Submitted by Cori Mae McClatchey in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology.

Accepted on behalf of the Faculty of the Graduate School by the thesis committee:

05 June 15

LTC Stacy Larsen, DDS — Date

AEGD Program Director

Jason B

5 June 15

CPT Jason Bullock, DMD, MS — Date

AEGD Assistant Program Director

05 JUN 15

LTC Manuel Pelaez, DMD, MS — Date

Research Director

The Author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

Effect of Setting Time on the Shear Bond Strength Between Biodentine and Composite

Is appropriately acknowledged and, beyond brief excerpts, is with the permission of the copyright owner.

CPT Cori M. McClatchey
2-Year AEGD Program, Ft. Bragg
Uniformed Services University
Date: 04/01/2015

Effect of Setting Time on the Shear Bond Strength Between Biodentine and Composite

CORI MAE MCCLATCHEY

B.S. Oregon State University, Corvallis, Oregon 2008

D.M.D. University of Nevada, Las Vegas, Nevada 2012

CPT United States Army

Submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Oral Biology in the Graduate School of the Uniformed Services University of the Health Sciences

Fort Bragg, North Carolina

2015

Submitted by Cori Mae McClatchey in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology.

Accepted on behalf of the Faculty of the Graduate School by the thesis committee:

LTC Stacy Larsen, DDS Date
AEGD Program Director

CPT Jason Bullock, DMD, MS Date
AEGD Assistant Program Director

LTC Manuel Pelaez, DMD, MS Date
Research Director

The Author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

Effect of Setting Time on the Shear Bond Strength Between Biodentine and Composite

Is appropriately acknowledged and beyond brief excerpts, is with the permission of the copyright owner.

Cori Mae McClatchey
2-Year AEGD Program, Ft. Bragg
Uniformed Services University
Date: 04/01/2015

Abstract

Objective: This in-vitro study investigated the shear bond strength (SBS) between the calcium silicate based material, Biodentine, and composite resin restorative material after Biodentine was allowed to set either 15 minutes, 1 hour, 24 hours, or 2 weeks.

Materials and Methods: Sample cylinders (n=134) and Biodentine capsules were randomly assigned to groups based on the setting time allowed for Biodentine (Group 1 = 15 minutes, Group 2 = 1 hour, Group 3 = 24 hours, Group 4 = 2 weeks). Biodentine was prepared and placed in the wells of the acrylic cylinders and stored at 37^{o}C with 100% humidity. After the designated setting time, Biodentine samples were etched, rinsed, and dried, followed by application of bonding agent (Scotchbond Universal Adhesive) and composite resin restorative material (Filtek Supreme Ultra). After bonding of the composite resin material to Biodentine, an Instron 5943 universal testing machine was used to evaluate the shear bond strength of samples from each group. Data was analyzed using one-way ANOVA and Tukey tests.

Results: Biodentine specimens bonded after 15 minutes exhibited the least SBS (mean 3.644MPa). Specimens allowed to set 1 hour, 24 hours, and 2 weeks before bonding showed significantly greater SBS. The greatest average SBS was attained from samples that were allowed set 24 hours before bonding (mean 14.294, p-value 0.00).

Conclusion: When bonding composite resin material to Biodentine, the practitioner may attain greater shear bond strength if Biodentine is allowed to set 24 hours before bonding, ruling out same-day definitive restoration placement.

Table of Contents

Thesis Committee Signatures i

Copyright Statement ii

Abstract iii

Table of Contents iv

List of Tables v

List of Figures v

List of Abbreviations vi

Introduction 1

Significance 2

Hypothesis 2

Background 3

Purpose 16

Materials and Methods 17

Results 21

Discussion 23

Conclusion 27

Appendix 28

References 29

List of Tables

Table 1. Biodentine Components and Properties……………………………….. 4

Table 2. Manufacturer Instructions Per Product………………………………… 18

Table 3. Summary of SBS by Test Group ………………………………………. 21

Table 4. One-Way ANOVA Amongst Groups …………………………………. 22

Table 5. Post-hoc Comparison Summary SBS Amongst Groups …………..….. 23

List of Figures

Figure 1. Ultradent Bonding Clamp and insert ………………………………… 19

Figure 2. Ultradent Test Base Clamp and Crosshead Assembly ……………….. 20

Figure 3. Shear Bond Strength by Setting Time ………………………………… 21

Figure 4. Mean Shear Bond Strength …………….………………………………… 22

Figure 5. Examples of Precipitate Found on Biodentine Samples of Group 4 ….. 25

List of Abbreviations

SBS	Shear bond strength
MPa	Mega Pascal
mm	Millimeter
N	Newton
mW	Milliwatt
cm	Centimeter
min	Minute
hr	Hour
SDev	Standard deviation
MTA	Mineral trioxide aggregate
WMTA	White mineral trioxide aggregate
GI	Glass ionomer
RMGI	Resin modified glass ionomer
ANOVA	Analysis of variance
HSD	Honest significant difference

Introduction

Biodentine is a relatively new calcium silicate-based material manufactured by Septodont, France. Similar to mineral trioxide aggregate (MTA), Biodentine has been shown to exhibit palliative effects on the pulp warranting investigation regarding its use for various dental procedures (Han *et al.* 2013). Septodont advertises Biodentine application as an endodontic restorative material, for direct and indirect pulp capping procedures, and for use as a dentin replacement alone or in conjunction with composite resin materials (Septodont, Saint Maur Des Fossés, France). Because multiple dental materials are often used in combination, it is important to know how the materials can be used together.

Regarding the use of Biodentine as a dental restorative material, it is important to examine its capacity for bonding to other dental materials. Composite resin materials have been advocated for concurrent or subsequent use with Biodentine (Septodont, Saint Maur Des Fossés, France). Because time management is a critical element in dental clinics, information is needed regarding the effect of Biodentine setting time on the resulting bond strength between Biodentine and the subsequent composite resin. The manufacturer of Biodentine claims that the necessary setting time is only 10-12 minutes (Atmeh *et al.* 2012). However, research is needed to determine the setting time required to achieve the strongest shear bond strength between Biodentine and composite resin restorations.

Significance

This study compared the shear bond strength achieved between Biodentine and composite resin after various setting times. This information is clinically significant because it could provide guidance regarding how much time the dental practitioner must wait before placing definitive composite resin restorations in approximation with Biodentine. Shear bond strength between Biodentine and composite was examined when Biodentine was allowed to set 15 minutes, 1 hour and 24 hours and 2 weeks. If shear bond strength was measured to be the same across all setting times, there would theoretically be no benefit to postponing bonding of the final restoration for another appointment. However, if greater bond strength can be achieved at 24 hours or 2 weeks setting time, it may be prudent for the dental professional to avoid placing the final restoration at the initial visit in order to attain a stronger bond to the composite. Because time is a commodity for the dental professional, this information could help the dentist determine the most time efficient and economical way to restore teeth with regard to the use of Biodentine.

Hypothesis

Because the manufacturer claims that Biodentine is fully set after 10-12 minutes, hypothetically, optimum shear bond strength should be achievable at that time (Septodont, Saint Maur Des Fossés, France). Alternatively, greatest shear bond strength may be achieved after a setting time longer than 10-12 minutes. The null hypothesis would imply that there is no difference in shear bond strength between Biodentine and composite at various Biodentine setting times.

Background

Indications for Use of Biodentine

French manufacturer Septodont released the material Biodentine in 2011 (Septodont, Saint Maur Des Fossés, France). According to the manufacturer, Biodentine has many potential uses. Biodentine has been advocated for use as an endodontic restorative material for procedures including apexification, root perforation repair, and for repair of internal or external root resorption. With regard to vital pulp therapy, it has been advocated as a direct and indirect pulp-capping medicament, as well as for pulpal coverage in pulpotomy procedures and apexogenesis. As a direct restorative material, Biodentine can be used as a temporary restoration or as a dentin replacement under definitive composite restorations (Bachoo *et al.* 2013). While it has many purported uses, further investigation is still needed to determine the long-term efficacy of Biodentine as a dental material.

Properties of Biodentine

Biodentine is distributed in single application units of the liquid and powder forms to be mixed immediately before placement (Koubi *et al.* 2013). According to the manufacturer, the powder is composed of tricalcium silicate, dicalcium silicate, calcium carbonate, iron oxide, and zirconium oxide while the liquid component is made up of water, calcium chloride, and a partially modified polycarboxylate to serve as a super-plasticizing and water reducing agent (Han *et al.* 2013, Raskin *et al.* 2012). First the liquid portion is added to the powder capsule, and then the capsule is triturated. Once mixed, Biodentine has a consistency that is dense and can be carried with a spatula and then gently condensed within the cavity preparation with amalgam condensers (Raskin *et al.* 2012).

Biodentine does not require dentin or enamel to be etched before application, nor does it require a bonding agent (Koubi *et al.* 2013). Biodentine is applied directly into the cavity preparation and, when used as a direct pulp-capping medicament, placed directly over the exposed pulpal tissues as well. For posterior teeth, Biodentine has been used for both class I (occlusal) or class II (interproximal) cavity preparations. As a layering material it is placed under composite restorations using a closed or open sandwich technique. A recent study reported that the occlusal surfaces of Biodentine restorations show degradation by abrasion that becomes significant after approximately six months (Koubi *et al.* 2013). Therefore, bulk filling of cavity preparations with Biodentine is only considered for temporary restorations.

Table 1. Biodentine Components and Properties

Component	Function
Tricalcium Silicate	Main component of the powder. Regulates setting reaction
Dicalcium Silicate	Additional core material
Calcium Carbonate and Oxide	Acts similar to filler
Iron Oxide	Contributes to shade/color
Zirconium Oxide	Radiopacifier
Calcium Chloride	Accelerator
Hydrosoluble Polymer	Reduces the viscosity to increase workability Water reducing agent

(Bachoo *et. al.* 2013, Septodont, Saint Maur Des Fossés, France)

Setting Reaction

The hydration setting reaction exhibited by Biodentine has been investigated and is similar to that of other tricalcium silicate-based materials including MTA. As a result of the hydration reaction, calcium hydroxide is formed as a bi-product (Camilleri *et al.* 2014). When applied to pulpal tissues (as in direct pulp capping), the formation of calcium hydroxide and the leaching of calcium ions are beneficial to the healing response of the dental pulp (Camilleri *et al.* 2014). A study by Grech used scanning electron microscopy to evaluate Biodentine and found that upon setting, Biodentine "displayed hydrating cement grains, surrounded by a matrix composed of calcium silicate hydrate and calcium hydroxide" (Grech *et al.* 2012). Although the initial setting time is only 12 minutes, the crystallization reaction may continue for approximately 2 weeks (Bachoo *et al.* 2013).

Interface Between Biodentine and Tooth Structure

Biodentine has an alkaline pH (pH=12) that is caustic and degrades the collagenous portion of the superficial area of dentin leaving the dentin porous (Atmeh *et al.* 2012, Septodont, Saint Maur Des Fossés, France). The altered porous surface of dentin facilitates the permeation of mineral ions into the dentin as well as the formation of tag-like structures with the phrase "mineral infiltration zone" (MIZ) used to describe the interface (Atmeh *et al.* 2012). The term MIZ is also used to describe the interaction of dentin with other calcium-silicate-based materials (including MTA). Through its interaction and ion exchange with enamel and dentin, Biodentine can obtain well-sealed interface with tooth structure. Laboratory studies have been conducted to analyze leakage at the dentin-Biodentine interface. Thus far, researchers have concluded that there is low penetrance of silver nitrate solution as well as low glucose diffusion apparent between the tooth structure and Biodentine restoration (Pradelle-Plasse et al. 2009, Koubi *et al.* 2012). Because leakage at the restoration-dentin interface can contribute to postoperative sensitivity as well as secondary caries and restoration failure, a well-sealed interface is critical for dental restorations of all types.

Comparative Material: Mineral Trioxide Aggregate (MTA)

Biodentine shares many similarities with white mineral trioxide aggregate (WMTA, MTA). Introduced in 1993, MTA was first available as a grey compound but then was adapted to be white in color (Atmeh *et al.* 2012, Atabek *et al.* 2012). MTA is considered the material of choice by many practitioners for multiple dental procedures including endodontic apexification, pulp-capping, and pulpotomies (Parirokh *et al.* 2010). Although MTA is well suited for some uses, its granular consistency, loose initial mixture, and poor handling characteristics make it unacceptable as a bulk restorative material (Ber *et al.* 2007). The American corporation Dentsply distributes MTA in a powder form to be mixed with sterile water immediately before use. MTA powder contains tricalcium silicate and dicalcium silicate (as does Biodentine), tricalcium aluminate, bismuth oxide, and calcium sulfate (Han *et al.* 2013).

Biodentine does have an overt advantage over the MTA, and that is in regard to setting time. MTA has a setting time of approximately 3 to 4 hours (Atmeh *et al.* 2012). In that time, the MTA also requires exposure to moisture in order for it to effectively set. Thus, when MTA is placed in a prepared tooth, a damp cotton pellet is usually placed between the MTA and the temporary restoration. After approximately seven days the MTA is fully set and the temporary restoration is removed, cotton pellet retrieved, and treatment proceeds with final restoration or any other additional procedures (Nowicka *et al.* 2013).

According to the manufacturer, Biodentine has a setting time of only 10 to12 minutes (Atmeh *et al.* 2012). The faster setting time can be attributed to the addition of calcium chloride as an accelerator, the hydrosoluble polymer which lessens the amount of the liquid component needed, and the greater surface size of particles (Bachoo *et al.* 2013). Because of the reduced setting time, Biodentine may be more readily applied in the clinical setting.

Clinically, Biodentine also has more preferential handling abilities over MTA. Reportedly, prepared MTA is more difficult to handle, has a sandy consistency, and is more time consuming than Biodentine (Norwicka *et al.* 2013, Atabek *et al.* 2012). Another possible advantage of Biodentine over MTA could be color stability. A recent study by Vallés noted MTA can discolor teeth in vivo and in vitro turning them dark after exposure to light and environments lacking oxygen. Additionally, they found that in comparison with MTA, Biodentine “maintained color stability in all conditions over time and showed no significant differences” in coloration of the restored teeth (Vallés *et al.* 2013). Acceptable color stability of Biodentine could make it a good restorative choice for esthetic regions. Paired with its beneficial effects on the pulp, Biodentine’s ease of handling and ability to keep teeth more natural in color increases its potential applications.

When immersed in biological fluids containing phosphate, MTA has been shown to form an effective seal with dentin and demonstrates the formation of an apatite layer with tag-like structures extending into the dentinal tubules (Han *et al.* 2013). Electron microscopy has also been used to explore the release of calcium and silica ions from MTA and their incorporation into the depths of surrounding dentin (Han *et al.* 2013). Calcium and silica ion release and subsequent uptake into the dentin is significant because calcium incorporation can yield higher strength and acid resistance while silica incorporation may induce remineralization of demineralized dentin (Han *et al.* 2013). Biodentine shares similar features with MTA including the extension of tag-like structures into the dentinal tubules and the ability for ions to be incorporated into the dentin itself (Han *et al* 2013). This similarity between MTA and Biodentine confers that Biodentine also has an exceptional ability to seal with dentin as well as exhibiting the added potential benefits of ion incorporation into dentin.

Comparative Material: Glass Ionomer

Glass ionomer (GI) is another dental restorative material that has uses similar to Biodentine. Glass ionomer and resin modified glass ionomer (RMGI) are used under composite resin restorations (with both open and closed sandwich techniques) as well as for temporary restorations. Note that RMGI can also be used as a definitive restoration of cervical preparations or as a large buildup material, both of which Biodentine has not been indicated for at this time. According to an article composed by Raskin *et al.* GI and RMGI "show a high percentage of gap-free interfacial adaptation to dentin" and bond spontaneously to dentin without the need for etching or bonding prior to placement

(Raskin *et al.* 2012). The study also examined the microleakage exhibited by both Biodentine and Fuji II (RMGI) and found that there was no statistically significant difference between the two with regard to microleakage at the interface between the material and dentin (Raskin *et al.* 2012). While GI and RMGI are often selected for placement due to their attributed fluoride release, they may not be as well suited for restorations in close proximity to the pulp. Glass ionomers contain polyacrylic and tartaric acids that degrade the dentin surface and facilitate bonding (Atmeh *et al.* 2012). Whereas Biodentine is biocompatible with pulpal tissues, GI and RMGI are acidic and potentially very irritating to the pulp. Because of their acidity and cytotoxicity, manufacturers indicate that GI and RMGI should not be used directly approximating pulp tissues (as in direct-pulp capping).

Comparative Material: Calcium Hydroxide

Calcium hydroxide is yet another material that can be used for some of the same purposes as Biodentine. Calcium hydroxide has been widely used as a temporary intracanal medicament during root canal therapy, as a liner, and for direct and indirect pulp capping procedures. Although it is considered a good product for direct pulp capping, it can cause some pulpal necrosis due to the caustic effects of its highly alkaline pH (pH=13) (Tran *et al.* 2012, Atmeh *et al.* 2012). Calcium hydroxide has other less desirable features as well, for example, it does not bond or adhere to dentin and is soluble over time (Norwicka *et al.* 2013). Because Calcium hydroxide is soluble in the oral environment, its potential uses are more limited than that of Biodentine.

Pulp-Capping Procedures

Pulp capping procedures involve cavity preparations that extend close to the pulp or even exposing a small area of pulpal tissue. Pulp capping is performed with the intention of maintaining pulp vitality. In reference to pulp capping, the two techniques utilized are the indirect and direct methods. Indirect pulp capping is performed when there is a small amount of dentin remaining over the pulp chamber and there has been no exposure of the pulp. Material is layered over the thin dentin in an attempt to insulate the pulp chamber from subsequent restorative materials and to induce reparative dentin formation thereby thickening the dentin layer protecting the pulp. Direct pulp capping is utilized when a cavity preparation exposes a small amount of pulpal tissue. After the exposure, material is placed directly over the pulpal tissue and dentin surrounding the area. If direct pulp capping is successful, the formation of a reparative dentin bridge can be observed reinforcing the area of the exposure (Tran *et al.* 2012). As stated by Norwicka *et al.*, pulp capping "protects the pulp-dentin complex against chemical irritation by operative procedures, toxicity of the material used, and bacterial penetration due to microleakage" (Norwicka *et al.* 2013).

Biodentine Effect on Dentin and Pulpal Tissues

Comparable to MTA, Biodentine has also been reported to induce biomineralization, as well as pulp cell differentiation and dentinogenesis when used as a direct pulp-capping medicament (Han *et al.* 2013). Biodentine and MTA do not have an acidic pH (glass ionomer for example, does), which would be irritating/cytotoxic to the pulp if placed as a direct or indirect pulp cap (Atmeh *et al.* 2012). A recent in vitro study reported, “human gingival fibroblasts in contact with Biodentine and MTA attached to and spread over the material surface after an overnight culture and increased in numbers after 3 and 7 days of culture” (Zhou *et al.* 2013). As a direct pulp-capping material, studies have been conducted to determine if Biodentine is an appropriate material of choice. Thus far, Biodentine has been shown to be very biocompatible and has low cytotoxicity similar to MTA (Tran *et al.* 2012). Researchers have found that proliferation of human dental pulp stem cells exposed to Biodentine decreased at high concentrations above 20mg/ml and that at normal dosing, Biodentine does not exhibit negative effects associated with cytotoxicity (Luo *et al.* 2013). The deposition of mineral ions and notable biocompatibility are very appealing properties of both MTA and Biodentine.

Primary dentin can only be formed in the presence of the enamel organ by primary odontoblasts. Therefore, in the case of pulp capping procedures, the goal is to induce a reparative dentin barrier (Tziafa *et al.* 2014). Various studies have determined that calcium silicate materials (like MTA and Biodentine) can cause recruitment of odontoblast-like cells within the pulp chamber that can propagate the formation of reparative dentin (Tran *et al.* 2012, Tziafa *et al.* 2014). Biodentine specifically has been found to induce effective dentinal repair and that the reparative structures were homogenous, and in continuity with primary dentin while the odontoblast like cells deposit matrix in a pattern similar to predentin (Tran et al 2012, Tziafa *et al.* 2014). Also, research conducted by Tran *et al.* concluded that pulp tissue adjacent to Biodentine appeared normal and free of inflammatory cells (Tran *et al.* 2012).

Research completed by Norwicka *et al.* found that as a direct pulp-capping material, Biodentine showed similar efficacy and influence on the dentin-pulp complex to

MTA and that both showed the absence of bacterial proliferation in the pulp tissues (Norwicka *et al.* 2013). Investigations have been conducted regarding the specific mechanism of human dental pulp stem cells being recruited for odontoblast differentiation. One such study performed by Luo *et al.* proposed that "Biodentine significantly increased alkaline phosphatase activity and mineralized nodule formation" likely through the mitogen-activated protein kinase and calcium/calmodulin-dependent protein kinase II pathways (Luo *et al.* 2014). Because of the many beneficial properties exhibited by Biodentine, it could be considered an acceptable alternative to MTA as a direct pulp-capping agent (Norwicka *et al.* 2013).

Layering Techniques

Biodentine has also been examined as a restorative material for layering under composite resin restorations. Techniques discussed for layering materials under composite are the open and closed sandwich techniques. In a closed sandwich technique, the base layer material does not extend to the cavosurface (does not have direct exposure to the oral cavity) of the preparation and can be compared to application of a traditional liner or base. The open sandwich technique implements a base layer of material that does extend to the cavosurface of the preparation and is directly exposed to the oral cavity. The open sandwich technique is useful for restoring cavity preparations where the cavosurface enamel is not of quality or is absent, hindering the bonding capability of composite restorations (Raskin *et al.* 2012).

Resin based composite materials show greater bond strength to enamel than to dentin. Therefore, when enamel is lacking, materials that bond well to dentin may be better suited for the cervical portion of the restoration. For this reason (among others), RMGI is commonly used for open sandwich technique restorations. Like RMGI, Biodentine can also be used with the open sandwich technique for posterior interproximal (class II) restorations following a series of steps. After cavity preparation is completed, a matrix band is placed and wedged appropriately and Biodentine is layered along the most cervical portion of the preparation including the proximal box area. Once fully set, a bonding agent is applied (per manufacturers protocol) followed by a composite resin restoration (Raskin *et al.* 2012). Another option for the open or closed sandwich techniques is to first temporarily bulk-fill the preparation completely with Biodentine and then later cut back the Biodentine for layering composite resin material on the occlusal surface.

Bonding Biodentine to Composite

Although Biodentine does not require dentin etching or the application of a bonding agent before application, composite resin materials do, which must be considered when using Biodentine in either the open or closed sandwich technique. Preparation of the Biodentine surface before composite resin placement is dependent on the bonding system selected, as different bonding systems require different placement protocols for use. While self-etch bonding systems do not require the initial use of a separate etchant, total-etch bonding systems do.

When total etch bonding systems are utilized the preparation must first undergo exposure to a phosphoric acid etchant, rinsing, and drying, before application of the primer/bonding agent(s). Research has been conducted investigating the effect of 37%

phosphoric acid on the surface of Biodentine. A study by Camilleri used scanning electron microscopy and light microscopy were used to examine the surface of Biodentine that was exposed to 37% phosphoric acid for 20 seconds and then rinsed and dried. Their results showed the calcium hydroxide on the surface of Biodentine reacted with atmospheric carbon dioxide and yielded a “heavily carbonated surface” that was also reduced in its calcium to silicon ratio (Camilleri *et al.* 2013). Microhardness was also examined and it was found that there was no significant difference between the etched and unetched Biodentine (Camilleri *et al.* 2013). While that study showed physical changes of the Biodentine surface after etching, it made no conclusions regarding the subsequent effect on bond strength to the etched surface.

Compressive strength of Biodentine after acid etching has also been investigated. A study conducted by *Kayahan et al.* examined the compressive strength of various calcium–silicate cements including Biodentine, ProRoot MTA, Angelus MTA and CEM cement. In that study, all materials tested were allowed to set for seven days before undergoing exposure to 37% phosphoric acid for 15 seconds followed by a water rinse (Kayahan *et al.* 2013). Researchers found that “regardless of the acid etch application, Biodentine showed significantly higher compressive strength values than other materials” (Kayahan *et al.* 2013). More recent research has deduced similar conclusions that Biodentine has higher compressive strength and microhardness and may also be more resistant to an acidic environment when compared to its MTA counterpart (Elnaghy 2014). Compressive strength of Biodentine is valuable because an effective restoration needs to be able to withstand compressive masticatory forces even after being exposed to the acid etchants required for bonding to composite resin materials.

Because they are used in conjunction, the interface between Biodentine and composite resin restorations must be examined. Optibond Solo Plus bonding agent is a two-step etch and rinse system that has been studied for many years and shown superior bonding ability while Septobond SE is relatively newer two-step self etching system that does not require rinsing (Raskin *et al.* 2012). A study completed by Raskin examined the in vitro microleakage between Biodentine and the composite resin Filtek Z250 using various bonding agents. When Biodentine was used in conjunction with Optibond Solo Plus and Filtek Z250, there was minimal microleakage between the Biodentine and composite with results statistically similar to bonding Fuji II (RMGI) and composite resin material. When Septobond SE bonding agent was used, more microleakage was noted compared to Optibond Solo Plus. They also evaluated silane porcelain primer application for use between the Biodentine and composite (after etching and before bonding agent applied) and found that it had no significant effect on microleakage (Raskin *et al.* 2012).

The bond strength between Biodentine and composite resin material is an important consideration because Biodentine is used in layered restorations. Shear bond strength is an indicator of how well the Biodentine and composite interface will withstand shear forces that are encountered by the teeth. Atabek compared the shear bond strength of multiple adhesive systems at various time intervals with MTA. The results showed a significantly higher shear bond strength (mean 13.103 MPa) with the two-step total etch and rinse bonding systems compared to both one-step self-etching and three-step total etch bonding systems (Atabek *et al.* 2012). The research also suggested that composite restorations should not be placed over MTA until at least 96 hours after placement (Atabek *et al.* 2012). However, contradicting results obtained by Odabas found that there was no statistically significant difference between the total etch, and the one and two step self-etch systems reviewed in their study (Odabas *et al.* 2013). Although Biodentine shares similar components and properties with MTA, more extensive research should be conducted to examine the shear bond strength between different composite resin materials and different adhesive systems with Biodentine.

More recently, different types of resin-based composites have been used for bonding to Biodentine. While most readily available composite resin materials in the United States are methacrylate based, there are some composite resin restorative materials that are silorane based. Silorane based composite resin materials, for example Filtek P90, are coming of interest because manufacturers claim less than 1% shrinkage (which is low for composite resin materials) (3M ESPE, St. Paul MN, USA). Though more investigation is needed, initial research conducted by Cantekin and Avci has determined that methacrylate-based resin composite showed significantly greater shear bond strength than silane-based composite when bonding to Biodentine—which could have significant clinical implications (Cantekin and Avci 2014).

Purpose

To investigate the effects of various Biodentine setting times on shear bond strength when bonding to resin based composite dental restorative material utilizing a two-step total etch bonding system.

Materials and Methods

The following study protocols were approved by the Womack Army Medical Center Internal Review Board for Research, Fort Bragg, North Carolina and by the Uniformed Services University of the Health Sciences, Bethesda, Maryland. Funding for this study was provided by the United States Army Dental Activity, Fort Bragg, North Carolina. No commercial/financial relationship, interest, or association that might pose a conflict of interest has been present.

Materials Used

The materials used in this study included the tricalcium silicate based restorative material Biodentine (Septodont, Saint Maur Des Fossés, France), Scotchbond Universal Adhesive (3M ESPE, St. Paul MN, USA), Scotchbond Universal Etchant (3M ESPE, St. Paul MN, USA), and Filtek Supreme Ultra Universal Restorative material, Shade A2B (3M ESPE, St. Paul MN, USA). Manufacturer's instructions were followed for preparation, placement, and curing for each of the materials used (See table 2.).

Table 2. Manufacturer Instructions Per Product

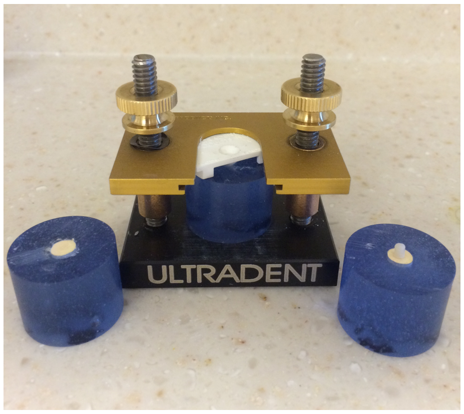
Product **(Manufacturer)**	**Manufacturer instructions for use**
Biodentine, tricalcium silicate restorative material (Septodont)	Open powder capsule, place upright in capsule holder Detach single dose container of liquid, dispense 5 drops of the liquid into powder capsule, replace top onto powder capsule to close Place capsule in triturator at speed of 4000-42000 rotations/min, mix for 30 seconds Remove from triturator, open capsule, collect Biodentine from capsule using manufacturer supplied plastic applicator Condense and smooth Biodentine into preparation and allow to set (initial set is 12 minutes) before bonding
Scotchbond Universal Etchant, 32% phosphoric acid (3M ESPE)	Apply for 15 seconds Rinse with water 15 seconds, dry with cotton pellet
Scotchbond Universal Adhesive, self-etch or total etch bonding agent (3M ESPE)	Apply for 20 seconds, rubbing in with microbrush Gentle air dry for 5 seconds to evaporate solvent Light cure 10 seconds
Filtek Supreme Ultra Universal Restorative, methacrylate based composite resin (3M ESPE)	Place into preparation using condensing instrument Incremental placement depth maximum 2mm Light cure with LED (output 400-1000mW/cm^2) 20 seconds

Specimen Preparation

Acrylic cylinders (n=134) with a central well measuring 2mm deep by 5mm diameter were utilized. Blocks were randomly divided into four groups for testing different setting times of Biodentine. Capsules of Biodentine were randomly distributed for use amongst the four groups and lot numbers were noted. After the Biodentine was prepared, the plastic instrument provided by Septodont was used to condense and smooth the Biodentine into the wells until fully filled and level with the acrylic.

Samples were stored/allowed to set at 37^{o}C in 100% humidity. Depending on the assigned grouping, samples were allowed to set either 15 minutes (Group 1, n=34), 1 hour (Group 2, n=41), 24 hours (Group 3, n=32), or 2 weeks (Group 4, n=27). After the allotted setting time, samples were etched using Scotchbond Universal Etchant then rinsed and dried. Scotchbond Universal Adhesive was then applied to the Biodentine surface. Using a bonding clamp and bonding mold inserts (Ultradent, South Jordan UT, USA), Filtek Supreme Ultra Restorative material was condensed and light cured onto the Biodentine prepared with adhesive. The dimensions of the composite cylinder bonded to the Biodentine were 2.3798mm diameter by 2mm height, yielding a bonded surface area of 4.45mm^{2}.

Figure 1. Ultradent Bonding Clamp and Insert

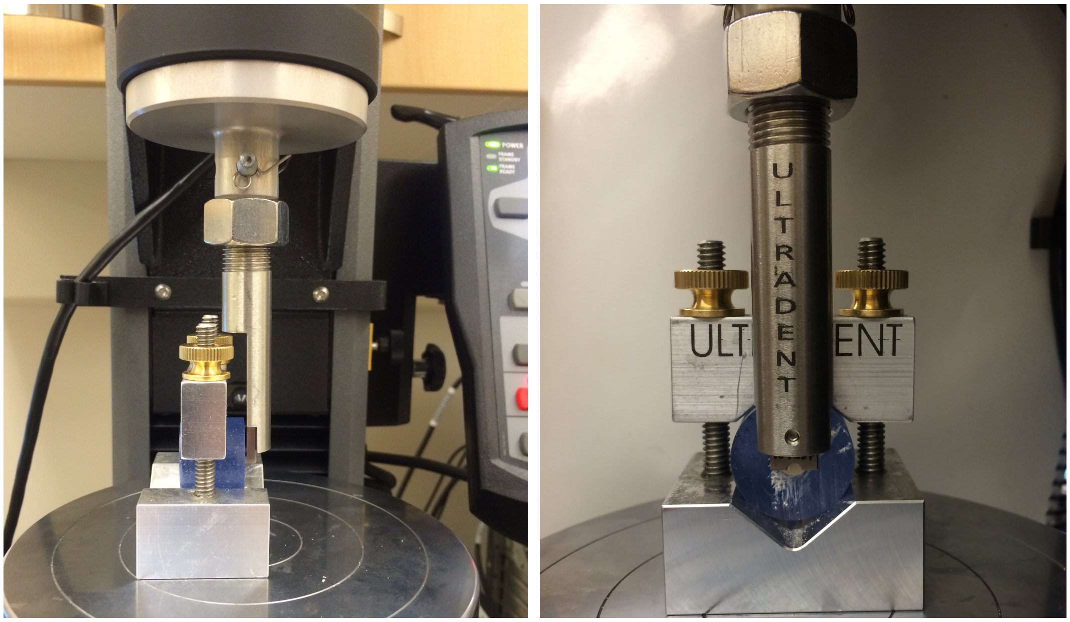


Shear Bond Strength Test

For Shear bond strength testing, the Instron 5943 universal testing machine was utilized (Instron, USA). The acrylic cylinders were secured to the test base clamp and placed on the static lower table of the universal testing machine while the crosshead assembly was secured to the mobile upper member (Ultradent, South Jordan UT, USA).

The acrylic cylinder mold, bonding clamp, bonding mold inserts, test base clamp, and crosshead assembly are products from Ultradent that are designed to be used together for precise shear bond strength testing (Ultradent, South Jordan UT, USA). The crosshead was positioned so that the leading edge would contact the bonded Biodentine—composite resin interface. The crosshead speed was set to 1mm/min extension.

Figure 2. Ultradent Test Base Clamp and Crosshead Assembly



Data Collection

For each sample, maximum force achieved before breaking the bond was recorded. Shear bond strength was then calculated by dividing the maximum force by the surface area of the bonded composite resin interface (Surface Area=4.45mm^2) (1N/mm^2= 1 MPa). Notes were also taken regarding date and time of Biodentine specimen preparation as well as the associated lot number for each Biodentine capsule used.

Statistical Analysis

For each group the mean, standard deviation, and confidence intervals were calculated. One-way analysis of variance tests as well as Post-Hoc Tukey tests were used to compare the groups and determine if statistically significant differences existed between the calculated shear bond strengths.

Results

Initially shear bond strength for each group was examined individually. The mean, standard deviation, and 95% confidence intervals were calculated for each group. A table of raw data collection is included in the appendix.

Table 3. Summary of SBS by Test Group

Group	N	Mean SBS (MPa)	SDev
1 (15 min)	34	3.644294	.3218755
2 (1 hour)	41	8.442634	.4416371
3 (24 hours)	32	14.29406	.6795069
4 (2 weeks)	27	7.017259	.7212786

Figure 3. Shear Bond Strength by Setting Time

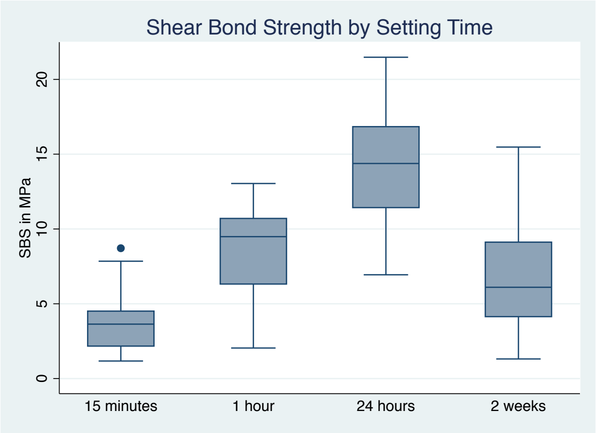
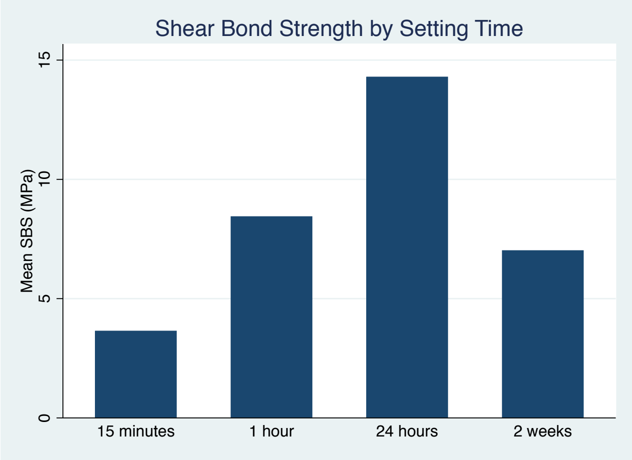


Figure 4. Mean Shear Bond Strength



To determine if there were significant differences across the groups, analysis of variance (ANOVA) was calculated. The F-statistic from the ANOVA was 66.47 with a p-value <0.0001, revealing that a significant difference in shear bond strength existed across the treatment groups. To compare the mean shear bond strength of each of the groups to one another, one-way ANOVA testing was used followed by post-hoc analysis with Tukey's honest significant difference testing.

Table 4. One-Way ANOVA Amongst Groups

Comparison of Groups	F-statistic	Prob > F
1 vs. 2	71.63	0.0000
1 vs. 3	208.36	0.0000
1 vs. 4	20.98	0.0000
2 vs. 3	56.16	0.0000
2 vs. 4	3.19	0.0788
3 vs. 4	53.69	0.0000

Table 5. Post-hoc Comparison Summary SBS Amongst Groups.

***Critical value at 5% significance**

Comparison of Groups	Difference in mean SBS	p-value	Tukey's HSD test*
1 vs. 2	4.79834	0.000	6.65
1 vs. 3	10.64977	0.000	13.89
1 vs. 4	3.372965	0.000	4.20
2 vs. 3	5.851428	0.000	7.97
2 vs. 4	-1.425375	0.256	-1.85
3 vs. 4	-7.276803	0.000	-8.95

Discussion

Results from this study support the conclusion that there is a difference in the bond strength achieved between Biodentine and composite resin restorative material at varied Biodentine setting times. Samples of Biodentine bonded after 15 minutes (Group 1) showed statistically significant less shear bond strength than samples bonded after a longer setting times. There was a significant increase in shear bond strength comparing Groups 2, 3, and 4 to Group 1 (p-values <0.000). These results indicate that even though the manufacturer of Biodentine states an initial setting time of 12 minutes is adequate, that time point may be too early to achieve optimal bonding to subsequent composite restorations.

In this study, samples of Biodentine that were allowed to set for 24 hours before bonding to composite resin restorative material (Group 3) exhibited the greatest average shear bond strength (14.29406 MPa). The maximum shear bond strength reported in this study was 19.677 MPa, associated with the Biodentine that was allowed to set for 24 hours. The lowest shear bond strength value recorded was 1.306MPa, associated with the Biodentine that was only allowed to set for 15 minutes.

With regard to Biodentine's use with composite resin, as in the open or closed "sandwich technique," it is prudent to compare the bond strength with resin modified glass ionomer (RMGI) or conventional glass ionomer (GI) which can also be used for the sandwich technique. A similarly conducted study completed by Nuttall reported an average shear bond strength of 14.50MPa could be attained between resin modified glass ionomer and the commonly used composite resin restorative material Filtek Z250 (Nuttall *et al.* 2013). The average shear bond strength for group 3 in this study was 14.29406MPa, very similar to the shear bond strength reported by Nuttall between RMGI and composite. These similar results could infer that Biodentine is equally suited for use in layering with composite as the conventionally recommended resin modified glass ionomer.

There was however no significant difference in shear bond strength when comparing Group 2 (1 hour) to Group 4 (2 weeks). This was an interesting finding because thoughts were that the bond strength would likely be greatest once the Biodentine was fully set. Resulting shear bond strengths recorded in Group 4 had the greatest variation ranging from 1.311MPa to 15.476MPa.

Although further investigation should be done to determine if this finding is consistent across other lab studies, possible explanations for the lower shear bond strength are as follows. The Biodentine samples that were allowed to set for two weeks had a notable precipitate on the surface of the Biodentine when removed from the controlled storage unit. The precipitate, which was macroscopically visible, had a crystalline appearance (See Figure 5). Keeping testing protocols consistent throughout the study, the precipitate was not removed in any way prior to the etching procedure. However, after etching, the apparent crystalline precipitate was no longer visible macroscopically. After completion of the study, and analysis of the shear bond strengths achieved, it became apparent that the noted precipitate might have had an effect on the results. In an article published in the journal Dental Materials, researchers made note that when exposed to simulated oral fluids, hydroxyapatite formed on the surface of the cement (Camilleri *et al.* 2013). However, in this study Biodentine samples were stored at 37^{o}C in 100% humidity but were not exposed to simulated body fluids. Therefore, it would be presumptive to conclude that the precipitate visualized in this study is the same compound (hydroxyapatite) and further investigation is needed to determine the actual identity of the material. Samples of the precipitate were collected for further analysis and microscopic evaluation. To investigate the precipitate further, new Biodentine samples were prepared and placed into controlled storage for two weeks. After storing for two weeks, the precipitate was photographed and samples of the precipitate were also collected for microscopic analysis.

Figure 5. Examples of Precipitate Found on Biodentine Samples of Group 4

Another possible reason for lower shear bond strength recorded between composite and Biodentine allowed to set for two weeks could be that it does not bond as well once the setting (hydration) reaction is fully complete. As mentioned earlier in this report, Biodentine's setting reaction is reported to be complete at approximately two weeks setting time (Bachoo *et al.* 2013). It could be possible that the Biodentine that is fully set has less bonding capacity to resin-based composite restorative materials.

With regard to the consistency of Biodentine, it was noted that some samples had different resulting textures than others. While all Biodentine samples were prepared per manufacturer's instructions (and with the same triturator), some samples were more dry/moist when compared across all capsules prepared. This could possibly be due to incomplete mixing of the powder and liquid within the capsule. Alternatively, the instructions call for "5 drops" of liquid into the capsule, and I did note that the drops emitted from the liquid dispensing units were not consistent in volume. Less likely reasoning would be variation in powder mass contained in the capsule, which is weighed and dispensed by manufacturer. The variation in moisture/consistency of the Biodentine samples could have affected the results of this study. One study completed by Fridland and Rosado examined the effect of water-to-powder ratio on MTA and found that both porosity and solubility increased with increased water content (Fridland and Rosado 2003). After fifteen minutes of setting time, some Biodentine samples were still slightly moist and softer in consistency than others (as noted on application of bonding agent with a microbrush and visible transfer of some Biodentine onto the fibers of the microbrush).

Conclusion

This study has elucidated information regarding Biodentine setting time and subsequent bonding to resin-based composite restorative material. The results from this study indicate that bonding to Biodentine after a setting time of 15 minutes achieves less shear bond strength compared to Biodentine that is allowed to set for a longer period of time. While a significant increase in bond strength can be seen from 15 minutes setting time to 1 hour setting time, an even greater increase was noted after allowing the Biodentine to set for 24 hours. For this study, the average shear bond strength attained after 24 hours setting time for Biodentine was 14.29406 MPa, which could be considered strong enough for clinical use of the "sandwich technique." Although time is a great consideration for clinicians, it may be advisable for the dentist to wait longer than 15 minutes setting time before bonding to Biodentine. While shear bond strength between Biodentine and resin-based composite was greater after 1 hour setting time, it may be prudent for the dentist to postpone bonding to Biodentine until 24 hours of setting has been reached. Even though it is not ideal economically for the dentist, same-day bonding composite to Biodentine may compromise results and lessen success of the definitive restoration.

Appendix: Raw data results

Shear Bond Strength in MPa for each sample

n	Group 1 MPa		Group 2 MPa		Group 3 MPa		Group 4 MPa
1	1.331		7.006		9.959		3.716
2	2.747		8.788		9.398		4.727
3	7.23		11.604		6.939		2.698
4	8.712		10.742		7.782		5.341
5	2.405		6.901		13.204		15.476
6	4.967		6.856		11.03		11.351
7	4.144		10.87		14.423		2.101
8	4.321		7.611		8.945		4.419
9	4.611		5.934		14.667		9.07
10	4.554		9.487		15.968		8.92
11	1.173		12.514		11.829		6.102
12	3.634		7.446		19.228		8.296
13	3.485		10.532		16.789		13.393
14	1.955		12.662		21.48		1.311
15	1.432		10.968		19.677		4.139
16	2.599		7.831		16.397		12.567
17	1.599		13.04		19.092		4.614
18	1.721		5.338		16.183		7.122
19	3.648		4.046		11.864		9.118
20	4.973		6.453		13.917		9.948
21	4.41		4.84		10.444		12.125
22	4.509		8.319		13.239		7.665
23	3.645		5.2		20.44		2.985
24	7.846		6.318		13.453		5.325
25	2.362		10.774		14.332		8.253
26	4.449		2.062		15.531		5.662
27	1.834		10.47		17.945		3.022
28	6.481		4.486		12.486		
29	1.306		10.44		14.942		
30	2.17		9.565		16.889		
31	3.829		11.622		9.926		
32	3.16		9.604		19.012		
33	3.813		6.26				
34	2.851		9.529				
35			2.042				
36			10.429				
37			9.702				
38			10.378				
39			10.738				
40			6.038				
41			10.703				

References

1. Atabek D, Sillelioglu H, Olmez A (2012) Bond Strength of Adhesive Systems to Mineral Troxide Aggregate with Different Time Intervals. Journal of Endodontics **38,** 1288—1292.

2. Atmeh AR, Chong EZ, Richard G *et al.* (2012) Dentin-cement interfacial interaction: Calcium Silicates and Polyalkenoates. Journal of Dental Research **91(5),** 454—459.

3. Bachoo IK, Seymour D, Brunton P (2013) A biocompatible and bioactive replacement for dentine: is this a reality? The properties and uses of a novel calcium-based cement. British Dental Journal 214: E5, 1—7.

4. Ber BS, Hatton JF, Stewart GP (2007) Chemical Modification of ProRoot MTA to Improve Handling Characteristics and Decrease Setting Time. Journal of Endodontics **33**, 1231—1234.

5. Camilleri J (2013) Investigation of Biodentine as dentine replacement material. Journal of Dentistry **41,** 600—610.

6. Camilleri J, Sorrentino F, Damidot D (2013) Investigation of the hydration and bioactivity of radiopacified tricalcium silicate cement, Biodentine and MTA Angelus. Dental Materials **29(5)**, 580—593.

7. Camilleri J, Laurent P, About I (2014) Hydration of Biodentine, Theracal LC, and a Prototype Tricalcium Silicate-based Dentin Replacement Material after Pulp Capping in Entire Tooth Cultures. Journal of Endodontics **40(11)**, 1846—1854.

8. Cantekin K, Avci S (2014) Evaluation of shear bond strength of two resin-based composites and glass ionomer cement to pure tricalcium silicate-based cement (Biodentine). Journal of Applied Oral Science **22(4),** 302—306.

9. Elnaghy AM (2014) Influence of Acidic Environment on Properties of Biodentine and White Mineral Trioxide Aggregate: A Comparative Study. Journal of Endodontics **40(7)**, 953—957.

10. Fridland M, Rosado R (2003). Mineral Trioxide Aggregate (MTA) Solubility and Porosity with Different Water-to-Powder Ratios. Journal of Endodontics **29(12)**, 814—817.

11. Grech L, MAllia B, Camilleri J (2012). Characterization of set intermediate Restorative Material, Biodentine, Bioaggregate and a prototype calcium silicate cement for use as root-end filling materials. International Journal of Endodontics **46(7)**, 632—641.

12. Kayahan MB, Nekoofar MH, McCann A, *et al.* (2013) Effect of Acid Etching Procedures on Compressive Strength of 4 Calcium Silicate-based Endodontic Cements. Journal of Endodontics **39,** 1646—1648.

13. Han L, Okiji T. (2013) Bioactivity evaluation of three calcium silicate-based endodontic materials. International Endodontic Journal **46,** 808—814.

14. Koubi G, Colon P, Franquin JC *et al.* (2013) Clinical evaluation of the performance and safety of a new dentine substitute, Biodentine, in the restoration of posterior teeth—a prospective study. Clinical Oral Investigations **17,** 243—249.

15. Koubi S, Elmerini H, Koubi G *et al.* (2012) Quantitative Evaluation by Glucose Diffusion of Microleakage in Aged Calcium Silicate-Based Open-Sandwich Restorations. International Journal of Dentistry, Hindawi Publishing Corporation **2012**.

16. Luo Z, Kohli MR, Yu Q, Kim S, Qu T, He W (2014) Biodentine Induces Human Dental Pulp Stem Cell Differentiation through Mitogen-activated Protein Kinase and Calcium-/Calmodulin-dependent Protein Kinase II. Journal of Endodontics **40(7)** 937—942.

17. Luo Z, Li D, Kohli MR, Yu Q, Kim S, He W (2013) Effect of Biodentine on proliferation, migration, and adhesion of human dental pulp stem cells. Journal of Dentistry **42,** 490—497.

18. Norwicka A, Lipski M, Parafiniuk M *et al.* (2013) Response of Human Dental Pulp Capped with Biodentine and Mineral Trioxide Aggregate. Journal of Endodontics **39,** 743—747.

19. Nuttall C, Vandewalle K, Casey J, Sabey K (2013) Bond strength of silorane- and methacrylate-based composites to resin-modified glass ionomers. Academy of General Dentistry, Dental Materials, November/December 2013, 73—78.

20. Odabas ME, Bani M, Tirali RE (2013) Shear Bond Strengths of Different Adhesive Systems to Biodentine. The Scientific World Journal **(2013)**, article ID 626103, 1—5.

21. Parirokh M, Torabinejad M (2010) Mineral Trioxide Aggregate: A Comprehensive Literature Review—Part I: Chemical, Physical, and Antibacterial Properties. Journal of Endodontics **36**, 16—27.

22. Pradelle-Plasse N, Tran XV, Colon P (2009) Physico-chemical properties of Biodentine. In Goldberg M, ed. Biocompatability or Cytotoxic Effects of Dental Composites, 1st ed. Oxford: Coxmoor Publishing Co, 2009: 222

23. Raskin A, Eschrich G, Dejou J *et al.* (2012) In Vitro Microleakage of Biodentine as a Dentin Substitute Compared to Fuji II LC in Cervical Lining Restorations. Journal of Adhesive Dentistry **14,** 535—542.

24. Septodont, Manufacturer of Biodentine, Saint Maur Des Fossés, France.
http://www.septodontusa.com/products/biodentine
http://www.septodontusa.com/sites/default/files/Biodentine.pdf
http://www.septodontusa.com/news/introducing-dentin-capsule-biodentine
http://www.septodont.fr/fichiers_upload/biodentine_publi___com_20110311_s.pdf

25. Tran XV, Gorin C, Willig C *et al.* (2012) Effect of a Calcium-silicate-based Restorative Cement o Pulp Repair. Journal of Dental Research **91(12),** 1166—1171.

26. Tziafa C, Koumpia EK, Papadimitriou S, Tziafas D (2014) Dentinogenic Response after Direct Pulp Capping of Miniature Swine Teeth with Biodentine. Journal of Endodontics **40(12)** 1967-1971.

27. Ultradent, Manufacturer of Utilized Shear Bond Testing Equipment, South Jordan, UT, USA

28. Vallés M, Mercadé M, Duran-Sindreu F, *et al.* (2013) Influence of Light and Oxygen on Color Stability of Five Calcium Silicate-based Materials. Journal of Endodontics **39**, 525—528.

29. Zhou H, Shen Y, Wang Z, *et al.* (2013) In Vitro Cytotoxicity Evaluation of a Novel Root Repair Material. Journal of Endodontics **39**, 478—483.