acid, hydroxyl, ester, amine or ether moieties in order to assist in encouraging the water miscibility of adhesive preparations. However, this renders them to be more vulnerable to hydrolysis in the oral environment [3] owing to water sorption in the adhesive layer, which then behaves as a permeable membrane [4]. In addition to encouraging a reduction in bond quality between the com8posite and the substrate, such perviousness of the adhesive layer appears to add up to the hydrolysis of resin polymers and the consequential degeneration of tooth-resin bond over time [5].

While dealing with materials sensitive to moisture, isolation remains one of the critical factors for ensuring good adhesion [6]. Despite this, due to various reasons when the isolation protocol is breached, especially when the operative site is near or at the gingival margin, the patient is unwilling, teeth are malpositioned or have cervical lesions, there is a high likelihood of the operative surface being exposed to a variety of substances, resulting in contamination [6]. This creates hindrances for proper infiltration of the adhesives that are required to offer the mechanical bonding and reduce the quality of the bond. Saliva is one such element existing in the oral cavity which has a high probability of contaminating the surface to be restored. It constitutes of 99.4% water and 0.6% solids. They are mainly aggregates of molecules like glycoproteins, sugar, proteins and amylase and inorganic components like sodium, chloride and calcium [7]. It has been observed

that an acid conditioned tooth surface absorbs salivary constituents and decreases the surface energy and ends up being detrimental for bonding [8]. Water, organic remains and biofilms present in a clinical setting might interfere with the wetting and spreading of the adhesives on the restorative surface [1]. The SEM evaluation of the restoration revealed that saliva contamination did not inhibit hybrid layer formation but, it reduced the adaptation of the restorative material to bonded surfaces [9].

In few of the prior studies, it was observed that 2-step etch and rinse adhesive were comparatively less susceptible to salivary contamination [10-13]. Nevertheless, while applying etch-and-rinse adhesives in dentin, the acid-etching demineralizes the superficial (5–8 μm) inter-tubular dentin matrix to produce porosities in the underlying collagen fibrillar matrix. This facilitates infiltration of co-monomers into collagen fibrils to secure retention for resin composite (RC) restorations [14] but, the collagen fibrils that gets demineralized by acid will collapse post air-drying and does not give the required support for the resin, resulting in a decreased bond quality [14].

The self-etching adhesive befits to be an idyllic adhesive for restorations in the dentin while it does not eliminate the entire moisture but modifies the smear layer to form a hybrid layer [15] making it a golden standard for bonding to dentin. It has been debated that the self-etching adhesives are more vulnerable to salivary contamination in dentin [16-22]. In contrast, few studies ascertained that there was no significant difference in bond quality while bonding to dentin [23-25]. A recent study investigated the effect of relative humidity and saliva contamination on bond strength in dentin after one year and noticed that the two self-etching adhesives showed stable bond strength over time [25]. The earlier findings had also verified that when some sort of decontamination procedure like rinsing the saliva or re-applying the adhesive system, the restoration attained improved adhesion [10, 18, 22, 26-33]. However, there is no consistency in the procedure of decontamination and findings thus varied [10].

Although immediate studies on the influence of bond strength post contamination have been discussed in great detail, it is also essential to understand the consequences of

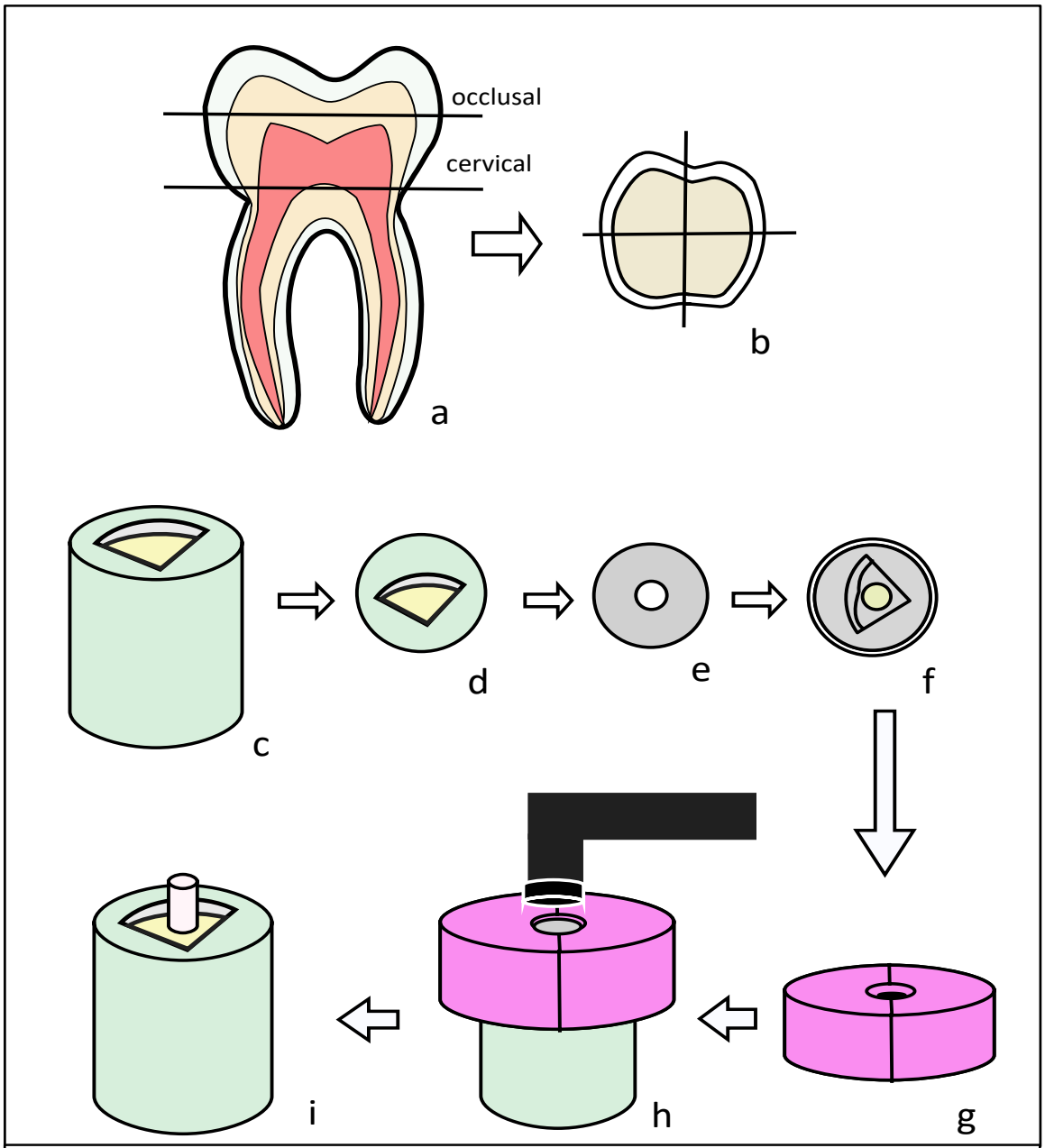
contamination of these modern formulations together with clinically possible decontamination methods post aging. Comprehending the altering structure of the interface and its faults over time remains a task at hand. The purpose of this study was to evaluate the long-term effects of salivary contamination on the bond strength of the self-etching and universal adhesive and also, to find the clinically possible remedies using decontamination procedures at various stages of application. The null hypotheses that were evaluated in this study are that the SBS in dentin is not affected by; a) the type of adhesive, b) aging (one week, one month, three months and one year), c) salivary contamination, d) decontamination methods and e) the stage of salivary contamination.

3.3 Materials and Methods

Extracted carious free human third molars were collected and stored in dilute sodium azide solution at 4°C. They were thoroughly cleaned and were sectioned mid-coronally, parallel to the occlusal plane using a low speed saw (Isomet, Buehler, Lake Bluff, IL, USA) to obtain two dentin segments labelled as "occlusal" and "cervical" (Fig.3.1a). The obtained segments were further divided into 2 or 4 parts depending on the size of the tooth, ensuring that there is enough (>3.2mm diameter) dentin to bond (Fig.3.1b). A total of 1120 dentin substrates obtained were embedded in cold-curing methacrylate resin (Technovit 4004, Heraeus Kulzer, Germany) with the help of stainless-steel cylindrical moulds (Fig.3.1c). The substrates were wet ground with 600-grit silicon carbide grinding paper (Leco, St. Joseph, USA) and a grinding system (Exakt 400 cs, Norderstedt, Germany) to obtain and flat dentinal surface (Fig.3.1d). They were then randomly allocated into 56 groups (n=20); 14 subgroups for four intervals; one week (1W), one month (1M), three months (3M) and one year (1Y). They were treated with two adhesives; Clearfil SE Bond 2(SE) and Clearfil Universal (U) (Table.3.1) (Fig. 3.3). A thin adhesive strip with a circular hole (3.2 mm diameter) (Fig.3.1e) was placed on the prepared surface, limiting the region to be bonded (Fig.3.1f). The exposed dentin surface was then treated with the

adhesive according to the group allocated (Fig.3.3). Groups with no contamination (NC) served as control and was treated as per the manufacturer's instructions (Table.3.1). The contamination(C) and decontamination (DC) treatment occurred in three stages; stage-1: after surface preparation, stage-2: after primer (only for SE) and stage-3: after adhesive curing (Fig 3.2). Detailed step wise process of specimen preparation for each group is explained in Fig.3.3 and Fig.3.4.

Figure 3.1: Diagrammatic representation of the overview of specimen preparation



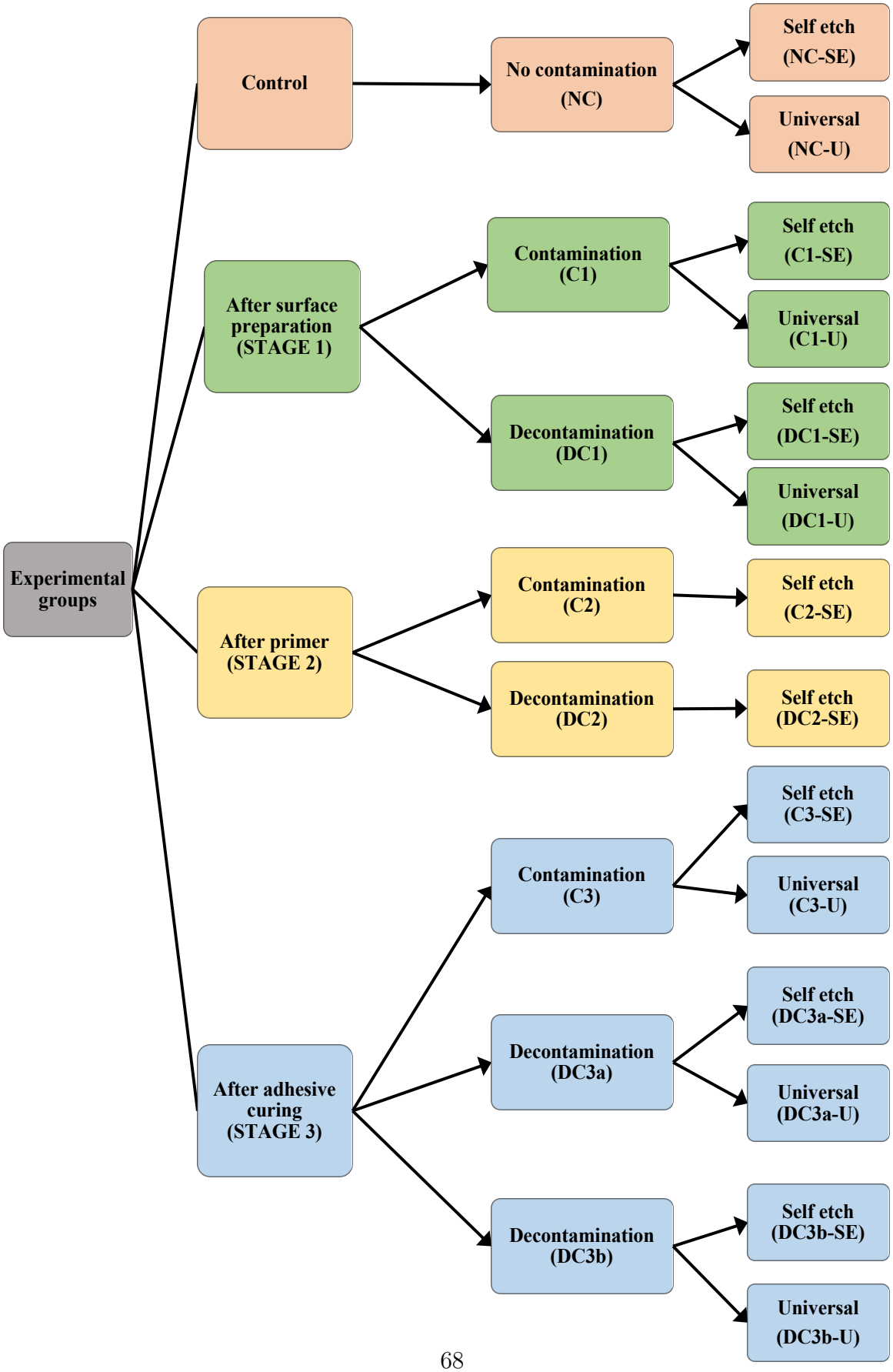
a) Diagrammatic representation of the cutting planes
b) Diagrammatic representation of cross-section each segment
c) Dentin substrate embedded in cold cure acrylic
d) Horizontal view of the surface of the specimen
e) Adhesive sheet with 3.2mm hole
f) Adhesive sheet applied on the substrate to delimit to area of bonding
g) Custom made vinyl polysiloxane split mold with a cylindric cavity of 3.2 mm diameter and 4 mm height
h) Illustration of composite placement and light curing
i) Design of restored final specimen

Table 3.1: Material composition and description

Material (ACRONYM) *Manufacturer (Lot no)*	**Type of material**	**Composition**	**Instructions for use**
Clearfil SE Bond 2 (SE) *Kuraray Noritake (000031)*	2-step self-etching adhesive	Primer • 2-hydroxyethyl methacrylate • 10-Methacryloyloxydecyl dihydrogen phosphate • Hydrophilic aliphatic dimethacrylate • dl-Camphorquinone • Accelerators • Water • Dyes Adhesive • bisphenol A diglycidylmethacrylate • 2-hydroxyethyl methacrylate • 10-Methacryloyloxydecyl dihydrogen phosphate • Hydrophobic aliphatic dimethacrylate • Colloidal silica • dl-Camphorquinone • Initiators • Accelerators	Apply Primer and leave for 20 Seconds. Dry with mild air. Apply bond. Make a uniform bond film using a gentle airflow. Light cure for 10 seconds.
Clearfil Universal (U) *Kuraray Noritake (000017)*	Universal adhesive	Adhesive • bisphenol A diglycidylmethacrylate • 2-hydroxyethyl methacrylate • ethanol • 10-Methacryloyloxydecyl dihydrogen phosphate • Hydrophilic aliphatic dimethacrylate • Colloidal silica • dl-Camphorquinone • Silane coupling agent • Accelerators • Initiators • Water	Apply bond liquid and rub for 10 seconds Blow mild air to make a uniform bond film. Light cure for 10 seconds
Admira Fusion X-tra (AFX) *Voco (1537600)*	Bulk fill resin composite	Matrix: Ormocer Fillers: Silicon dioxide	Dispense an increment of 4-mm and light cure for 20 seconds

Fresh unstimulated human saliva from a single individual was collected. It was made sure to be collected at least one hour after the consumption of any food or drink, and just before the substrate preparation. The contamination and decontamination procedures simulated the clinical situation during the process of restoration. In all the contamination and decontamination groups (C & DC), the salivary contamination was done with one drop (0.025ml) of saliva for 20 seconds. In stage-1 contamination groups (C1), the surfaces were contaminated after surface preparation with saliva (20 seconds) and air-dried (5 seconds) and in decontamination group (DC1), saliva was applied (20 seconds), and then rinsed with water (10 seconds) and air-dried (5 seconds). In stage-2 (only in SE), the saliva was applied (20 seconds) after the primer application (C2) and was decontaminated by rinsing with water (10 seconds), air-dried (5 seconds) and the primer was re-applied (20 seconds) (DC2). In stage-3, the saliva was applied after the adhesive system was cured (C3) and was decontaminated in two ways, either by only rinsing with water (10 seconds) and air-drying (5 seconds) (DC3a) or by rinsing with water (10 seconds) air-drying (5 seconds) and re-applying the bonding liquid and curing (10 seconds) (DC3b). Except for the experimental modifications wherever mentioned, the rest of the procedures in both SE and U adhesives were as per the manufacturer's instructions (Table 3.1) using the self-etch bonding method and cured for 10 seconds (Bluephase; Ivoclar-Vivadent; Schaan, Lichtenstein) with a radiant emmitance of 1316 $\pm$ 5.1 mW/cm^2 as measured with MARC simulator (BlueLight Analytics Inc., Halifax, Canada). A custom-built vinyl polysiloxane split mould (Regisil PB, Dentsply Caulk; USA) with a cylindrical cavity (3.2 mm in diameter and 4 mm in height) (Fig.3.1g) was positioned on the specimen. An ormocer based bulk-fill resin composite, Admira fusion x-tra (Voco, Cuxhaven, Germany) was then placed in one 4-mm increment, followed by polymerizing it for 20 seconds (Bluephase; Ivoclar-Vivadent; Schaan, Lichtenstein) (Fig.3.1h).

Figure 3.2: Description of the experimental groups



The prepared specimens (Fig.3.1i) were stored vertically immersed in distilled water at 37 °C for four different time intervals (1W, 1M, 3M and 1Y). The distilled water was periodically changed every week without disturbing the specimens. After storing the specimens for the pre-determined durations, they were subjected to SBS test with a broad chisel head in a universal testing machine (MCE 2000ST; Quicktest Prüfpartner GmbH, Langenfeld, Germany) at a constant crosshead speed of 0.5 mm/min until fracture. Subsequently, the loaded force at fracture was recorded. Post fracture, the diameter of the fractured specimens was measured to a precision of 0.01mm using a digital micrometre scale at two perpendicular positions (to calculate an average) and then the bonded area was determined. The SBS was calculated by dividing the loaded force by the bonded area. The fractured fragments were then closely examined with a 10x magnification. Fracture patterns were categorized as an adhesive fracture if the failure occurred along the adhesive interface, in a mixed failure a fracture line ran along of adhesive surface together with the resin composite or dentin and cohesive failure occurred when fracture occurred within the resin composite or dentin.

Figure 3.3: Flowchart explaining the experimental procedure of each group in SE adhesive

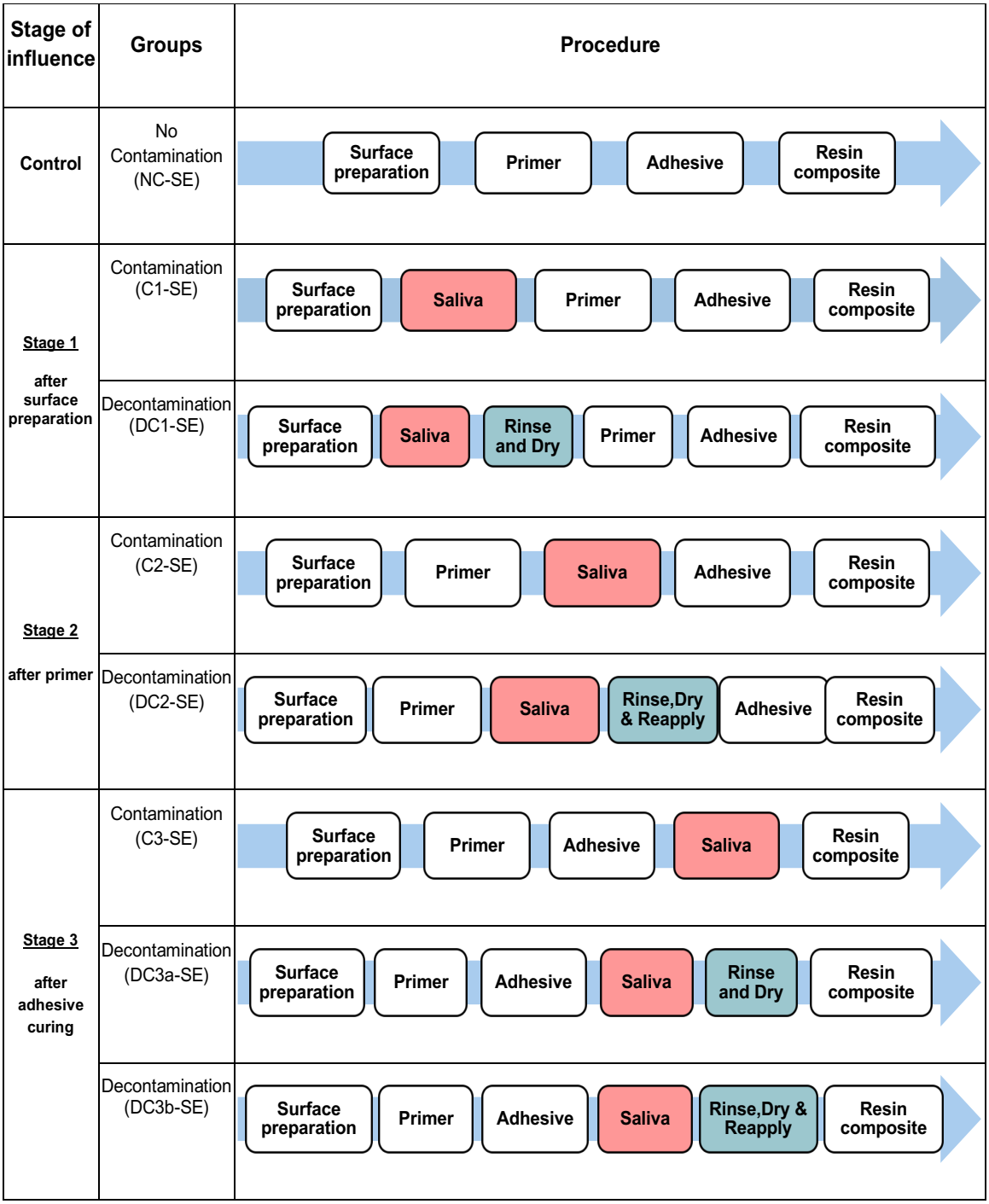
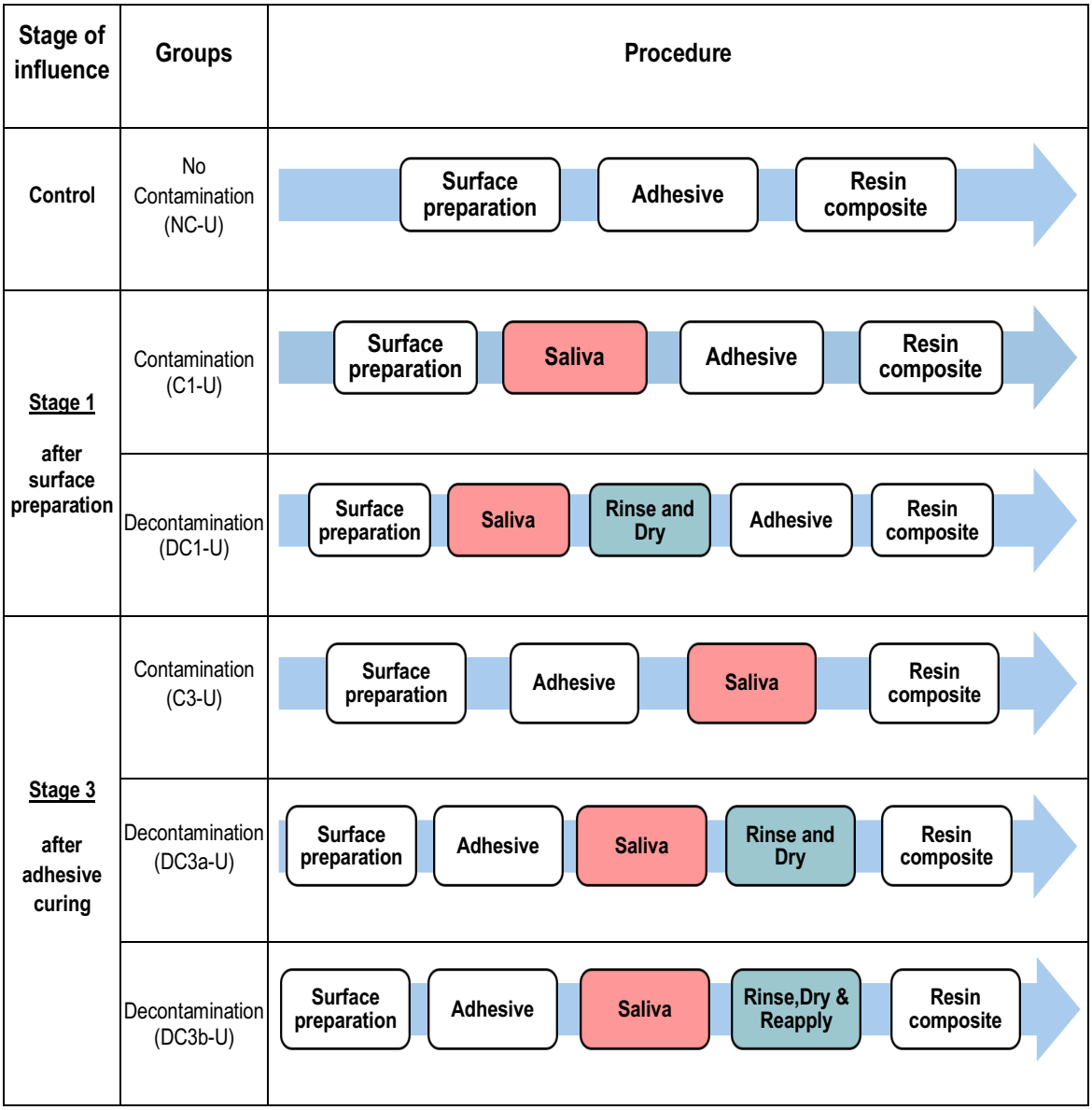


Figure 3.4: Flowchart explaining the experimental procedure of each group in U adhesive



3.4 Statistical Analysis

The SBS results were statistically analyzed (Version 25.0; IBM SPSS Statistics. USA) for normality and homogeneity of variance using the Kolmogorov-Smirnov Test and Levene's test, respectively. The SBS data of individual experimental groups over time were evaluated using a one-way analysis of variance (ANOVA) with the Tukey HSD post-hoc test (α=0.05). The univariate analysis (general linear model with partial eta squared (η_p^2))(α=0.05) was used to analyse the influence of the factors; treatment procedures, type of adhesive, aging, stage of influence, and experimental groups on the bond strength. Additionally, SBS of specimens obtained from occlusal and cervical parts of the tooth were compared within each control (NC) experimental group in order to assess a possible influence of dentin substrate obtained from different areas of the tooth.

To assess the reliability of each experimental group, Weibull analysis was performed based on the SBS data to determine the Weibull modulus and characteristic strength (n=20), at a confidence level of 95%. The expression of Weibull distribution: $P_f\left(\sigma_c\right) = 1 - exp\left[- \left(\frac{\sigma_c}{\sigma_0}\right)^m \right]$ where P_f is the probability of fracture at applied stress, σ is the measured strength, σ_0 is the characteristic strength at which probability of fracture is 63.2%, and m is the Weibull modulus [34]. The double logarithm of this expression gives: $\left(\frac{1}{1-F}\right) = mln(\sigma) - mln(\sigma_0)$. By mapping $1\left(\frac{1}{1-F}\right)$ versus $\ln(\sigma)$, a straight ascending slope m and its intersection with the x-axis gives the logarithm of the characteristic strength (σ_0). The scatter in the computed Weibull parameters as well as the bias were analyzed and compared to results at 95% confidence level using $P_f = \frac{(1-0.5)}{n}$ estimator [35].

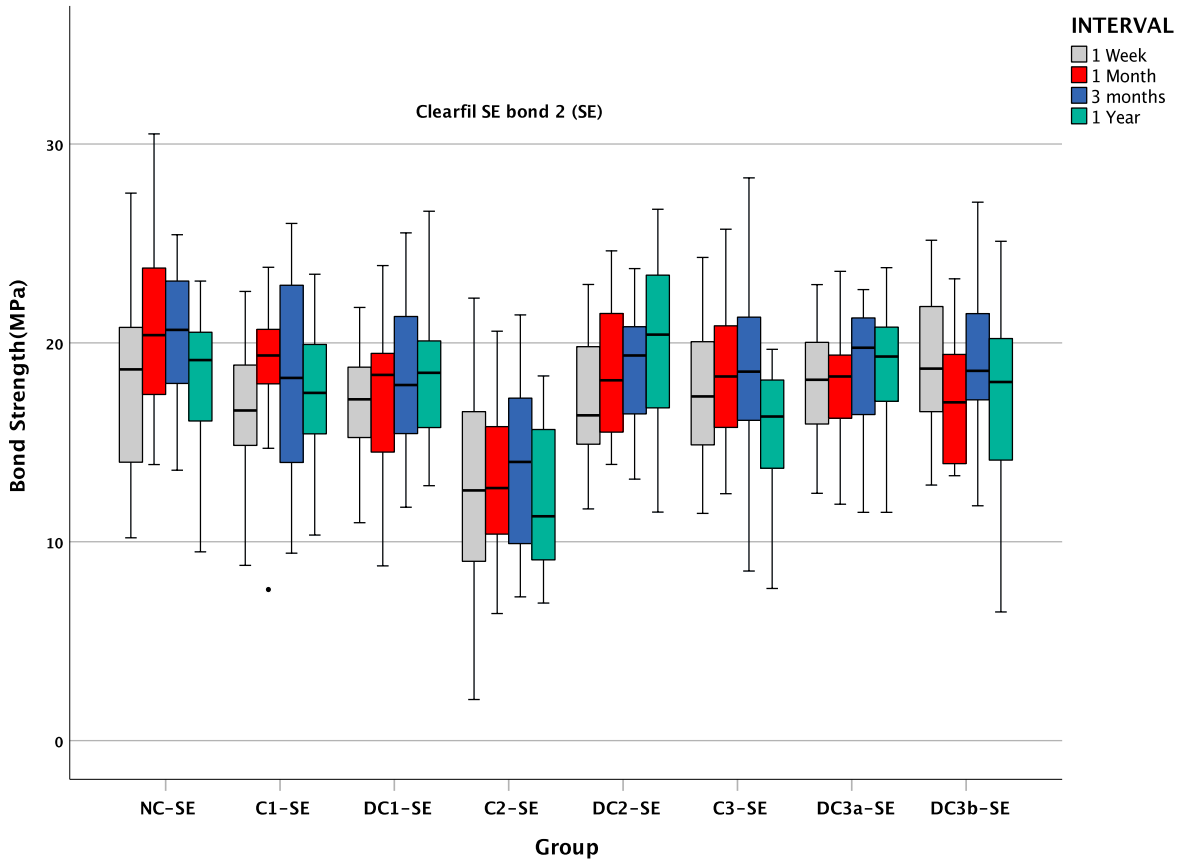
3.5 Results

SBS data of SE adhesive (Table.3.2) (Fig.3.5) and U adhesive (Table.3.3) (Fig.3.6) depicts the aging behaviour of different groups pre and post contamination with saliva. It can be observed that among the control groups, there is a drastic reduction in SBS in the NC-U, while a stable bond strength was observed in NC-SE groups all throughout one year of the aging period. Although, immediate bond strength comparison of the control groups of both adhesives NC-SE and NC-U showed no significant difference (p=0.186) at 1W. There was a significant difference in the SBS of the control group over time.

Table 3.2: Shear bond strength (SBS) of self-etching adhesive (SE)

Stage of Influence	**Groups**	**Shear bond strength (SE)** MPa (SD)				
		1 week	**1 month**	**3 months**	**1 year**	**p-value**
No influence	**NC-SE**	18.53 (5.27)^{A}	20.29 (5.27)^{B}	20.44 (3.40)^{E}	18.51 (3.37)^{D}	0.146
Stage 1	**C1-SE**	16.58 (3.28)^{A}	18.61 (3.40)^{B}	18.40 (5.20)^{E}	17.51 (3.10)^{D}	0.330
	DC1-SE	16.92 (4.03)^{A}	17.31 (4.02)^{B}	18.18 (3.60)^{E}	18.08 (4.56)^{D}	0.546
Stage 2	**C2-SE**	12.73 (5.28)^{a}	13.20 (3.49)^{b}	13.91 (4.34)^{e}	12.01 (3.48)^{d}	0.170
	DC2-SE	17.02 (3.25)^{A}	18.65 (3.49)^{B}	18.87 (4.07)^{E}	19.87 (4.29)^{D}	0.720
Stage 3	**C3-SE**	17.43 (3.31)^{A}	18.25 (4.17)^{B}	18.17 (4.98)^{E}	15.74 (3.20)^{D}	0.148
	DC3a-SE	18.04 (2.97)^{A}	17.5 (3.56)^{B}	18.61 (3.33)^{E}	18.93 (2.89)^{D}	0.504
	DC3b-SE	19.12 (3.59)^{A}	17.09 (3.23)^{B}	18.9 (3.45)^{E}	16.99 (5.02)^{D}	0.172
Same superscripts letters show mean values with no statistically significant differences within the respective interval. ($p<0.001$; $\alpha = 0.05$)						

Figure 3.5: SBS of all groups in SE adhesive over time



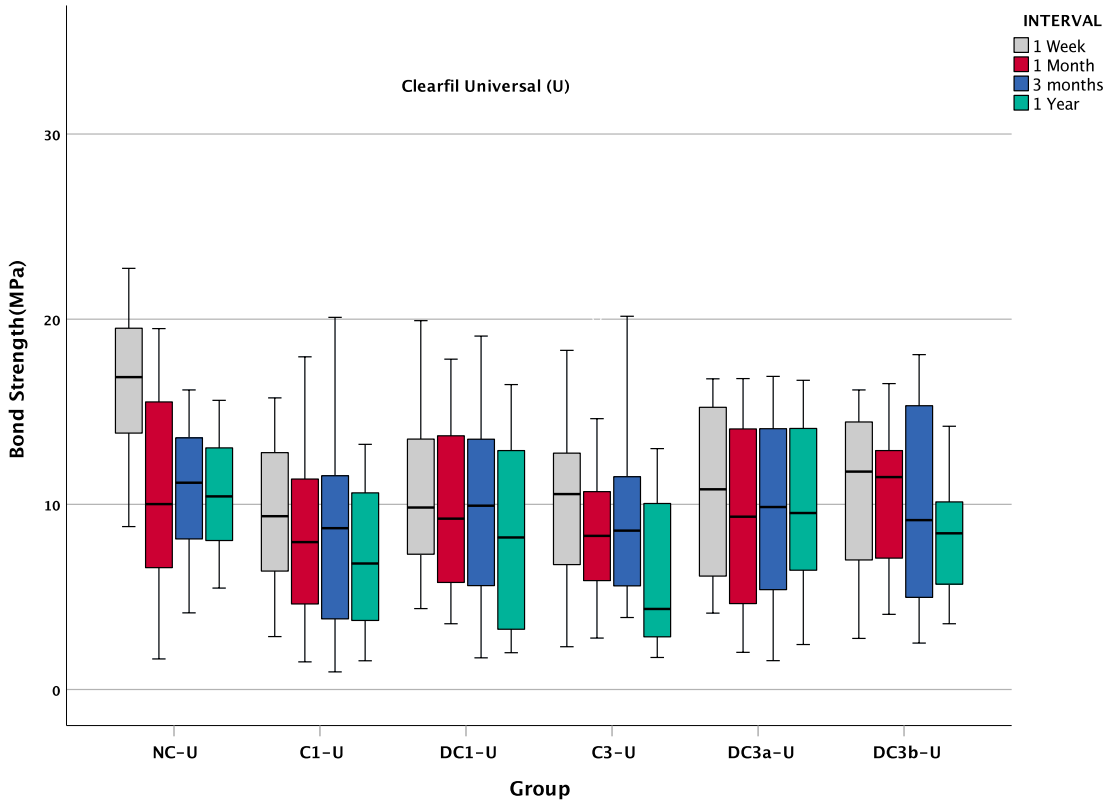
All the control groups in U adhesive had significant reduction in SBS compared to the control group of 1W storage (NC-U) ($p<0.0001$) (Fig.3.6). Whereas, there was no significant influence of aging on the SBS in dentin on the control groups of SE adhesive (NC-SE) ($p=0.517$) (Fig.3.5). At the 1W storage period, it can be observed that the salivary contamination significantly reduced the SBS (C1-U, C3-U) in U adhesives compared to the NC-U and the decontamination procedures (DC1-U, DC3a-U and DC3b-U) could not restore the SBS to control levels (NC-U) (Table.3.3) (Fig.3.6). Nonetheless, in the 1M and 3M intervals, the influence of contamination although lower was not significantly different as compared to the SBS values of control (NC-U) group. The lowest mean SBS was recorded for the group C3-U (6.27 ± 4.06 MPa) at 1Y interval which was significantly lower SBS compared to NC-U (10.51 ± 3.11 MPa) at 1Y (Table.3.3) (Fig.3.6)

Table 3.3: Shear bond strength (SBS) of Universal adhesive (U)

Stage of Influence	Groups	Shear bond strength (U) MPa (SD)				
		1 week	1 month	3 months	1 year	p-value
No influence	**NC-U**	16.56 (3.92)^{a}	10.56 (5.63)^{B}	10.69 (3.52)^{C}	10.51 (3.11)^{D}	0.000
Stage 1	**C1-U**	9.64 (3.84)^{A}	8.24 (4.58)^{B}	8.33 (4.88)^{C}	7.24 (3.67)^{Dd}	0.372
	DC1-U	10.40 (4.31)^{A}	9.72 (4.41)^{B}	9.84 (4.73)^{C}	9.53 (4.99)^{Dd}	0.470
Stage 3	**C3-U**	10.16 (4.04)^{A}	8.77 (4.28)^{B}	9.16 (4.29)^{C}	6.27 (4.06)^{d}	0.030
	DC3a-U	10.68 (4.40)^{A}	9.65 (4.86)^{B}	9.77 (5.04)^{C}	8.30 (3.08)^{Dd}	0.816
	DC3b-U	10.55 (4.51)^{A}	10.20 (3.97)^{B}	9.67 (5.38)^{C}	9.68 (4.32)^{Dd}	0.375

Same superscripted letters show mean values with no statistically significant differences within the respective interval. ($p<0.001$; $\alpha = 0.05$)

Figure 3.6: SBS of all groups in U adhesive over time

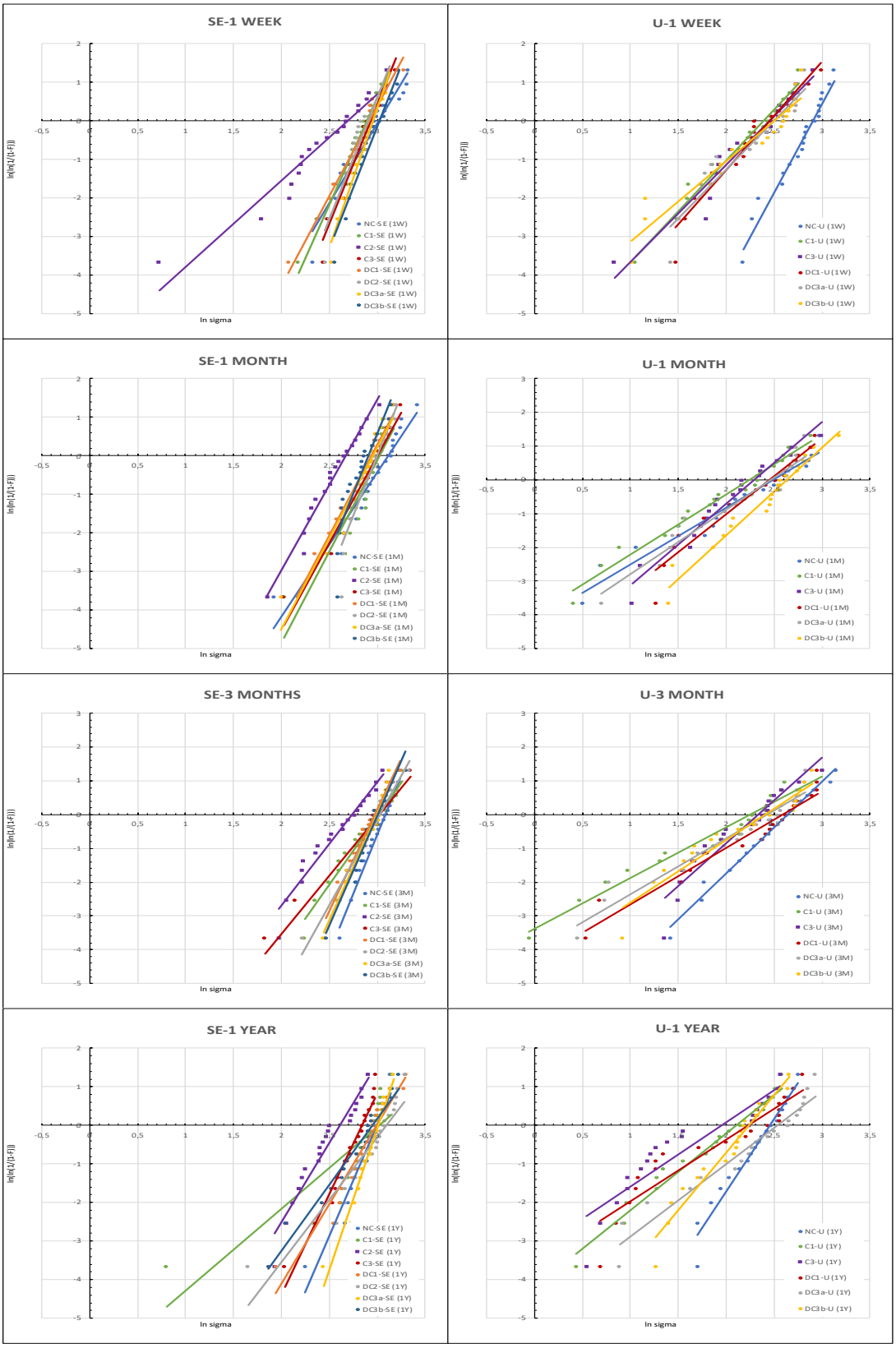


SE group showed a statistically significant reduction in the SBS only when the contamination occurred after the application of primer (C2-SE) in all the intervals of aging. (1W,1M,3M and 1Y) (Table.3.2) (Fig.3.5) Though, decontaminating the surface by rinsing, drying and replying the primer and adhesive considerably improved the SBS and was similar to the control group levels at all the intervals of aging (DC2-SE) (Table.3.2) (Fig.3.5). The general linear model with partial eta squared statistics revealed that there was significant influence seen by the stage of influence ($\eta_p^2 = 0.600, p < 0.0001$), experimental groups ($\eta_p^2 = 0.518, p < 0.0001$), type of adhesive ($\eta_p^2 = 0.328, p < 0.0001$), aging ($\eta_p^2 = 0.130, p = 0.003$) and the treatment procedure ($\eta_p^2 = 0.075, p < 0.0001$). The part of the tooth (occlusal or cervical) exhibited no significant influence (p =0.527) on the SBS when the control groups (NC) for both the adhesives across the aging process was observed (Fig. 3.10 The Weibull analysis data of SE adhesive and U adhesive (Table 3.4) (Fig.3.7), illustrates the Weibull modulus (m) at 95% confidence level and characteristic strength (σ_0) of each experimental group over time. The m values of U adhesive were lower than SE in all the intervals irrespective of stages of contamination. The m values in SE adhesive varied from 2.12 $\pm$ 0.3 to 7.39 $\pm$ 0.09 and in U adhesive, they ranged from 1.50 $\pm$ 0.11 to 4.60 $\pm$ 0.10.

Table 3.4: Weibull parameters of both SE and U adhesives over time

Weibull Parameters									
Group		1 Week		1 Month		3 Months		1 Year	
		SE	U	SE	U	SE	U	SE	U
NC	m ± CI	4.12 ±0.13	4.60 ± 0.10	3.75 ±0.15	1.69 ± 0.11	7.07 ± 0.15	2.73 ± 0.07	5.81 ± 0.13	3.78 ± 0.11
	σ_0 (MPa)	12.44	13.32	11.69	4.21	21.81	7.21	17.39	9.29
C1	m ± CI	5.53 ±0.11	2.63 ± 0.07	4.75 ± 0.24	1.78 ± 0.08	4.02 ± 0.10	1.50 ± 0.11	2.12 ± 0.3	2.00 ± 0.09
	σ_0 (MPa)	15.98	6.23	14.36	3.98	12.12	3.39	6.38	4.20
DC1	m ± CI	4.66 ±0.10	2.81 ± 0.13	4.71 ± 0.09	2.27 ± 0.14	6.02 ± 0.10	1.68 ± 0.13	4.13 ± 0.14	1.60 ± 0.19
	σ_0 (MPa)	13.61	6.90	13.85	5.54	17.91	4.33	12.37	3.50
C2	m ± CI	2.24 ±0.14	-	4.42 ± 0.09	-	3.68 ± 0.13	-	4.16 ± 0.14	-
	σ_0 (MPa)	6.01	-	11.8	-	10.07	-	10.86	-
DC2	m ± CI	6.28 ±0.15	-	6.31 ± 0.18	-	5.12 ± 0.10	-	3.24 ± 0.20	-
	σ_0 (MPa)	18.26	-	18.92	-	15.48	-	10.03	-
C3	m ± CI	6.27 ±0.10	2.53 ± 0.13	4.50 ± 0.12	2.42 ± 0.11	3.47 ± 0.16	2.53 ± 0.17	5.19 ± 0.10	1.67 ± 0.21
	σ_0 (MPa)	18.36	6.18	13.50	5.55	10.47	5.90	14.75	3.27
DC3a	m ± CI	7.22 ±0.08	2.55 ± 0.16	4.73 ± 0.17	1.92 ± 0.15	6.28 ± 0.10	1.66 ± 0.13	7.39 ± 0.09	1.88 ± 0.13
	σ_0 (MPa)	21.35	6.30	13.97	4.70	18.81	4.02	22.20	4.76
DC3b	m ± CI	6.38 ± 0.11	2.10 ± 0.16	6.20 ± 0.22	2.60 ± 0.11	6.52 ± 0.11	1.86 ± 0.15	3.46 ± 0.10	3.01 ± 0.13
	σ_0 (MPa)	19.26	5.24	18.07	6.81	19.62	4.45	10.19	6.71

Figure 3.7: Weibull plot of all groups of SE and U adhesive over time



The fracture pattern analysis indicated a low ratio of cohesive failures (0.9%) suggesting a relatively decent set of SBS test results. U adhesive groups showed 89%of adhesive, 11% of mixed failures and no cohesive failure (Fig.3.9). Whereas, SE adhesive showed 52.6% of adhesive, 45.8% of mixed and 1.6% of cohesive failures (Fig.3.8). Higher SBS values were associated with higher ratio of mixed and cohesive failures. Mean SBS of adhesive failures (12.35 $\pm$ 5.70 MPa) were significantly lower compared to cohesive (18.72 $\pm$ 4.50 MPa) and mixed failures (18.04 $\pm$ 4.30 MPa). There were no pre-test failures observed.

Figure 3.8: Fracture pattern observed in SE adhesive over time

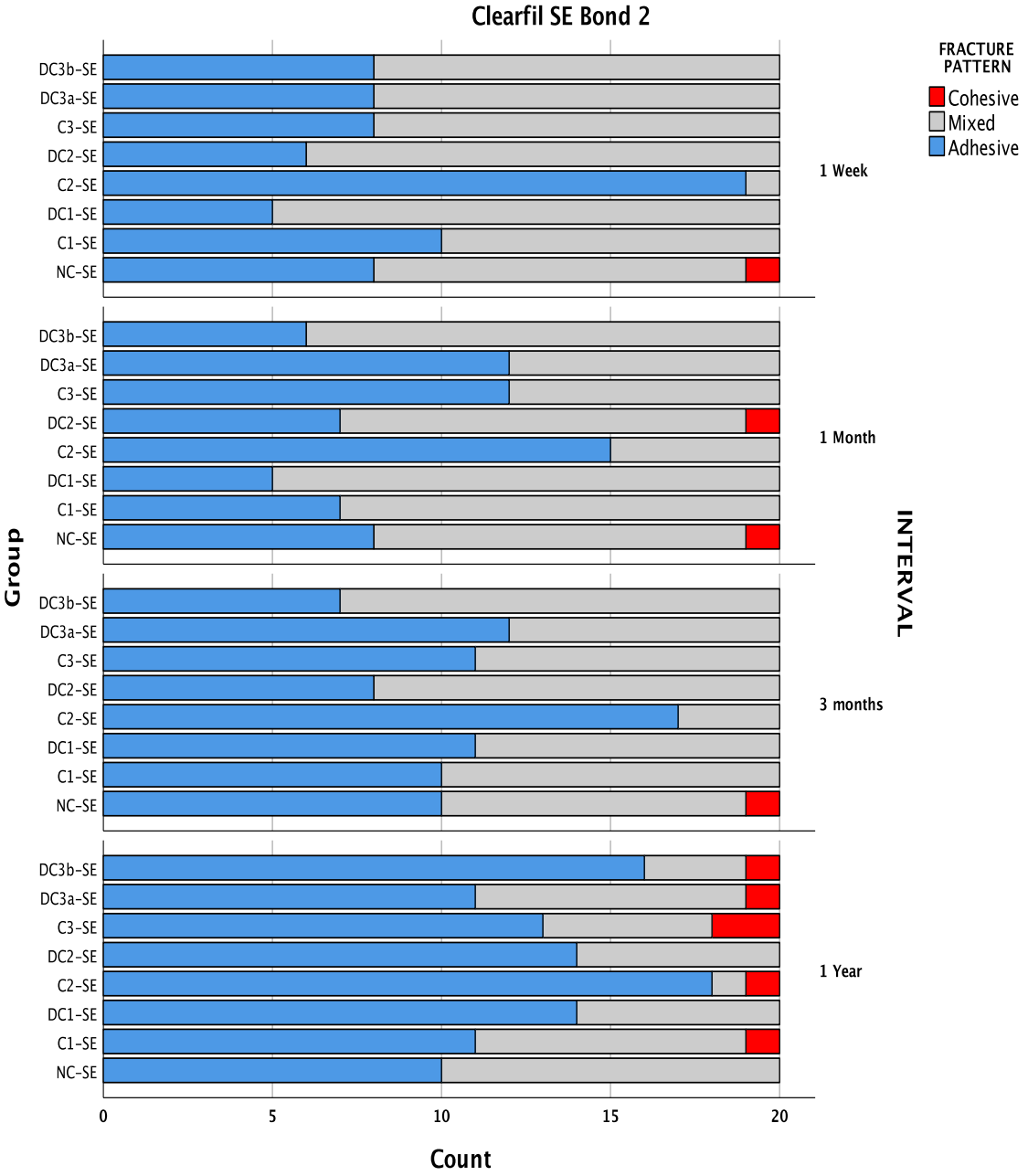


Figure 3.9: Fracture pattern observed in U adhesive over time

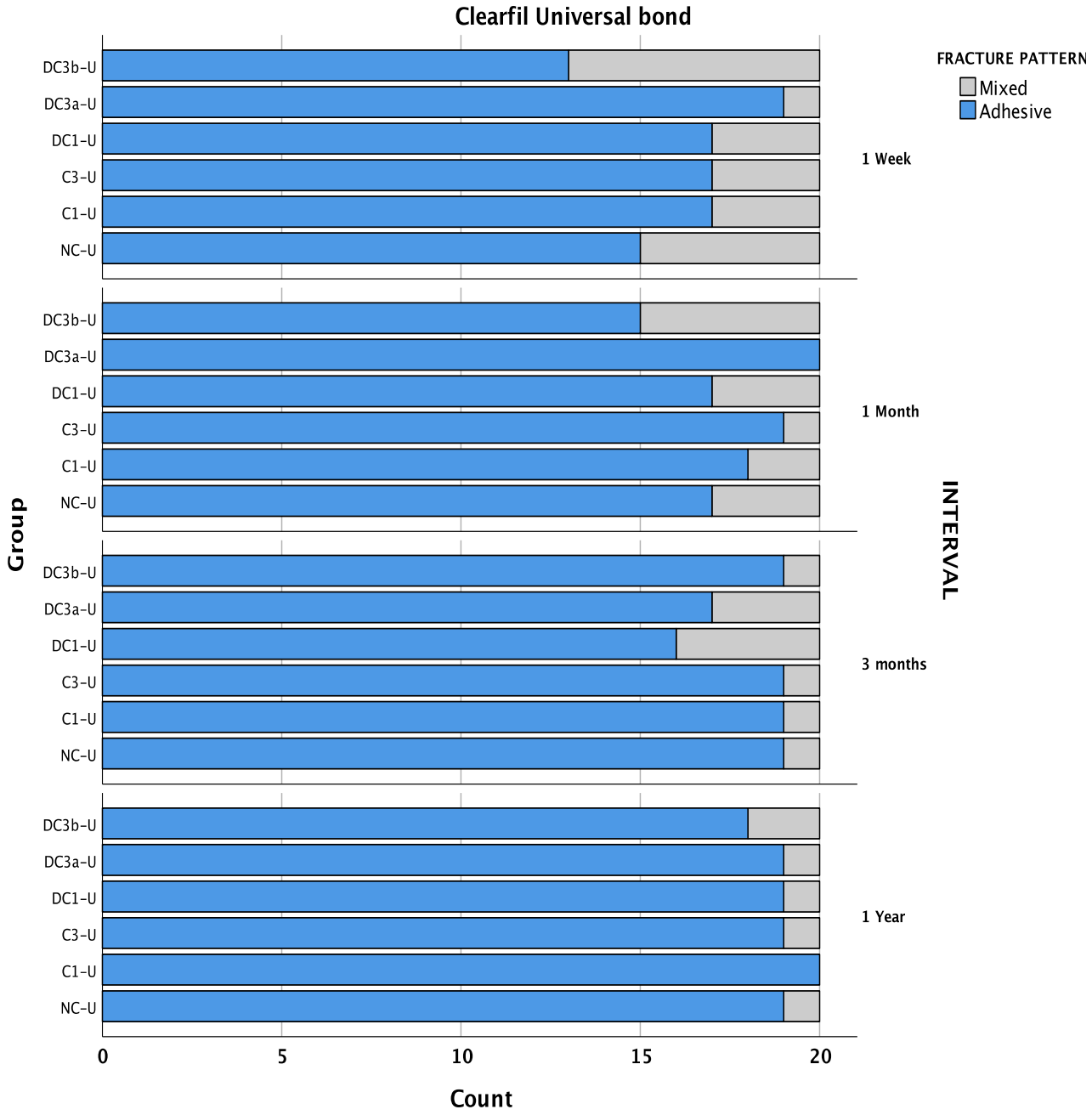
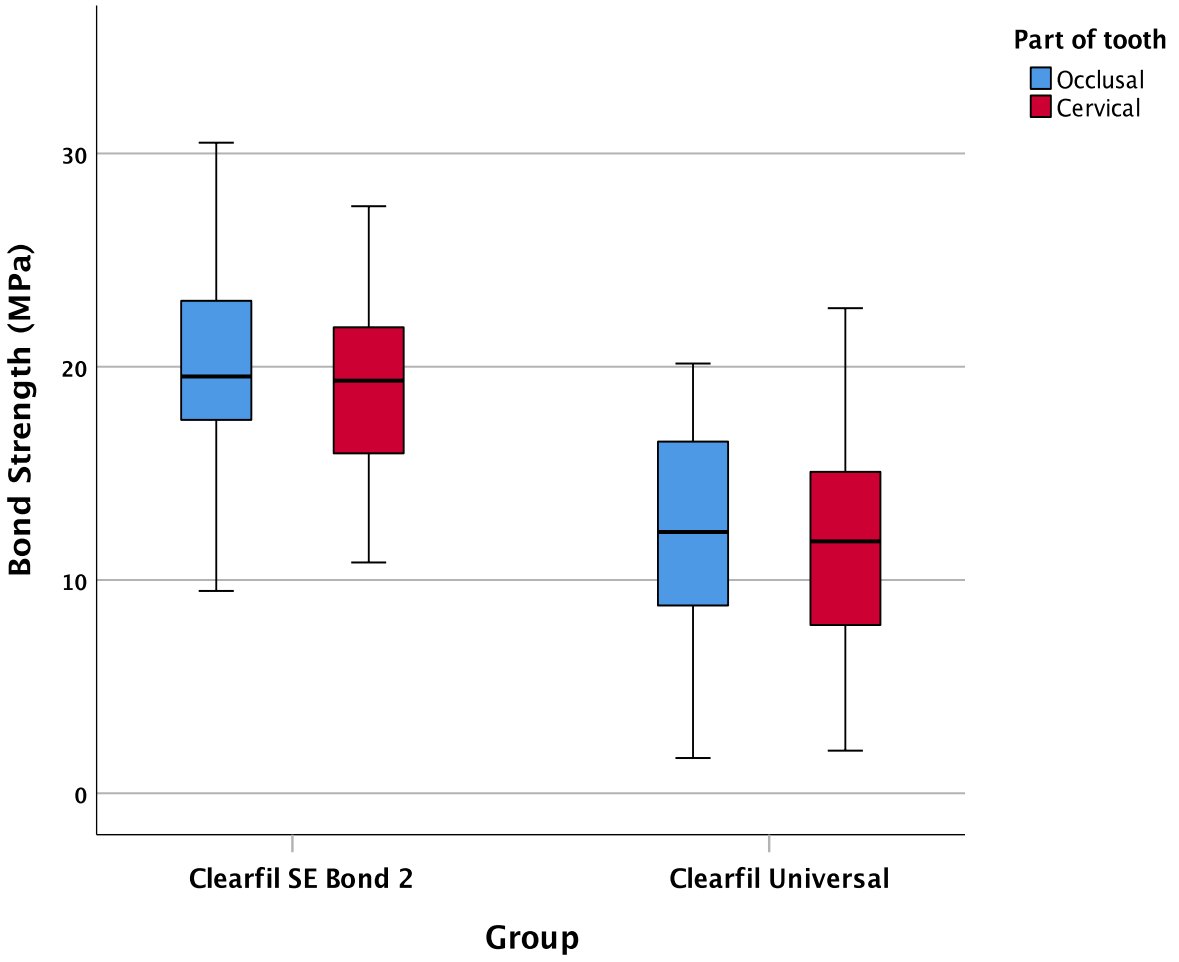


Figure 3.10: The shear bond strength measured in occlusal and cervical parts of the teeth in both adhesive



3.6 Discussion

The quintessential goal of obtaining a good adhesion in restorative dentistry is to produce an interface that is stable over time, guarantee adequate bond strength, good marginal seal, assure clinical durability and have minimal imperfections [36].The structural and morphological differences in dentin challenge the understanding of attaining a durable bond between adhesive resin and dentin [37]. It is acknowledged that moisture trapped within the adhesive during polymerization may cause an inferior polymerization of the adhesive monomers [38]. Through this study, our intention was to recognize unfavourable consequences of salivary contamination on the SBS of two contemporary adhesives over time in dentin. Also, if the effect of contamination is found to be substantial, which stage in the adhesive application is more vulnerable. Furthermore, does clinically feasible decontamination procedures regain their original bond quality.

When bonded to dentin, the U adhesive applied in self-etching mode differed significantly in their SBS compared to the SE adhesive over time. Thus, the null hypothesis that there is no difference in SBS of adhesives used for bonding to dentin has to be rejected. The parameter "type of adhesive" showed significant influence on the SBS ($\eta_p^2 = 0.328, p < 0.0001$). Hence, the type of adhesive used is proved to be crucial when observed over time. The complexity of the dental substrate and the different characteristics of enamel and dentin necessitates the availability of diverse dental adhesive systems to contain various components that prepare the surface and interact with the different components of the tooth surface efficiently [39] and therefore they react differently in the oral environment.

The composition of universal adhesives is complex as it contains both hydrophobic as well as hydrophilic monomer mixtures, due to which the presence of any residual moisture can cause phase separation and result in blister formation [40]. Adhesive penetration of the dentin is vital for the maintenance of durable bonds. The phase separation in BisGMA/HEMA adhesives can end up in lower bond quality as the adhesive tries to diffuse

into the moist dentin matrix, the constituents split into hydrophilic HEMA-rich and hydrophobic BisGMA-rich phases [40]. The low cross-linking potential of HEMA makes it unstable in aqueous environments which tends to degrade with exposure to oral fluids. Consequently, this phase becomes the weak point for the adhesive bonding and adversely affect their durability [5].

The effects of aging were evidently perceived on the U adhesive when irrespective of the treatment group all groups were considered for each interval, the combined mean SBS significantly reduced over time ($p<0.0001$). This result has also been established in another study where the U adhesive showed a deteriorated micro tensile bond strength after 1 year of aging [41]. Whereas, in SE adhesive there was no significant reduction in SBS ($p=0.085$) over time. So, the null hypothesis that aging does not have a significant influence on SBS is partially accepted.

The proposed null hypothesis that there will be no effect of salivary contamination for both the adhesives were rejected as there were significant differences in the SBS values exhibited by both the tested adhesives post contamination. In the SE adhesive, the contamination was critical post primer application(C2-SE), but the contamination at stage 1 and stage 3 (C1-SE and C3-SE) did not show any detrimental effect on the bond quality. Decreased SBS values significantly increased after decontamination (DC2-SE). It conveys that if a noticeable salivary contamination is spotted at the priming stage, just a simple water rinsing for 10 seconds, air-drying for 5 second and re-priming the area followed by the adhesive application will bring the bond quality to control levels and also maintain it long-term.

This finding is in accordance with previous researches deliberating the influence of saliva contamination on the bond quality of self-etching adhesives which revealed that contamination after primer application decreased the bond strength significantly[18,20,42,43]. The cause for this reduction is presumed to be due to the rinsing away of the hydrophilic monomer (HEMA) in the SE primer with the saliva along with the moisture from the dentin, that may have resulted in the collapsing of the collagen. The monomers in the

adhesive could have failed to efficiently infiltrate into the dentin due to collapsed collagen. In one of the study, the LV-SEM micrographs showed contaminant deposited on dental surfaces, when saliva was applied after primer application, creating a physical barrier to monomer diffusion and resulting in a deteriorated adhesion [43].

In the U adhesive, contamination (C1-U and C3-U) at all the intervals (1W, 1M, 3M AND 1Y) reduced the mean SBS significantly as compared to the bond strength of control (NC-U) in 1W. The decontamination procedures (DC1-U, DC3a-U and DC3b-U) did not bring back the SBS values to the control levels. The natural pH of saliva is between 6 to 7. The pH in salivary flow can range from 5.3 (low flow) to 7.8 (peak flow)[44]. In stage 1 contamination, the contaminated saliva on the prepared surface can act as a buffer and reduce the etching capacity of monomers in the U adhesive whose pH is more acidic (pH=2) compared to SE which result in reduced penetration into the dentinal tubules and resulting in a decreased bond quality over time. This observation conforms to the earlier studies [31, 33, 45-47] which established that the salivary contamination in universal adhesives could be detrimental. The adverse effect of presence of saliva in stage 3 may be partly justified due to the adsorption of glycoproteins on the polymerized adhesive surface, which consequently inhibits oxygen and reduces bonding capacity [33]. The most substantial impact on the SBS was exercised by the parameter "stage of contamination" ($\eta_p^2 = 0.600$) followed by the "experimental group" (η_p^2 =0.518) and then the "type of adhesive"(η_p^2 =0.328). The stage at which the contamination or decontamination occurred was the most critical in this study. It is evident from the C2-SE group, the contamination occurring at stage 2 (after primer application) was significantly damaging to the SBS in dentin. aging of the specimens had a significant, but relatively low influence (η_p^2 =0.130) on the SBS and treatment procedure (contamination or decontamination) of the specimens had the least influence (η_p^2 =0.075).

The scattered data cannot be exclusively attributed to the experimental error, it is likewise suggestive of the intrinsic material property. The Weibull analysis enables evaluation of data scattering by relating the probability of failure to applied stress. Defining the SBS

data only with mean and standard deviation does not convey the information about the distribution of stresses at which the individual specimens failed, as these stresses could be formed due to the distribution of the flaws, like the inconsistencies or interferences in the adhesive layer, air bubbles, size and amount of filler particles, areas of inadequate conversion and separated phases within the material [48].

In the Weibull analysis (Table.3.4), the lower values of m are indicative of an unreliable underlying defect in the group, supposing that the specimen was examined accurately and it fractured in a brittle manner. In contrast, a higher Weibull modulus is suggestive of narrow distribution and resonates too closely placed stress values at which the specimens failed indicative of a consistent flaw. It can be seen from the Weibull plot (Fig.3.7) that the slopes (Weibull moduli) indicate the strength distribution at a given interval (1W, 1M, 3M and 1Y) for both the adhesives. The slopes in SE suggest imply that the flaw in post contamination groups C2 were more inconsistent, hence a lower m value compared to the control group (NC). It is very evident from the data that the U adhesives were less reliable compared to SE adhesives, based on their overall Weibull modulus. The deviances within the slopes in Weibull plot are not unpredicted and they are frequently witnessed in small size sample sets. When comparing the Weibull parameters of the control groups at 1W, it can be observed that the U adhesive (m=4.60 $/pm$ 0.10, σ_0 = 13.32 MPa) showed higher m and characteristic strength than the SE (m= 4.12 $/pm$ 0.13, σ_0 = 12.44 MPa). Nevertheless, the reliability of the U adhesive reduced over time. In our study, since we have introduced the flaw of salivary contamination, it is evident that this variability makes the result inconsistent among the various contamination and decontamination groups.

Bond strength values have been previously reported to be sensitive to the depth of the dentin used as a substrate, as it is influenced by the diameter of the dentinal tubules and the water content [37]. While preparing the substrate, the tooth was cut mid coronally to obtain two portions, an “occlusal” and a “cervical” segment. These parts differed in depth of the dentin roughly by the thickness of the diamond saw used to cut the

tooth (0.270 mm). When the bond strengths obtained in the control groups (NC-SE and NC-U) of both these parts were analyzed, there was no statistically significant difference (p= 0.527) between the occlusal or cervical parts even after one year of aging in both the adhesives (Fig. 3.10). This implies that the incongruity due to the difference in the dentin substrate was negligible. The substrate was prepared in such a manner in order to maximize the potential of the available dental substrate as the study required a sizeable number of specimens.

In general, the failure mode distribution correlated quite well with the bond strengths of SE and U adhesives. The predominant failure mode was an adhesive failure, irrespective of saliva contamination and aging (SE-52.6%; U-88.9%) (Fig.3.8 and Fig.3.9). This primarily indicates a good set of data for SBS, as the critique of the methodology is often the higher percentage of cohesive failures, because of non-uniform stress distribution. When the break occurs cohesively in the composite resin or dentin, the value attained conveys the cohesive strength. However, in the assessment of adhesive systems on substrates, the intention is to analyze the bond of the adhesive with dentin, and not cohesive strength of other regions such as the dentin or composite resin [49]. However, unlike micro-tensile bond strength testing, SBS is conventional and does not require vast stress inducing procedure during specimen preparation, which often results in pre-test failures [49]. In this study, no pre-test failures were recorded.

It is not surprising to see that the SE adhesive was more resilient to hydrolytic degradation over time as they offer a distinct hydrophobic resin layer with their final step of application unlike the U adhesive. Although, bond strength happens to be an essential assessment, the lifespan of a bonding is the most important indicator of clinical success. However, our findings in this study must not be generalized and should not be applied to the whole class of universal adhesives because each material features different composition and unique modifications to achieve their functional capability.

3.7 Conclusion

Within the limitations of the study, the results indicate that when the universal adhesives were used in the self-etching strategy on dentin, the bond strength deteriorated over time. Regardless of contamination or decontamination, the universal adhesive couldn't regain the immediate bond strength of control group after aging. In self-etching adhesive, the saliva contamination was most critical when the contamination occurred after primer application. Decontaminating by rinsing, air-drying and re-applying the primer regained the bond strength to control levels and maintained it over time.

3.8 References

1. Baier, R.E., Principles of adhesion. Oper Dent, 1992. Suppl 5: p. 1-9.

2. Marshall, S.J., et al., A review of adhesion science. dental materials, 2010. 26(2): p. e11-e16.

3. Ferracane, J.L., Hygroscopic and hydrolytic effects in dental polymer networks. Dental Materials, 2006. 22(3): p. 211-222.

4. Tay, F.R., et al., Single-bottle adhesives behave as permeable membranes after polymerization. I. In vivo evidence. Journal of Dentistry, 2004. 32(8): p. 611-621.

5. Van Landuyt, K.L., et al., The role of HEMA in one-step self-etch adhesives. Dent Mater, 2008. 24(10): p. 1412-9.

6. Cajazeira, M.R., T.M. De Saboia, and L.C. Maia, Influence of the operatory field isolation technique on tooth-colored direct dental restorations. Am J Dent, 2014. 27(3): p. 155-9.

7. Eiriksson, S.O., et al., Effects of saliva contamination on resin-resin bond strength. Dent Mater, 2004. 20(1): p. 37-44.

8. Buonocore, M.G., Caries prevention in pits and fissures sealed with an adhesive resin polymerized by ultraviolet light: a two-year study of a single adhesive application. J Am Dent Assoc, 1971. 82(5): p. 1090-3.

9. Duarte, S.J., et al., SEM analysis of internal adaptation of adhesive restorations after contamination with saliva. J Adhes Dent, 2005. 7(1): p. 51-6.

10. Nair, P., R. Hickel, and N. Ilie, Adverse effects of salivary contamination for adhesives in restorative dentistry. A literature review. Am J Dent, 2017. 30(3): p. 156-164.

11. Abdalla, A.I. and C.L. Davidson, Bonding efficiency and interfacial morphology of one-bottle adhesives to contaminated dentin surfaces. Am J Dent, 1998. 11(6): p. 281-5.

12. el-Kalla, I.H. and F. Garcia-Godoy, Saliva contamination and bond strength of single-bottle adhesives to enamel and dentin. Am J Dent, 1997. 10(2): p. 83-7.

13. Yazici, A.R., et al., The effect of saliva contamination on microleakage of an etch-and-rinse and a self-etching adhesive. J Adhes Dent, 2007. 9(3): p. 305-9.

14. Pashley, D.H., et al., State of the art etch-and-rinse adhesives. Dental Materials, 2011. 27(1): p. 1-16.

15. Van Meerbeek, B., et al., State of the art of self-etch adhesives. Dental materials, 2011. 27(1): p. 17-28.

16. Aboushelib, M.N., Clinical performance of self-etching adhesives with saliva contamination. J Adhes Dent, 2011. 13(5): p. 489-93.

17. Ari, H., N. Donmez, and S. Belli, Effect of artificial saliva contamination on bond strength to pulp chamber dentin. Eur J Dent, 2008. 2(2): p. 86-90.

18. Cobanoglu, N., et al., Bond strength of self-etch adhesives after saliva contamination at different application steps. Oper Dent, 2013. 38(5): p. 505-11.

19. Hegde, M.N., P. Hegde, and S.K. Shetty, The influence of salivary contamination on the shear bond strength of two newer generation dentin bonding agents - An in vitro study. J Conserv Dent, 2008. 11(3): p. 127-30.

20. Hiraishi, N., et al., Effect of artificial saliva contamination on pH value change and dentin bond strength. Dent Mater, 2003. 19(5): p. 429-34.

21. Munaga, S., et al., Effect of saliva contamination on the shear bond strength of a new self-etch adhesive system to dentin. J Conserv Dent, 2014. 17(1): p. 31-4.

22. Neelagiri, K., et al., Effects of saliva contamination and decontamination procedures on shear bond strength of self-etch dentine bonding systems: An in vitro study. J Conserv Dent, 2010. 13(2): p. 71-5.

23. Fakhri, M., et al., Effect of salivary contamination on microleakage of resin composites placed with a self-etch adhesive in primary teeth: an in vitro study. Pediatr Dent, 2009. 31(4): p. 334-9.

24. Yalçin, M., et al., Effect of salivary contamination on micro-tensile bond strength of self-etch adhesives systems after bonding procedure. Journal of Restorative Dentistry, 2013. 1(2): p. 55.

25. Amsler, F., et al., Long-Term Bond Strength of Self-Etch Adhesives to Normal and Artificially Eroded Dentin: Effect of Relative Humidity and Saliva Contamination. J Adhes Dent, 2017.

26. Darabi, F., M. Tavangar, and R. Davalloo, Effect of different decontamination procedures from a saliva-contaminated cured bonding system (Single Bond). Dent Res J (Isfahan), 2012. 9(4): p. 399-403.

27. Elkassas, D. and A. Arafa, Assessment of post-contamination treatments affecting different bonding stages to dentin. Eur J Dent, 2016. 10(3): p. 327-32.

28. Jiang, Q., et al., Effect of saliva contamination and decontamination on bovine enamel bond strength of four self-etching adhesives. Oper Dent, 2010. 35(2): p. 194-202.

29. Pinzon, L.M., et al., Effect of mucoprotein on the bond strength of resin composite to human dentin. Odontology, 2011. 99(2): p. 119-28.

30. Powers, J.M., W.J. Finger, and J. Xie, Bonding of composite resin to contaminated human enamel and dentin. J Prosthodont, 1995. 4(1): p. 28-32.

31. Santschi, K., et al., Effect of salivary contamination and decontamination on bond strength of two one-step self-etching adhesives to dentin of primary and permanent teeth. J Adhes Dent, 2015. 17(1): p. 51-7.

32. Suresh, B. and R. Pushpa, Effect Of Saliva Contamination And Different Decontamination Modes On Dentin Bond Strength During Bonding With Single Bottle Adhesive. Annals and Essences of Dentistry. , 2010. 2(3): p. 11-16.

33. Yoo, H.M., T.S. Oh, and P.N. Pereira, Effect of saliva contamination on the microshear bond strength of one-step self-etching adhesive systems to dentin. Oper Dent, 2006. 31(1): p. 127-34.

34. Weibull W (1951) A Statistical Distribution Function of Wide Applicability. ASME Journal of Applied Mechanics (June):293-297

35. Quinn JB, Quinn GD (2010) A practical and systematic review of Weibull statistics for reporting strengths of dental materials. Dental materials : official publication of the Academy of Dental Materials 26 (2):135-147. doi:10.1016/j.dental.2009.09.006

36. Tjaderhane, L., Dentin bonding: can we make it last? Oper Dent, 2015. 40(1): p. 4-18.

37. Perdigão, J., Dentin bonding—Variables related to the clinical situation and the substrate treatment. Dental Materials, 2010. 26(2): p. e24-e37.

38. Cadenaro, M., et al., The role of polymerization in adhesive dentistry. Dental Materials, 2019. 35(1): p. e1-e22.

39. Van Landuyt, K.L., et al., Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials, 2007. 28(26): p. 3757-85.

40. Spencer, P. and Y. Wang, Adhesive phase separation at the dentin interface under wet bonding conditions. J Biomed Mater Res, 2002. 62(3): p. 447-56.

41. Zhang, Z.-y., et al., Defying aging: An expectation for dentine bonding with universal adhesives? Journal of Dentistry, 2016. 45: p. 43-52.

42. Park, J.W. and K.C. Lee, The influence of salivary contamination on shear bond strength of dentin adhesive systems. Oper Dent, 2004. 29(4): p. 437-42.

43. Vieira, S.N., et al., Longitudinal evaluation of the effect of saliva contamination during the bonding protocol with a self-etch adhesive system. Braz J Oral Sci, 2010. 9(2): p. 98-103.

44. de Almeida Pdel, V., et al., Saliva composition and functions: a comprehensive review. J Contemp Dent Pract, 2008. 9(3): p. 72-80.

45. Bhatia, T.K., et al., Influence of salivary contamination on the dentin bond strength of two different seventh generation adhesive systems: In vitro study. J Conserv Dent, 2015. 18(6): p. 467-70.

46. Kermanshah, H., S. Ghabraei, and T. Bitaraf, Effect of salivary contamination during different bonding stages on shear dentin bond strength of one-step self-etch and total etch adhesive. J Dent (Tehran), 2010. 7(3): p. 132-8.

47. Taskonak, B. and A. Sertgoz, Shear bond strengths of saliva contaminated 'one-bottle' adhesives. J Oral Rehabil, 2002. 29(6): p. 559-64.

48. Par, M., et al., Dentin Bond Strength of Experimental Composites Containing Bioactive Glass: Changes During Aging for up to 1 Year. J Adhes Dent, 2018. 20(4): p. 325-334.

49. Van Meerbeek, B., et al., Relationship between bond-strength tests and clinical outcomes. Dent Mater, 2010. 26(2): p. e100-21.

Summary and Conclusion

Summary of Thesis

The literature review on the influence of salivary contamination of contemporary adhesives was done through a broad assessment in PUBMED, Cochrane Library, Google Scholar and Web of Science to isolate publications from 1990-2017 (March) and a total of 6202 articles were obtained. After title inspection for relevance and abstract reading, 54 publications were acquired that were relevant to salivary contamination of dental adhesives in restorative dentistry. These articles were thoroughly evaluated in various parameters like the year of publishing, type of adhesive, type of contaminant, type of test, parameters of the test, results, surface preparation, method of contamination, quantity and details of contaminant, stages of contamination, decontamination procedure, time between contamination and testing, type of aging, size of bonding area, type of substrate and number of specimens. The review revealed that 64.6% of the articles showed an adverse effect on adhesives when there was salivary contamination occurring at one or many points in restoration process. However, methodological variations hindered the direct comparison of the selected studies. The study conveyed that, 2-step etch and rinse adhesives were relatively less vulnerable to salivary contamination than the others. Decontamination procedure of some kind delivered improved bonding performance in 65% of the studies. However, the specificities of the procedures are not standard in all the evaluations. It was concluded that long term studies are required to assess the aging behaviour of the contaminated adhesives as well as understand the decontamination procedure.

The main aim of this thesis was completed with a study that examined the consequences of salivary contamination on the long-term shear bond strength of adhesive in dentin. A total of 1120 human dentin substrates were prepared by cutting the tooth mid coronally and embedding them in cold-cure acrylic resin. They were then randomly allocated to 56 groups (14 groups x 4 intervals) (n=20). The two adhesives evaluated were a 2-step self-etching adhesive and Universal adhesive. The experimental procedures were executed after surface preparation, after primer application (for self-etching) and after adhesive system curing. The area to be bonded was delimited using an adhesive sheet. One group with no contamination served as a control in both the adhesives. The saliva was freshly collected by a single donor just preceding the experimental procedure. The contamination process was done by using a drop (0.025ml) of saliva at every step of the restorative process. The decontamination procedure was done either by rinsing with water and air drying the surface or by rinsing with water, air drying and re-applying the adhesive/primer. A custom made polysiloxane mould was used to restore an ormocer based bulk-fill RC. The prepared samples were stored at 37°C in distilled water for four intervals; one week, one month, three months and one year. They were subjected to shear bond strength (SBS) test at a crosshead speed of 0.5 mm/min, and the force at the fracture was recorded. The diameter of the fractured fragments was measured, and the fracture pattern was examined using 10x magnification.

The data obtained were statistically analyzed with one-way ANOVA with Tukey's HSD test ($\alpha = 0.05$). A significant reduction in SBS in all the groups in universal adhesive compared to the control group (no contamination) at one week ($p < 0.0001$) was observed. In self-etching adhesive, when the contamination took place after primer application, a significant reduction in bond strength was seen ($p < 0.0001$). However, decontamination procedures improved the SBS in self-etching adhesive but not in universal adhesive. The Weibull analysis showed the reliability of universal adhesive reduced over time compared to the self-etching adhesive. The univariate analysis established significant influences ($p < 0.0001$) seen by the stage of influence ($\eta_p^2 = 0.600$), experimental

groups ($\eta_p^2 = 0.518$), type of adhesive ($\eta_p^2 = 0.328$), ageing ($\eta_p^2 = 0.130$) and treatment procedure ($\eta_p^2 = 0.075$).

The results indicate that when the universal adhesives were used in the self-etching strategy on dentin, the SBS depreciated over time. Regardless of contamination or decontamination, the universal adhesive could not regain the immediate bond strength of control group after ageing. In self-etching adhesive, the saliva contamination was most critical when the contamination occurred after primer application. Decontaminating by rinsing, air-drying and re-applying the primer regained the bond strength to control levels and maintained it over time.

A small preliminary study was done to evaluate the testing methodology as well as to understand if there are any variances in SBS of the high viscosity and low viscosity bulk-fill RC when compared to conventional RC. The focus was directed to find whether the differing viscosities and filler concentration affects the adhesion and SBS of the RCs. Four high viscosity bulk-fill restorative materials, five low viscosity BF-RC were dispensed in one 4 mm increment and polymerized for 20 seconds. One conventional resin composite was dispensed in two 2 mm increments. The SBS was measured at a crosshead speed of 0.5 mm/min after storing for seven days in distilled water at 37°C. The data were statistically analyzed using one-way ANOVA with Tukey HSD post-hoc ($\alpha = 0.05$) and Weibull statistical analysis. It was found that the bulk-fill RC functions comparable to the conventional RC. Also, the type of the restorative material, filler content or their viscosity had no significant effect on the SBS. Under proper polymerization conditions, a 4-mm increment placed with high viscosity or low viscosity materials in bulk would present comparable outcomes in terms of their bond strength.

Conclusion

Within the limitations of the studies performed for this doctoral thesis, the following conclusions were made:

a. Saliva contamination was detrimental for universal adhesive at all the stages of application.

b. In universal adhesive, decontamination of saliva by rinsing and air-drying or rinsing, air-drying and re-applying adhesive did not regain the SBS in dentin.

c. In self-etching adhesive, most vulnerable step for salivary contamination is after primer application.

d. Decontaminating the saliva by rinsing, air drying and re-applying the primer redeemed the SBS of self-etching adhesive and maintained it throughout the one year.

e. Salivary contamination was not detrimental for self-etching adhesive before and after adhesive application.

f. The SBS of universal adhesive used in self-etching strategy in dentin deteriorated over one year

g. The SBS of self-etching adhesive in dentin showed no significant difference after one year

h. Based on the literature review, 2-step etch and rinse adhesive performance was comparatively less susceptible to salivary contamination than the other type of adhesives.

i. The type of RC does not have a significant influence on the SBS in dentin.

j. The bulk-fill RCs perform similar to the conventional RC applied in an incremental technique.

k. The viscosity and filler content of bulk-fill RCs does not influence the SBS in dentin.

Acknowledgement

At the end of my thesis, it is a pleasure to thank those who made this thesis possible by their support and help. It had been an intensive period of learning for me on both the scientific and personal level. I would like to express my sincere gratitude and thanks to my supervisor Prof. Dr. Nicoleta Ilie for being an exceptional mentor. It has been truly an honor and a privilege working with her over the last five years. I am grateful to her for understanding me, encouraging me and allowing me to grow as a responsible researcher. I would like to thank her for her patience, motivation, and her immense knowledge and for having taught me innumerable lessons in research and life.

There are no proper words to convey my deep appreciation and respect for Prof. Dr. Jan Kühnisch for his guidance and support. I would like to thank him for being there when I had tough times and problems during my PhD journey and providing me with constructive criticism, which helped me develop a wider perspective to my thesis. I have been thoroughly benefitted by his productive discussions. I am particularly thankful for his support and advise when everything seemed impossible. Without his valuable help, it would be stressful to complete this PhD.

I am also hugely indebted to Prof. Dr. Reinhard Hickel, for inspiring me, giving me guidance and for having faith and confidence in me. Thank you for always willing to discuss things with me even if you didn't have enough time.

I also thank Prof. Dr. Bogna Stawarczyk for being supportive and understanding throughout this PhD.

I cannot miss to thank my colleagues who were there with me through the difficult times. Thank you all for rooting for me and helping me transform to a stronger and confident

individual. Special thanks also to my friend Dr. Ana Elisa Kauling who created such a good atmosphere in the University and for making time to have endless discussions over coffee.

I would like to express my gratitude to my parents Dr. N. Suresh Kumar and Dr. Jalaja Suresh Kumar for inspiring me to choose this path and encouragement they have provided me through my entire life and in particular, during my PhD. I would also like to convey my gratitude to my parents in law K.V.Sasidharan and M.Mini for being there for me whenever I need their help and support, especially during the final stages of my thesis. A very special thanks goes to my brother Sunjay Nair for his incredible support and constant motivation. Thanks for keeping me laughing while things went haywire and for celebrating each accomplishment with me.

The dream of doing a PhD would have just stayed a dream if not for my husband, Shyam Narayanan. I am forever thankful to him for providing me with unfailing support, trusting in my potentials and continuous encouragement throughout my years of study and through the process of writing this thesis. Last, but by no means least, I am grateful to my Son Neil, without whose unconditional love and understanding, the final stages of my thesis would have been impossible.

Finally, a special mention for all my friends who were constantly motivating me. Thank you for your thoughts, prayers, phone calls, emails, texts, visits and being there whenever I needed a friend. Many thanks to all who supported me during my stay in Germany. This accomplishment would not have been possible without all of them.