PROSPECTIVE ANALYSIS OF PRE-OPERATIVE PROGNOSTIC INDICATORS OF VITAL
PULP THERAPY ON MATURE PERMANENT TEETH

William S. Yeung

A thesis submitted to the faculty at the University of North Carolina at Chapel Hill in Partial
fulfillment of the requirements for the degree of Masters of Science in the Department of
Endodontics in the School of Dentistry.

Chapel Hill
2017

Approved by:

Asma Khan

Peter Tawil

Carol Haggerty

ABSTRACT

William S. Yeung: Prospective Analysis of Pre-operative Prognostic Indicators of Vital Pulp
Therapy on Mature Permanent Teeth
(Under the direction of Asma Khan)

Vital pulp therapy (VPT) is a conservative treatment option for exposed pulps with the goal of preserving the vitality of the pulp (1). Historically, VPT with calcium hydroxide had inconsistent success rates when used for carious exposures. Since that time, newer materials have resulted in a more favorable success rate for this treatment option. Factors in addition to the material type may affect the outcome that previous studies with small sample sizes did not identify (2, 3). This prospective, observational study aims to examine the correlation between the outcome of vital pulp therapy and pre-operative factors such as patients' gender, age, tooth type, and the site of pulp exposure. Written informed consent was obtained from asymptomatic patients experiencing a pulp exposure on a vital, mature permanent tooth. Caries removal was completed and hemorrhage was controlled with sodium hypochlorite. Mineral trioxide aggregate was placed on the exposed pulp and the tooth was restored permanently. The patients were followed up with a series of phone calls and in person examinations at 6 months and 1 year postoperatively. The data was analyzed by logistic regression using a generalized linear model to evaluate associations between pre-operative factors and outcome of treatment. A total of 73 patients were treated with ages between 15 and 78 years old. 51 patients were available for 6 month recall with a success rate of 79%. This study found that vital pulp therapy on mature, permanent teeth was a viable treatment and there was no association between the examined pre-operative factors and the outcome.

To Mom, Dad, & Sally.
Thank you for all your support along the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

REVIEW OF LITERATURE

Section 1.1 Introduction

Endodontic disease manifests as a variety of signs and symptoms. If untreated, it can lead to devastating outcomes with intense pain and swelling. Despite the different presentations, most endodontic diseases share the common etiology of microbial infection (4, 5). The most common route for infection is through carious lesions which permit microorganisms from the oral cavity to infect the pulp. Other routes of infection can be trauma, or mechanical exposures.

Pulpal exposures can occur under three different scenarios: caries, trauma, or mechanical causes. Caries, a common chronic disease, allows for bacteria from the oral environment to penetrate through the tooth's enamel and dentin into the pulp (6). Nearly 80% of dentists report encountering caries to the pulp at least once per month in their practice (7). Traumatic exposures are not as commonly encountered, making up only 10% of total dental traumas experienced (8). These can occur during physical accidents where the coronal part of the tooth becomes chipped in a fashion that exposes the pulp. Mechanical exposures are the least frequently encountered. These occur during a dental procedure where healthy tooth structure is removed erroneously, exposing the pulp. This study focuses on treating carious exposures which is the most commonly encountered by clinicians. Regardless of the method of pulpal exposure, all three scenarios allow for microbial invasion into a delicately formed tissue.

Microbial invasion of the pulp can elicit a dual immune response: innate and adaptive immunity. Innate immunity is the pulp's primary method of defense and consists of the outward

flow of dentinal fluid, infiltration of phagocytes, and chemical mediators (9-11). Adaptive immunity plays a role if innate immunity is unable to resolve the microbial infection.

Intrapulpal pressure causes an outward flow of dentinal tubule fluid which assists in the diffusion of noxious elements away from the pulp: a protective response. This underscores one of the arguments for maintaining a healthy vital pulp since non-vital teeth do not have this outward pressure. Non-vital teeth have increased invasion of noxious stimuli compared to their vital counterparts (12). In addition to fluid flow, the composition of the fluid aids in pulpal defense. Dentinal fluid has been found to contain immunoglobulins that change in intensity as caries progression advances (13). As caries forms and progresses, increased levels of immunoglobulins are detected in the dentinal tubules (14).

The cells that function as part of the pulp's innate immunity consists of neutrophils, macrophages, and dendritic cells. The primary goal of these cells is to phagocytize foreign bodies. Neutrophils are mainly for short-term responses. They phagocytize bacteria and their byproducts by engulfing them and digesting them into small peptides. Neutrophils and chemokines are attracted to the area by lipopolysaccharides on gram negative cell walls. They recognize foreign bodies via antibodies as well as bacterial cell walls. Macrophages are recruited to the site relatively slower than neutrophils but also engulf and digest antigens as well. Macrophages are developed from monocytes in the blood and recognize glycoproteins on the surface of bacteria. Dendritic cells function as antigen presenting cells which are used to activate T and B cells. They are derived from hematopoietic stem cells. Immature dendritic cells possess phagocytic activity while mature dendritic cells have less ability to phagocytize.

Chemical mediators such as cytokines, chemokines, arachidonic acid metabolites, nitric oxide, and neuropeptides function together with the cells to make up the innate immunity.

Cytokines are proteins that are produced mainly by immune cells and assist in regulation. They have three main categories: proinflammatory, Th1, and Th2. Proinflammatory cytokines are produced mainly by macrophages and cause elevation in temperatures which limits bacterial growth and promotes adaptive immunity. Th1 cytokines are produced by Th1 type helper cells. Their function includes assistance in macrophage and natural killer cell and antiviral activity while also downregulation of Th2 type reactions. Th2 cytokines are utilized in the activation, growth, and differentiation of B cells as well as inhibition of macrophage activation. The main function of chemokines is to induce exudation and translocation of leukocytes. Arachidonic acid metabolites are involved in different inflammatory and homeostastic processes while nitric oxide regulates the synthesis of various chemical mediators. Neuropeptides such as Substance P and calcitonin gene-related peptides function to regulate vasculature and control local blood flow. In the event where innate immunity is insufficient to defend the pulp, the pulp's other defense, adaptive immunity, comes into play (10).

Unlike innate immunity, adaptive immunity is more specific toward exogenous antigens and is composed primarily of T cells and B cells. T cells are lymphocytes that are activated to secrete cytokines and differentiate into various other cells. They require antigen presenting cells to capture and present the antigens to function. In the pulp, B cells along with immature dendritic cells bind to antigens and present them to T cells (15). The B cells are formed in the bone marrow and differentiate to become plasma cells to function as antibody producing cells. As the carious lesion approaches the pulp, the number of B cells in the pulp increases significantly. Their main function is to produce antibodies which then bind to the antigens tagging the antigen for other parts of the immune system to attack (9).

If the pulpal irritant is not removed, irreversible damage ensues and the pulp progresses to necrosis. It was once believed that the body's response to chronic inflammation increases fluid movement into the pulp as a part of the immune response. This increase in fluid was thought to increase the pressure inside the root canal system due to its non-compliant environment. The increase in pressure would eventually lead to strangulation of the vessels at the apex causing overall necrosis (16). Newer findings have refuted this, showing that the tissue pressure only rises in the immediate area. It is now believed that local necrosis occurs in an area immediately beneath the source of inflammation. If not treated, then the area of necrosis progresses gradually throughout the pulp and into the periapical tissues causing apical periodontitis (9).

The goal of endodontic treatment is to diagnose, prevent and/or treat apical periodontitis. There are two main treatment options to accomplish this goal: root canal therapy and vital pulp therapy. Traditionally, the standard method of approach to accomplish is root canal therapy. Root canal therapy is commonly achieved by chemo-mechanical debridement while maintaining aseptic technique (17). It is a predictable and effective treatment for teeth with vital pulps with a success rate of around a 90%. Despite these high success rates, it is not without its own drawbacks (18).

With the average cost of root canal therapy in private practice ranging from $700 to over $1,000, over 80% of dentists consider it to be a barrier to treatment for the general public (7). In addition to this high cost, there are risks associated with the procedure. Despite attempts to thoroughly clean the root canal system, micro CT studies have shown that nearly 40% of the surfaces remain untouched by instruments (19, 20). In the areas that are instrumented, errors can create perforations which can lower the prognosis of the treatment. Instrument failures can also leave segments of broken instruments that block the root canals from being cleaned out effectively. Today, root canal therapy is generally the treatment of choice for carious pulp exposures or pulpal

necrosis on mature permanent teeth. In the event of pulpal necrosis, it is currently not within our capability to regenerate this complex tissue. While vast amounts of efforts have been carried out to revitalize a necrotic pulp, a simpler solution may be to preserve and sustain a vital pulp.

Vital pulp therapy is a procedure aimed at preserving the vitality of the pulp. Although root canal treatment can prolong the tooth's survival, there are indications that the loss of the pulp can decrease the survival time of the tooth (21). Some theories have pointed to the damping properties of the pulp and minor proprioceptive functions that can be lost along with the pulp (22, 23). As stresses traverse the tooth, the pulp acts as a kind of shock absorber resulting in less forces overall. This allows the tooth to dissipate more strain than a non-vital tooth. Mechanoreceptors in the periodontal ligament monitor touch and pressure forces subjected by the tooth. No such structures are present in the pulp, however, non-vital teeth have higher pain thresholds that vital teeth. This can lead to more forces subjected by non-vital teeth. These extra forces can lead to early failure of the tooth. When appropriate, vital pulp therapy can be utilized to retain the pulp's vitality and maintain these benefits.

Vital pulp therapy includes procedures such as full coronal pulpotomies, partial pulpotomies, and pulp capping. A full coronal pulpotomy is a procedure to completely remove the coronal portion of a vital pulp to the orifice level. The pulp in the radicular portion is left untouched and a medicament is placed at the canal orifice(s). In an immature permanent tooth with a diseased coronal pulp, a pulpotomy can be used to promote continuing development and formation of the tooth's root (24). An immature tooth lacks a constriction at the apex which is challenging to hermetically seal with conventional root canal therapy. Since the pulp is necessary for continued development of the tooth, losing its vitality before maturation leaves the root end open.

Maintaining the pulp's vitality is preferred in these cases so that the root apices can continue development.

Partial pulpotomy is a procedure for teeth with exposed pulps. It involves the aseptic removal of a small section of the pulp and dentin immediately surrounding the exposure site. The goal is to remove irreversibly inflamed tissue as well microbial organisms while leaving healthy, clean tissue (25). Afterwards, hemorrhage from the site is controlled with an irrigant and dried. The partial pulpotomy creates a space for a pulp capping procedure to be carried out.

There are two kinds of pulp caps: indirect and direct. Indirect pulp capping is a technique for cases of deep carious lesions without direct exposure of the root canal system. It is based on the belief that there are two zones of dentin: the infected outer layer, and affected inner layer (26). The infected layer is between the carious lesion and the affected layer is closer to the pulp. When the infected layer is removed, it is believed that the affected dentin has the ability to remineralize. This is facilitated by placement of a medicament over the remaining dentin to reduce the bacteria. Indirect pulp capping is controversial with some studies reporting repair in up to 99% of cases while others report high failures that increase with time (27, 28). Direct pulp capping is a procedure where the pulp is exposed but covered with a medicament that allows for healing and repair while being protected from further noxious stimulus. Historically, direct pulp caps were placed were placed on tissue where inflammation was still present (29). This led to inflammation beneath the pulp caps and low success rates (30). Over the years, this technique has changed to improve the outcome for vital pulp therapy.

Vital pulp therapy gained popularity in the 1960s for traumatic pulpal exposure (25). Historically, this treatment has only been popular for traumatic pulpal exposures (31). If a pulp exposure occurs before all the carious tooth structure is completely removed, then the tooth

undergoes what is called a carious pulp exposure. In the past, vital pulp therapy on carious exposures have been considered controversial and conventional endodontic therapy has been recommended in many cases (31). This was due in part to the lower success rates of direct pulp capping compared to conventional root canal treatment which was reported to be 64% and 83% respectively (32). Since then, the advent of newer materials and treatment protocols have raised success rates of vital pulp therapy to levels comparable to root canal therapy in specific cases (25, 33, 34).

Previous direct pulp capping protocols involved placement of medicaments directly on inflamed and contaminated pulpal tissue (31). Newer protocols involve attempts to disinfect or remove the superficial inflamed tissue via partial pulpotomy prior to placement of a direct pulp cap (25, 34, 35). Histology and clinical studies support evidence that only localized irreversible damage occurs initially (36). Removal of this superficial layer of inflamed pulp and contaminated dentin has been associated with better outcome (31). This new protocol along with better capping materials have brought back interest vital pulp therapy as a viable treatment.

Many materials have been used for direct pulp caps. These have included hydrophilic resins, zinc oxide eugenol and antibiotics mixed with glucocorticoids (37, 38). In the 1930s, a compound known as calcium hydroxide was discovered to be an effective material to cover a pulp exposure site with relatively high success rates (39). Calcium hydroxide is a white, crystalline, soluble salt that dissociates into calcium and hydroxyl ions when placed in a solution (40). In the first week after placement of calcium hydroxide on the pulp, a zone of pulpal necrosis is formed adjacent to the capping material while the rest of the pulp remains vital with few inflammatory cells (41). The zone of necrosis consists of three layers. The most superficial zone is formed due to pressure during application of the material and edema from the middle layer. The middle layer

consisted of edema and necrosis caused by chemical injury from the medicament. The tissue and plasma partly neutralizes the hydroxyl ions from the calcium hydroxide leading to a weaker effect in the deepest layer (38). The alkaline effect from calcium hydroxide is of short duration. In vitro testing of the medicament on cell cultures showed that the pH drops to a range conducive for cell growth after 24 hours and did not have any lasting negative effects on cell proliferation. There is initial inflammation followed by migration and proliferation of pulpal cells to areas adjacent to the necrotic zone. New collagen is laid down after 4 days with increased DNA synthesis in the cells indicating increased cellular activity. This activity leads to formation of a calcific barrier known as a dentin bridge.

The dentin bridge formed by calcium hydroxide has been extensively studied (42, 43). It is not entirely known how the dentin bridge is formed however several theories exist. The high alkalinity environment produced by calcium hydroxide creates a favorable environment for the activation an enzyme, alkaline phosphatase, used for mineralization (44). Even though calcium ions are a component of the dentin bridge, it is not from calcium hydroxide. In a transmission electron microscope study, it was found that mineralization begins in the deepest layer of necrosis after 7 days (45). Degenerated pulp cells were seen near the necrotic zone with more collagen located more apically. Matrix vesicles like the ones that assist in mineralization of cartilage and bone were observed in the area. After 1 month, an irregular bone-like barrier was found with pre-dentin-like tissue incorporated into the bridge. After 3 months, the barrier had two layers: the coronal layer consisted of irregular dentin-like tissue with irregular tubules while the deeper layer had pre-dentin characteristics with collagen fibrils. These two layers make up the dentin bridge. Although the dentin bridge is not an indication of healing, it does serve as a barrier to microleakage which affects the outcome of vital pulp therapy.

One characteristic that has been scrutinized are structural defects in the dentin bridge known as tunnel defects. These defects increase the permeability of the dentin bridge and allow for infection due to microleakage leading to inflammation of the pulp (46). Despite this drawback calcium hydroxide in the form of a powder or paste is still used in many direct pulp capping procedures. Although it was once the gold standard medicament, its association with bacterial leakage and tunnel defects have led to the development of newer materials.

Tricalcium silicate based materials such as mineral trioxide aggregate (MTA) were first approved by the FDA in 1998 (47). Mineral trioxide aggregate is a variation of Portland cement and was found to have success rates comparable or better than calcium hydroxide (47, 48). Tricalcium silicates are typically composed of tricalcium silicate and dicalcium with varying amounts of silicate, tricalcium aluminate, tetracalcium aluminoferrite, gypsum and bismuth oxide (49). It typically comes in a powder form. Hydration of the powder turns the material into a gel which solidifies into a hard structure. Like calcium hydroxide, tricalcium silicates have been shown to induce dentinal bridge formation but at a faster rate with less pulpal side effects (50). Although the exact mechanism by which it induces dentinal bridge formation is not known, it is theorized that the tricalcium oxide component reacts with fluids from the pulp to form calcium hydroxide and then dentinal bridge formation ensues in a similar manner as calcium hydroxide (51). Unlike calcium hydroxide, tricalcium silicates are not associated with a high incidence of tunnel defects in the dentinal bridge (52). This results in better resistance to microleakage. Unlike calcium hydroxide, it functions well in the presence of blood. Tricalcium silicates have replaced calcium hydroxide as the gold standard in pulp capping material (53). Today, both materials are commonly used in direct pulp capping procedures. Despite the advances in new material, the

procedure itself has changed very little with one of the largest difficulties being diagnosis of the pulp status.

Accurate diagnosis of the pulpal status is paramount to the success of any endodontic treatment and direct pulp caps are no different. The status of the pulp is a major factor in determining whether the treatment will be successful or not. In teeth where there is progressive inflammation, this will lead to total necrosis of pulp. These cases are regarded as irreversibly inflamed pulps and should not be treated with vital pulp therapy. If the pulp is still able to recover from the inflammation after treatment, then the pulp is classified as reversible pulpitis. Historically, this diagnosis has been difficult to determine clinically (54, 55). The gold standard of diagnosing the pulpal status is histological examination of the pulpal tissue. Unfortunately, this is not practical in a clinical setting. Instead of histology, dentists use five methods to obtain information to form a preoperative diagnosis of the pulp. These methods include patient symptoms, radiographic exam, thermal testing, electric pulp testing, and percussion testing.

Patient symptoms and tests such as percussion and palpation are an integral part of endodontic diagnosis. Percussion testing involves the application of a slight tapping force to each tooth to evaluate for tenderness. This test can be used to detect signs of apical periodontitis in conjunction with symptoms the patient may have (56). Palpation testing, on the other hand, can provide valuable information indicating whether symptoms have spread to the overlying periodontium.

Thermal testing involves placing either a hot or cold stimulus on the surface of the tooth to evaluate for a response form the patient. Even though the mechanism by which temperature is transmitted through dentine are not completely understood, it is believed that at some point, thermal stimulus is converted into electrical signals by sensors in the tooth. Light and electron

microscopy have shown that the dentin is porous. The neuron is the most basic cell of the nervous system and is able to transmit electrical signals known as nerve impulses to other cells (57). Its mechanism is achieved by changes in the cell membrane's ability to separate positive and negative ions resulting in a resting potential. Stimulation of the neuron will cause a depolarization in the cell membrane along the length of the neuron known as an action potential thus lending to the cell's ability to transmit the nerve impulse to the next cell. The presence of nerve fibers in dentinal tubules has been detected but it does not extend throughout the entire length of the tubule (58). In addition to the nerve endings, cells known as odontoblasts that formed the dentin of the tooth remain in the periphery of the pulp. These cell have processes that extend into the dentinal tubules (59). One theory is direct stimulation of the nerve fibers from the thermal stimulus. It states that the temperature from the thermal testing travels through the tooth and eventually reaches the nerve where the nerve senses the temperature change and transmits the signals further to the brain. It has been noted that the sensory response to thermal stimulation occurs before there is a temperature change in the region of the nerve fibers (60). Another theory is the odontoblast acts as a transducer between the thermal stimulus and the nerve endings that transmit the signal to the brain. However, studies have indicated odontoblasts are not physically capable of transmissions of these kinds (61). The most widely accepted theory is the hydrodynamic theory. This theory predicts that the movement of fluids through the dentinal tubules stimulate the nerve endings that are located some distance away from the dentinal tubules (58). Temperature change and air flow across exposed dentinal tubules can cause movement of fluid. The hydrodynamic theory answers the question as to why the part of the dentine containing no nerve fibers can be sensitive to thermal stimuli as well as why there is a response to temperature when the temperature change has not yet reached the nerve endings.

The electric pulp test is another method that can measure pulp vitality (62). This method consists of conducting an electrical current to the tooth being evaluated. Vital receptors inside the pulp can detect the current and transmit sensations to the brain. Non-vital pulps lack these vital receptors therefore should produce no response. This method can be a useful supplement to thermal testing but unfortunately cannot be used if there is not any dentin or enamel exposed on the tooth being tested.

Radiographic examination can also be useful in diagnosis of endodontic pathology. Endodontic pathology with necrotic pulps can be associated with radiolucencies on periapical radiographs if enough bone loss has occurred (63, 64). Lack of a periapical radiolucency does not necessarily indicate a healthy pulp and presence of a periapical radiolucency does not always indicate endodontic pathology. However this is still a very important diagnostic test that is a part of an endodontic evaluation (65).

In most cases when a carious pulp exposure is encountered, the patient is offered two options: root canal therapy or extraction. As mentioned previously, root canal therapy can be very costly for the patient causing many to become focused on the high initial cost of the procedure and lead them to choose extraction (66). Extraction of the tooth can lead to a short term financial gain but can become very costly down the road. It can be very costly to replace the missing tooth and lead to morbidities such as nonfunctional edentulous spaces and compromise the integrity of the dental arch. Vital pulp therapy can have a far reach with a low cost especially in underserved areas but is rarely offered as an option. This can change if the procedure becomes more predictable by identifying pre-operative factors that influence the outcome.

There are indications that factors such as the patient's gender, age, and exposure site may affect the outcome of direct pulp caps (2, 34, 35, 67). While these studies provide valuable insight

into the topic, most of them are retrospective and have small sample sizes (1-3, 33, 67, 68). A prospective investigation of these factors can provide more evidence on the matter. By providing clinicians the information on which factors are associated with the outcome of the procedure, they can make a better clinical decision as well as have a more thorough discussion with the patient. This can lead to better and more efficient care of the patient. We hypothesize that the patient's age, gender, tooth type, and exposure site are pre-operative factors that influence the outcome of vital pulp therapy.

MANUSCRIPT

Section 1.1 Introduction

Vital pulp therapy such as direct pulp caps on mature, permanent teeth have been shown to be successful with recalls up to 9 years postoperatively (33). It is a cost effective, minimally invasive procedure that saves time, and effort for both the clinician and the patient (69). The procedure was once only commonly accepted in traumatic pulp exposures. However, most clinicians treat carious pulpal exposures with conventional root canal therapy (31). This is due in part to the erratic long term success rates reported for the procedure ranging from 13% to 97% (33, 68). Successful treatment relies on treating reversible pulpal inflammation, removing the source of the inflammation, and preventing future microbial leakage. There has been mounting interest in the topic with the advent of newer materials that are more biocompatible and improves the seal against future leakage (39).

Calcium hydroxide was once the gold standard with its ability to prevent bacterial growth and high pH (70). It initially induces a zone of necrosis in the pulp and stimulates formation of a dentinal bridge. Unfortunately, calcium hydroxide has been associated with bacterial leakage with pores in the dentinal bridge known as tunnel defects (50). Tunnel defects allow for leakage of microbial elements leading to pulpal inflammation (42). Tricalcium silicate based materials such as mineral trioxide aggregate (MTA) have since replaced calcium hydroxide as the gold standard material. Like calcium hydroxide, these newer materials are able to induce hard-tissue formation but studies have indicated better biocompatibility with thicker dentinal bridge formation that have less tunnel defects (50). Most importantly, the ability of tricalcium silicates to resist bacterial

penetration and remain effective in the presence in blood make it a superior material to calcium hydroxide. Recent studies comparing the two materials show better outcome with tricalcium silicates compared to calcium hydroxide (2, 3).

While the introduction of tricalcium silicates has resulted in better outcomes, it is yet to be determined whether there are other factors that affect the outcome of vital pulp therapy. Other factors have been suggested however most of these studies are retrospective or have small sample sizes (2). The objective of this prospective, observational study is to evaluate the association between pre-operative factors associated with the outcome of direct pulp capping.

Section 1.2 Materials and Methods

This prospective study was approved by the Office of Human Ethics at our institution. Written informed consent was obtained from each participant. Patient demographics, history of odontogenic pain, treatment notes, and postoperative data including symptoms, and follow-up examinations were recorded.

The inclusion criteria were healthy men and women (aged 15 years or older) undergoing routine treatment who experienced a vital pulp exposure on a mature, permanent tooth during caries removal. Confirmation of a vital pulp was established visually after caries removal. Exclusion criteria includes history of pain indicative of irreversible pulpitis (e.g. patient presents with or reports history of spontaneous or exaggerated pain from the tooth), inability to obtain hemostasis at the exposure site, and medically compromised patients ASA Class 3 or higher.

Section 1.3 Capping Procedure

The procedure was carried out with a standardized protocol using calibrated clinicians. Each case was isolated prior to the procedure and caries removal was standardized using Sable™ Seek® caries indicator (Ultradent Products, Inc, South Jordan, UT, USA). Slight pressure was

applied to the exposure site with a sterile cotton pellet slightly moistened with approximately 4.125% dilution of sodium hypochlorite for 60 seconds which was then removed and the site dried gently with an indirect gentle spray of air from the air water syringe. If hemorrhage was not controlled from the exposure site, a 1 mm diameter diamond round bur in a high speed handpiece was used to prepare 1 mm circumferentially into and around the exposure site to remove additional inflamed pulpal tissue. Pressure from a fresh sterile cotton pellet was applied and the site was dried again. This cycle could be repeated up to a total of 3 times to control any hemorrhage if needed. If the hemorrhage was not controlled after the 3 cycles, the patient was excluded from the study. White MTA Angelus® (Angelus, Londrina, Brazil) was prepared by following the manufacturers recommendation. It was transported using either an amalgam carrier or Dovgan MTA carrier. The exposure site was sealed with a layer of MTA approximately 3 mm thick. The MTA was then covered with a layer of Vitrebond™ Plus (3M™, St. Paul, MN, USA) and the tooth was restored immediately. A periapical radiograph of the tooth was taken and the patient was given verbal expectations about post-operative symptoms and follow-up.

Section 1.4 Follow up

Each patient was followed up with a phone call at 24 hours to collect data on postoperative symptoms and any analgesics used. Pain levels were evaluated using a standardized questionnaire. Data included a rating of pain on an 11-point numerical scale and information on the quality of the pain. Additionally, data regarding analgesic intake including the type, quantity, and frequency was obtained. The same telephone questionnaire was repeated at 1 week and 3 months post-operatively.

At 6 months and 1 year, each patient was asked to return for a clinical exam involving standard endodontic testing (percussion, palpation, mobility, probing, cold testing, and electric

pulp testing) as well as a periapical radiograph to evaluate for any pathology. The examination was conducted by study investigators who were calibrated.

Section 1.3 Statistical Analysis:

Sample size analysis was based on prior studies on vital pulp therapy (71). The main outcome variable for this study was success or failure at 6 months. Data was analyzed using the "R" function "glm" for generalized linear model with family (success/failure) as "binomial". Success was defined as a tooth that exhibited a positive response to the pulp sensibility testing without evidence of irreversible pulpitis or pulp necrosis. The procedure was considered a failure if additional treatment such as root canal therapy or extraction was indicated.

The data was also examined for association between selected peri-operative predictors and post- treatment pain. For size of the exposure, the data was log transformed and linear regression was fit with the predictor and outcome variable. All the statistical analysis was performed in R statistical software (version 3.2.3, www.cran.r-project.org).

Section 1.4 Results:

Over a period of 15 months between 2015 and 2017, a total of 73 healthy men and women were enrolled (Figure 1). 71 of the 73 patients were available for the 24-hour follow-up and 1-week follow-up. 55 out of 68 patients were available for 3-month phone calls. 51 out of 59 patients were available for the 6-month clinical follow-up and 23 out of 36 patients were available for the 1 year follow-up. The patients were between the ages of 15-78 years old (Figure 2). The mean age was 46.7 years old with a standard deviation of 18.8. 32 subjects were 40 years old or younger and 41 subjects were over the age of 40. 10 subjects were between 70 and 80 years old. Subjects consisted of 24 males and 49 females (Figure 3). Teeth that were treated consisted of 19 incisors, 19 premolars, and 34 molars (Figure 4). The site of exposure was examined in this study. 30 cases

had exposure sites that involved the proximal surface of the tooth while 43 did not (Figure 5). Failures were documented with notes indicating suspected cause of failure as well as the timespan between when the VPT was completed and when the failure was detected (Table 1). 3 failures were detected at 1 week, the next 2 failures did not occur until 4 months postoperatively. 2 failures were detected at 6 months and 2 more at 7 and 8 months.

Using the generalized linear model with predictors – pain at 24 hours, pain at 1 week and pain at 3 months, tooth type and patient' age, pain was found to be significantly associated with failure at 3 months (p=0.028). We also noted marginal significance between patients age and failure (p=0.059) with failure rates being higher in older patients. On analyzing our data for an association between exposure size and post-treatment pain, a marginal significance (p=0.0596) was noted between exposure size and pain at 24 hours.
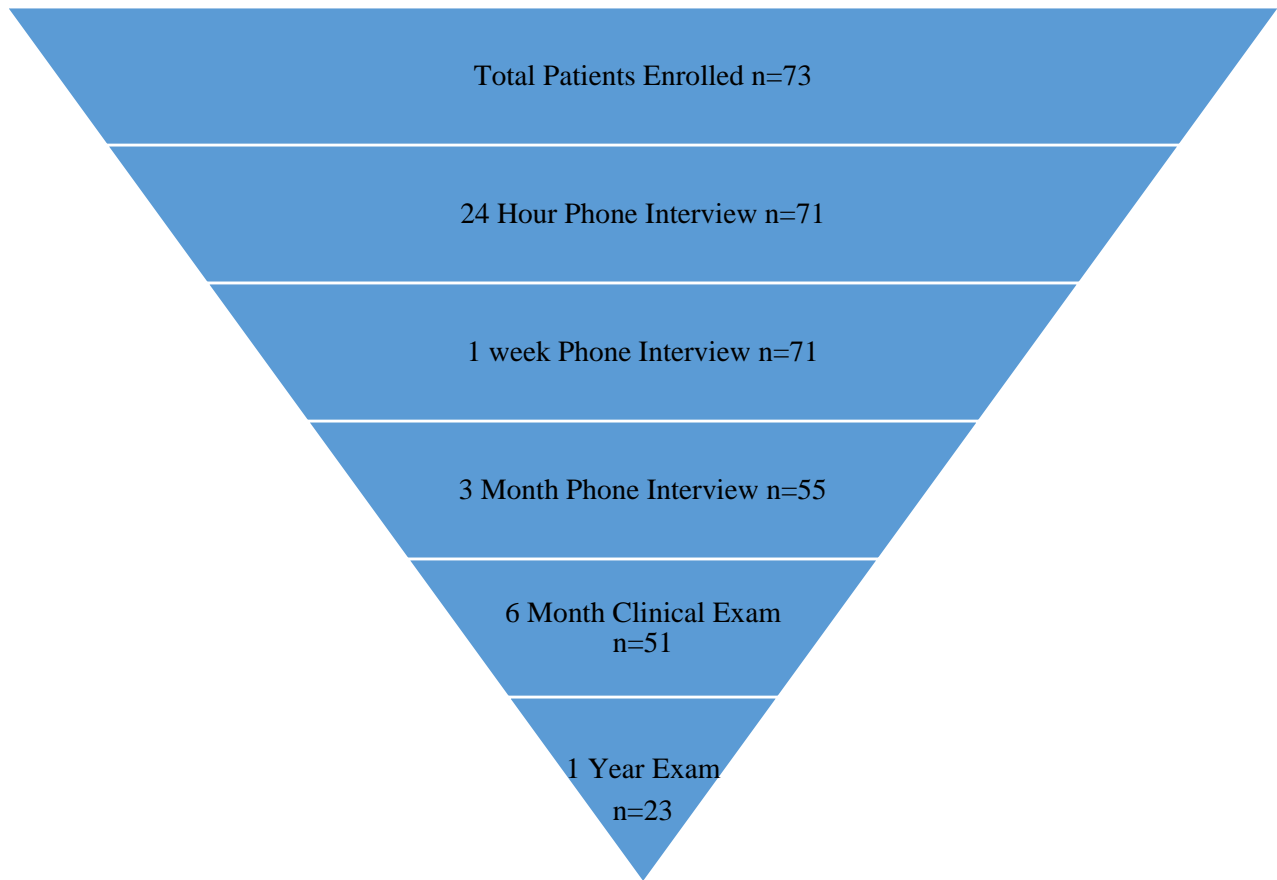
Figure 1: Subject Flow. 73 total subjects participated in the pulp capping procedure. 71 were available for 24-hour follow-up and 1-week follow-up. 55 patients were available for 3-month phone calls. 51 returned for a 6-month in person exam and 23 subjects were available for the 1-year exam.
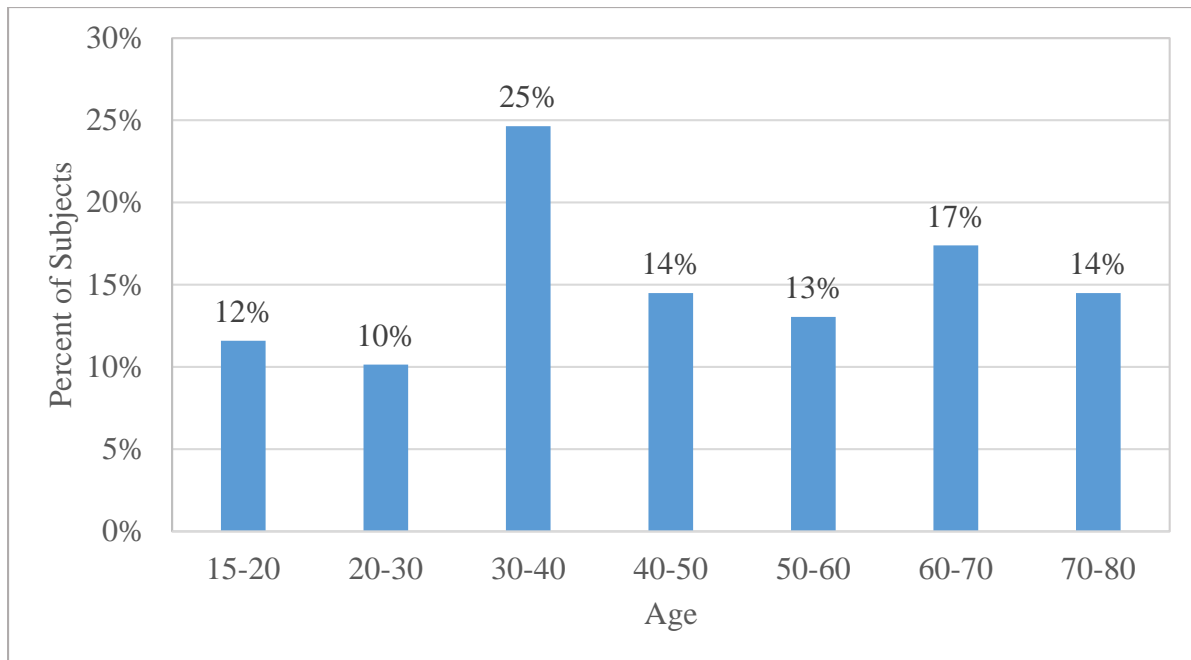
Figure 2: Age Group Distribution. Ages of subjects ranged from 15 to 78 years old with 32 subjects 40 years old and younger, and 41 subjects older than 40 years old.
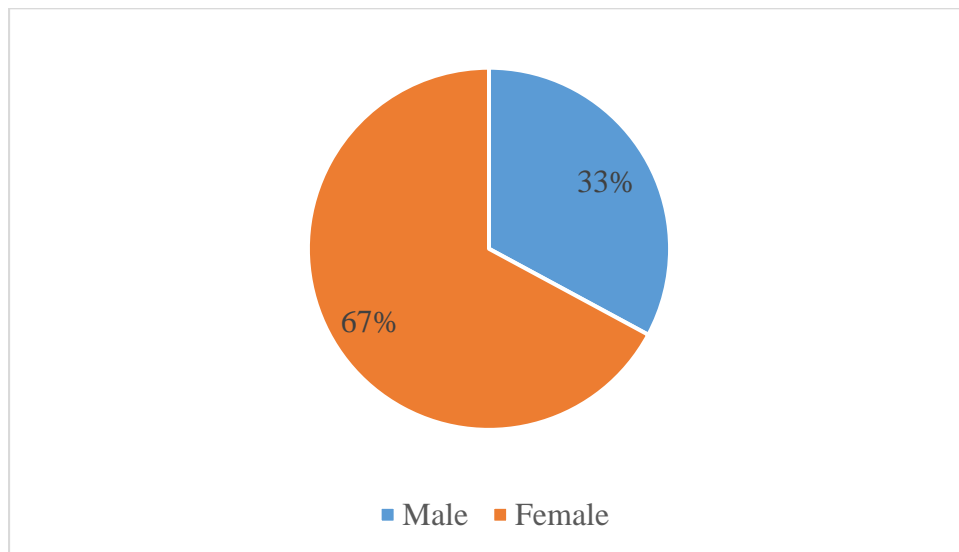

Figure 3: Gender Distribution. A majority of patients were female with 33% (n=24) male and (n=49) 67% female.
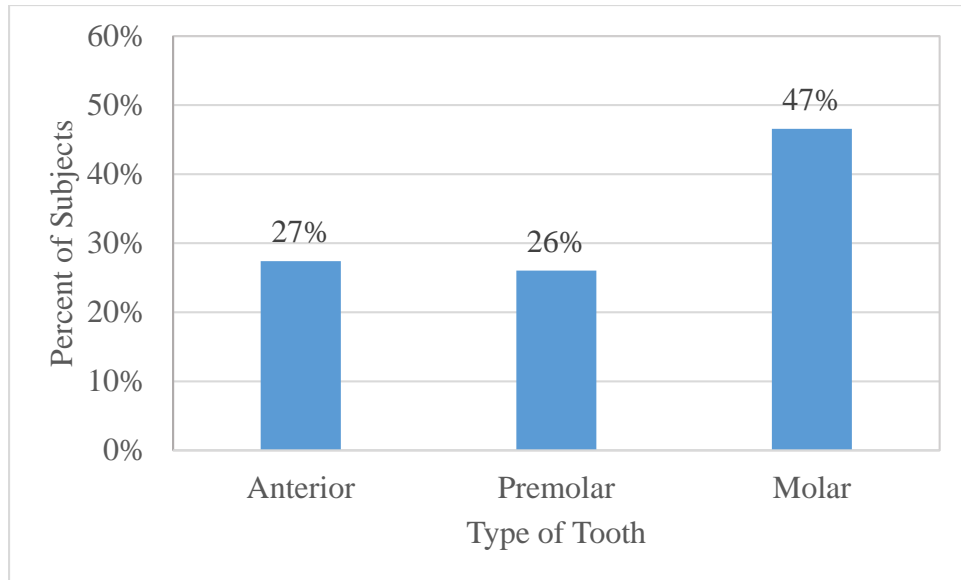
Figure 4: Tooth Types Treated. All tooth types were evaluated with a majority of cases being molars. There were 20 anterior teeth, 19 premolars, and 34 molars treated over the course of this study.
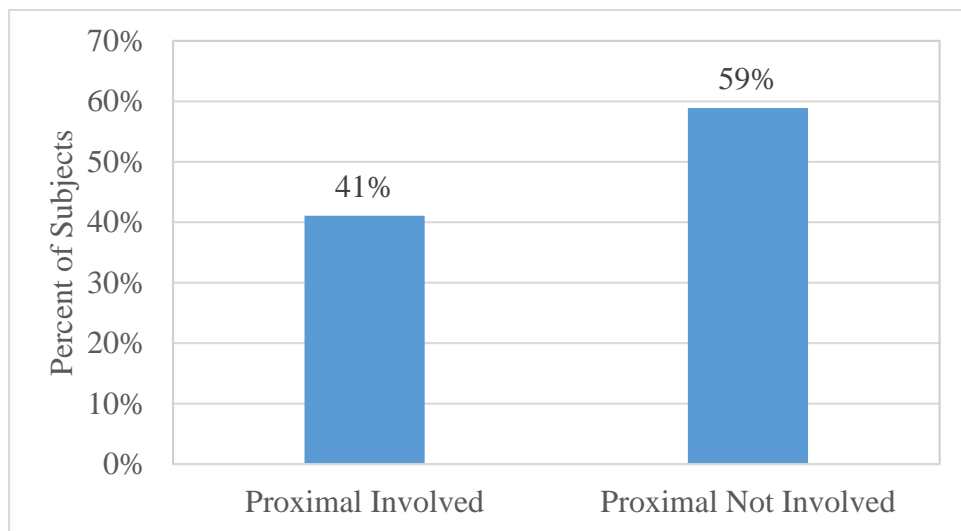

Figure 5: Proximal Surface Involvement. 30 cases had pulp exposures involving the proximal surface while 43 cases did not.
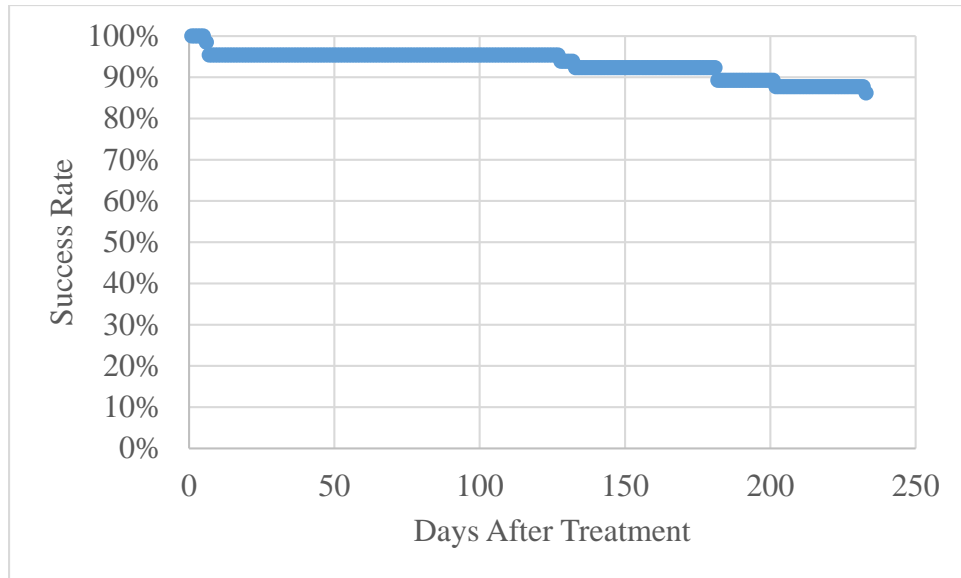
Figure 6: Success Related to Time. Showing the number of success as related to time. 3 failures were detected at 1 week, the next 2 failures did not occur until 4 months postoperatively. 2 failures were detected at 6 months and 2 more at 7 and 8 months.

Case	Age	Tooth#	Sex	Days til Failure Detected	Exposure Site	Notes
1	62	31	female	6	O	Crack
2	76	3	male	182	MF	Pulpal necrosis
3	33	14	male	7	D	No notes
4	72	15	female	202	ML	Exposure site left open for several hours
5	33	30	female	182	O	Spontaneous pain reported prior to VPT
6	78	22	female	182	D	PARL PN/AAP
7	78	23	female	133	D	PARL PN/AAP
8	39	4	female	233	OM	SIP/SAP
9	33	15	female	128	MO	PN/SAP, Caries under restoration
10	45	14	male	7	M	PN/AAA

Table 1: Distribution of Teeth. Details showing each failed case including any notes regarding findings during treatment. PARL=Periapical radiolucency, PN=Pulpal necrosis, AAP=Acute apical periodontitis, SIP=Symptomatic irreversible pulpitis, SAP=Symptomatic apical periodontitis, AAA=Acute apical abscess.

Section 1.5 Discussion:

Many clinicians believe that vital pulp therapy for teeth with carious exposures has a low success rate (31). This is due in part to prior studies done with calcium hydroxide (68). Recent studies based on tricalcium silicates report higher success rates in the range of 80%-90% (2, 34, 35). The purpose of the current study was to build upon prior studies to examine factors associated with outcome of VPT when capped with tricalcium silicates. This study was not designed to evaluate success rates but instead it focused on preoperative factors that may associate with outcome.

The capping material we used in this study was MTA Angelus. While there are many different formulations of tricalcium silicates, MTA is the most extensively studied to date (39).

Studies comparing different formulations of MTA did not find any significant differences in the pulpal responses (72). MTA Angelus has the beneficial therapeutic effects of traditional MTA with the added benefit of a faster setting time. Traditional MTA takes up to 4 hours to set while MTA Angelus was reported to set in 15 minutes (39). The quicker setting time allowed for placement of the pulp cap and restoration in one appointment rather than two appointments thus removing the risk of leakage through the temporary restoration.

Inability to obtain hemostasis from the exposure site was one of the exclusion criteria in this study. Excess bleeding was used as a surrogate measure of inflammation based on prior studies which report a 40% increase in blood flow in inflamed pulps (73). Systemic factors such as medications, herbs, or systemic disease can cause excess bleeding as well. Medications and herbs such as clopidogrel, rivaroxaban, and ginseng are becoming more popular. Systemic conditions such as vitamin K deficiency is a factor as well. These can cause dramatic effects on the flow of blood through the body (74). While these conditions can lead to increased bleeding, there are factors that can reduce bleeding.

The local anesthetic commonly used prior to dental treatment contains epinephrine, a vasoconstrictor. Epinephrine from the local anesthetic can affect regional blood vessels leading to a reduction in blood flow of up to 40% compared to anesthetics without epinephrine (75). Additionally, the patient's age can affect blood flow. In older teeth, there is a general decrease in the number of blood vessels (76). The size of the pulp chamber reduces in size with deposits of calcifications in the root and coronal pulp. These factors as well as a partially necrotic pulp can lead to less blood flow in the region. Even though we used hemorrhage as a measure of inflammation, we are aware that many factors can influence this characteristic.

This study evaluated tooth type and site of the exposure as a possible factor of outcome. Since the size and configuration of each pulp chamber is different from one tooth type to another, the two factors may influence the pulp's ability to heal as suggested by several studies (77, 78). The thickness of pulp immediately beneath the pulpal exposure site can vary. This variation can play a role in the healing potential. For example, mandibular anterior teeth have narrow pulp chambers mesiodistally compared to the labiolingual dimension. A pulp exposure on the proximal surface would affect the pulp in an area that is thinner than a pulp exposure facially or lingually. Although this is the case for mandibular incisors, it is quite the opposite in maxillary incisors. Maxillary incisors typically have pulp chambers which are wider mesiodistally while being narrower labiolingually. Additionally, as we move posteriorly, it becomes more difficult to isolate and access certain areas. Interproximal areas in posterior teeth are typically significantly more difficult to treat than the facial of an anterior tooth. This could affect the clinician's ability to properly adapt restorations or medicaments to the area thereby affecting the outcome.

The results of this study suggest that none of the preoperative factors examined are correlated with failure of the treatment. Like many other studies, age was evaluated as a possible factor influencing the outcome however we were unable to find any correlation. Other factors examined including site of exposure as well as tooth type did not affect the outcome. There could be three possibilities for this explanation. The number of subjects may not have been sufficent, the number of failures may not have been at a level high enough to detect a difference, or there simply may not be a significant association between these factors and outcome.

A goal of the procedure is to maintain the vitality of a healthy pulp. Obtaining the correct pulpal diagnosis can be challenging (55, 79). A correct diagnosis is crucial in order to provide the appropriate treatment plan and prognosis of the tooth. Standard endodontic tests used today are

used to reproduce symptoms and help distinguish between reversible pulpitis, irreversible pulpitis, and pulpal necrosis. This distinction is paramount since vital pulp therapy will only be effective in reversible pulpitis and not the other two. It was traditionally believed that in cases of carious exposures on mature permanent teeth, the pulp is irreversibly inflamed and there is little chance of healing. Histologically, previous studies have indicated that although there is a pulpal exposure, only the area immediately adjacent to the exposure site has irreversible damage (36). The other areas have the potential to heal. In several of our cases that resulted in failure, some multi-rooted teeth were found to have developed partial necrosis (Table 1). The tissue in one root canal was necrotic while the rest still had vital tissue. This reinforces the notion that the pulp can compartmentalize itself and try to separate diseased from healthy pulp tissue. Standard diagnostic terms and techniques used today have limitations in practice. A diagnostic technique that may have future potential is chairside molecular assessment of pulpitis.

We are not yet able to fully comprehend the changes of the pulp at the molecular level. We know that specific mediators have been associated with inflammation such as irreversible pulpitis. These mediators include elevation in CGRP, Substance P, Neurokinin A, TNF-α, bradykinin, Prostaglandin E_2 and F_2 (80, 81). Although there are many other inflammatory mediators, measuring a few key markers may potentially predict the outcome of vital pulp therapy (82). It is possible that in the future instead of relying on the subjective endodontic testing we use today, we can rely on a chairside sampling of mediators from the pulp for a clear diagnosis.

Conclusion:

Based on our findings, we confirmed that vital pulp therapy is a viable treatment. Age should be factored into the preoperative prognosis for vital pulp therapy. Patients with mild to

moderate postoperative pain be managed with analgesics first, before additional treatment. If there's still pain at 3 months, then further evaluation is needed.

DISCUSSION

Many clinicians believe that vital pulp therapy for teeth with carious exposures has a low success rate (31). This is due in part to prior studies done with calcium hydroxide (68). Recent studies based on tricalcium silicates report higher success rates in the range of 80%-90% (2, 34, 35). The purpose of the current study was to build upon prior studies to examine factors associated with outcome of VPT when capped with tricalcium silicates. This study was not designed to evaluate success rates but instead it focused on preoperative factors that may associate with outcome.

The capping material we used in this study was MTA Angelus. While there are many different formulations of tricalcium silicates, MTA is the most extensively studied to date (39). Studies comparing different formulations of MTA did not find any significant differences in the pulpal responses (72). MTA Angelus has the beneficial therapeutic effects of traditional MTA with the added benefit of a faster setting time. Traditional MTA takes up to 4 hours to set while MTA Angelus was reported to set in 15 minutes (39). The quicker setting time allowed for placement of the pulp cap and restoration in one appointment rather than two appointments thus removing the risk of leakage through the temporary restoration.

In many studies on VPT with MTA, the treatment was completed using a 2-step protocol to confirm setting of the MTA (3, 34, 35, 83, 84). The first visit consisted of caries removal, capping the pulp with MTA, placing a cotton pellet moistened with saline, and sealing the tooth with a temporary restoration. After allowing several days for setting, the patient would return to have the temporary restoration removed to evaluate if the MTA was set. After setting is confirmed,

the tooth is then restored permanently. Temporary materials like the ones used for the 2-step protocols have the potential to leak (85). Leakage from an inadequate seal violates one of the goals of vital pulp therapy which is to prevent future leakage. Requiring a second visit is also time consuming for the patient as well as the clinician. This study utilized a one-step approach to treatment. Immediate sealing of the tooth with a permanent restoration has indicated better outcome (3, 68). We did not believe setting of the MTA was a concern. Studies indicate moisture content in the dentin as well as from the pulp provides sufficient moisture for setting of the material (86). Prior studies utilizing a 2-step protocol indicated no difficulty in setting of the MTA suggesting that a 2-step protocol is unnecessary (1, 34).

Inability to obtain hemostasis from the exposure site was one of the exclusion criteria in this study. Excess bleeding was used as a surrogate measure of inflammation based on prior studies which report a 40% increase in blood flow in inflamed pulps (73). Systemic factors such as medications, herbs, or systemic disease can cause excess bleeding as well. Medications and herbs such as clopidogrel, rivaroxaban, and ginseng are becoming more popular. Systemic conditions such as vitamin K deficiency is a factor as well. These can cause dramatic effects on the flow of blood through the body (74). While these conditions can lead to increased bleeding, there are factors that can reduce bleeding.

The local anesthetic commonly used prior to dental treatment contains epinephrine, a vasoconstrictor. Epinephrine from the local anesthetic can affect regional blood vessels leading to a reduction in blood flow of up to 40% compared to anesthetics without epinephrine (75). Additionally, the patient's age can affect blood flow. In older teeth, there is a general decrease in the number of blood vessels (76). The size of the pulp chamber reduces in size with deposits of calcifications in the root and coronal pulp. These factors as well as a partially necrotic pulp can

lead to less blood flow in the region. Even though we used hemorrhage as a measure of inflammation, many factors can influence this characteristic.

This study evaluated tooth type and site of the exposure as a possible factor of outcome. Since the size and configuration of each pulp chamber was different from one tooth type to another, the two factors may influence the pulp's ability to heal as suggested by several studies (77, 78). The pulp thickness beneath the pulpal exposure site can vary. This variation can play a role in the healing potential. For example, mandibular anterior teeth have very narrow pulp chambers mesiodistally compared to the labiolingual dimension. A pulp exposure on the proximal site could affect the pulp in an area that is thinner than a pulp exposure facially or lingually. Although this is the case for mandibular incisors, it is quite the opposite in maxillary incisors. Maxillary incisors typically have pulp chambers which are wider mesiodistally while being narrower labiolingually. Additionally, as we move posteriorly, it becomes more difficult to isolate and access certain areas. Interproximal areas in posterior teeth are typically significantly more difficult to treat than the facial of an anterior tooth. This could affect the clinician's ability to properly adapt restorations or medicaments to the area thereby affecting the outcome.

Long term follow-up periods provide valuable information on treatment success. However, this study only evaluates follow-up at 6 months and 1 year. Most failures of vital pulp therapy have been suggested to occur in the first 3 months after the procedure and most will remain relatively stable afterwards until 18 months (2). Many cases in this study that failed had the failures detected in the first several days when the patient came back in severe pain. Failures at the 6 month in-person examination were patients that only had mild or no discomfort. There was not enough data to determine if there is significant correlation between these two factors, however it may be

underscore the importance of an in-person examination for follow-up at 6 months to ensure the treatment was successful.

A previous study reported high success rates of the VPT for up to 9 years (33). This indicates that vital pulp therapy can have long term results provided the treatment is rendered in ideal circumstances. In that study, the procedures were performed by a single operator who was a specialist. Preoperative testing was performed and all teeth treated had no previous restorations. The study was also conducted on a younger age group with the mean age of 16 years old. Although this study was well controlled, it does not reflect the reality of dental care.

Our study was designed in a way to reflect the realities of a dental clinic. In many cases, a pulpal exposure was not evident until caries removal had already begun. In these cases, the patient was already anesthetized rendering pulpal testing unavailable. This scenario reflects real world dentistry where unanticipated pulp exposures occur without prior pulp testing. Although pulp testing was unavailable, a thorough history of symptoms was reviewed with each patient. This could still be accomplished after anesthesia is administered.

Vital pulp therapy could have a profound impact on the quality of life of patients especially those in underserved areas. As mentioned previously, most practitioners recommend either root canal therapy or extraction as options for carious pulpal exposures. Both have high costs overall while vital pulp therapy is often unmentioned. This is an enormous disservice to patients especially in clinics serving lower income patients. Vital pulp therapy can make a tremendous impact in these areas where root canal therapy is not affordable by many patients and extraction is commonly elected. Vital pulp therapy can satisfy the goal of preventing apical periodontitis and maintaining a functioning tooth for the patient for very little cost.

The results of this study suggest that none of the preoperative factors examined are correlated with the treatment's outcome. Like many other studies, age was evaluated as a possible factor influencing the outcome however we were unable to find any significant correlation. Other factors examined including site of exposure as well as tooth type did not affect the outcome of the procedure as well. There could be three possibilities for this explanation. The number of subjects may not have been adequate, the number of failures may not have been at a level high enough to detect a difference, or there simply may not be a significant association between these factors and outcome. Future studies could add more to the literature by including more cases as well as for a longer time period.

A goal of the procedure is to maintain the vitality of a healthy pulp. Obtaining the correct pulpal diagnosis can be challenging (55, 79). A correct diagnosis is crucial in order to provide the appropriate treatment plan and prognosis of the tooth. Standard endodontic tests used today are used to reproduce symptoms and help distinguish between reversible pulpitis, irreversible pulpitis, and pulpal necrosis. This distinction is paramount since vital pulp therapy will only be effective in reversible pulpitis and not the other two. It was traditionally believed that in cases of carious exposures on mature permanent teeth, the pulp is irreversibly inflamed and there is little chance of healing. Histologically, previous studies have indicated that although there is a pulpal exposure, only the area immediately adjacent to the exposure site has irreversible damage (36). The other areas have the potential to heal. In several of our cases that resulted in failure, some multi-rooted teeth were found to have developed partial necrosis (Table 1). The tissue in one root canal was necrotic while the rest still had vital tissue. This reinforces the notion that the pulp can compartmentalize itself and try to separate diseased from healthy pulp tissue. Standard diagnostic

terms and techniques used today have limitations in practice. A diagnostic technique that may have future potential is chairside molecular assessment of pulpitis.

Our study measures success by both clinical and radiographic signs. One study that is commonly quoted as evidence vital pulp therapy reports a 10-year success rate of 13% (68). Interestingly, it considers success only to be in teeth that respond to both thermal testing and electric pulp testing. This may be too strict a measure of success because clinicians rarely require a positive response to both tests in order to rule our disease (36). Our study, like many others, only requires a positive response to either cold or EPT. If the tooth responds to one or the other, it indicates that there is vital tissue which is one of the goals.

We are not yet able to fully comprehend the changes of the pulp at the molecular level. We know that specific mediators have been associated with inflammation such as irreversible pulpitis. These mediators include elevation in CGRP, Substance P, Neurokinin A, TNF-α, bradykinin, Prostaglandin E_2 and F_2 (80, 81). Although there are many other inflammatory mediators, measuring a few key markers may potentially predict the outcome of vital pulp therapy (82). It is possible that in the future instead of relying on the subjective endodontic testing we use today, we can rely on a chairside sampling of mediators from the pulp for a clear diagnosis.

REFERENCES

1.   Caliskan MK, Guneri P. Prognostic factors in direct pulp capping with mineral trioxide aggregate or calcium hydroxide: 2- to 6-year follow-up. Clin Oral Investig 2016.

2.   Cho SY, Seo DG, Lee SJ, Lee J, Lee SJ, Jung IY. Prognostic factors for clinical outcomes according to time after direct pulp capping. J Endod 2013;39(3):327-331.

3.   Mente J, Hufnagel S, Leo M, Michel A, Gehrig H, Panagidis D, et al. Treatment outcome of mineral trioxide aggregate or calcium hydroxide direct pulp capping: long-term results. J Endod 2014;40(11):1746-1751.

4.   Kakehashi S, Stanley HR, Fitzgerald RJ. The Effects of Surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats. Oral Surg Oral Med Oral Pathol 1965;20:340-349.

5.   Moller AJ, Fabricius L, Dahlen G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res 1981;89(6):475-484.

6.   Fluoridation USDoHaHSFPoCW. U.S. Public Health Service Recommendation for Fluoride Concentration in Drinking Water for the Prevention of Dental Caries. Public Health Reports 2015;130.

7.   Richardson S. Access to Endodontic Care in North Carolina Public Health and Medicaid Settings. 2012.

8.   Ritwik P, Massey C, Hagan J. Epidemiology and outcomes of dental trauma cases from an urban pediatric emergency department. Dent Traumatol 2015;31(2):97-102.

9.   Hahn CL, Liewehr FR. Update on the adaptive immune responses of the dental pulp. J Endod 2007;33(7):773-781.

10.   Hahn CL, Liewehr FR. Innate immune responses of the dental pulp to caries. J Endod 2007;33(6):643-651.

11.   Hahn CL, Liewehr FR. Relationships between caries bacteria, host responses, and clinical signs and symptoms of pulpitis. J Endod 2007;33(3):213-219.

12.   Nagaoka S, Miyazaki Y, Liu HJ, Iwamoto Y, Kitano M, Kawagoe M. Bacterial invasion into dentinal tubules of human vital and nonvital teeth. J Endod 1995;21(2):70-73.

13.   Knutsson G, Jontell M, Bergenholtz G. Determination of plasma proteins in dentinal fluid from cavities prepared in healthy young human teeth. Arch Oral Biol 1994;39(3):185-190.

14.   Hahn CL, Best AM. The pulpal origin of immunoglobulins in dentin beneath caries: an immunohistochemical study. J Endod 2006;32(3):178-182.

15.   Lanzavecchia A. Receptor-mediated antigen uptake and its effect on antigen presentation to class II-restricted T lymphocytes. Annu Rev Immunol 1990;8:773-793.

16.   Van Hassel HJ. Physiology of the human dental pulp. Oral Surg Oral Med Oral Pathol 1971;32(1):126-134.

17.   Peters OA PC. Cleaning and Shaping of the Root Canal System. Pathways of the Pulp. 10th Edition 2011:283-348.

18.   Marquis VL, Dao T, Farzaneh M, Abitbol S, Friedman S. Treatment outcome in endodontics: the Toronto Study. Phase III: initial treatment. J Endod 2006;32(4):299-306.

19.   Peters OA, Schonenberger K, Laib A. Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography. Int Endod J 2001;34(3):221-230.

20.   de Chevigny C, Dao TT, Basrani BR, Marquis V, Farzaneh M, Abitbol S, et al. Treatment outcome in endodontics: the Toronto study--phase 4: initial treatment. J Endod 2008;34(3):258-263.

21.   Caplan DJ, Cai J, Yin G, White BA. Root canal filled versus non-root canal filled teeth: a retrospective comparison of survival times. J Public Health Dent 2005;65(2):90-96.

22.   Ou KL, Chang CC, Chang WJ, Lin CT, Chang KJ, Huang HM. Effect of damping properties on fracture resistance of root filled premolar teeth: a dynamic finite element analysis. Int Endod J 2009;42(8):694-704.

23.   Randow K, Glantz PO. On cantilever loading of vital and non-vital teeth. An experimental clinical study. Acta Odontol Scand 1986;44(5):271-277.

24.   Goldstein S, Sedaghat-Zandi A, Greenberg M, Friedman S. Apexification & apexogenesis. N Y State Dent J 1999;65(5):23-25.

25.   Cvek M. A clinical report on partial pulpotomy and capping with calcium hydroxide in permanent incisors with complicated crown fracture. J Endod 1978;4(8):232-237.

26.   Dannenberg JL. Pedodontic endodontics. Dent Clin North Am 1974;18(2):367-377.

27.   Nirschl RF, Avery DR. Evaluation of a new pulp capping agent in indirect pulp therapy. ASDC J Dent Child 1983;50(1):25-30.

28.   Stanley HR. Criteria for standardizing and increasing credibility of direct pulp capping studies. Am J Dent 1998;11 Spec No:S17-34.

29.   Ørstavik D, Pitt Ford TR. Essential endodontology : prevention and treatment of apical periodontitis. Oxford ; Malden, MA: Blackwell Science; 1998.

30.   Cohen S, Burns RC. Pathways of the pulp. 6th ed. St. Louis: Mosby; 1994.

31.   Ward J. Vital pulp therapy in cariously exposed permanent teeth and its limitations. Aust Endod J 2002;28(1):29-37.

32.   Strindberg LZ. The dependence of the results of pulp therapy on certain factors; an analytic study based on radiographic and clinical follow-up examinations. [Tr. from the Swedish manuscript]. Stockholm,; 1956.

33.   Bogen G, Kim JS, Bakland LK. Direct pulp capping with mineral trioxide aggregate: an observational study. J Am Dent Assoc 2008;139(3):305-315; quiz 305-315.

34.   Marques MS, Wesselink PR, Shemesh H. Outcome of Direct Pulp Capping with Mineral Trioxide Aggregate: A Prospective Study. J Endod 2015;41(7):1026-1031.

35.   Jang Y, Song M, Yoo IS, Song Y, Roh BD, Kim E. A Randomized Controlled Study of the Use of ProRoot Mineral Trioxide Aggregate and Endocem as Direct Pulp Capping Materials: 3-month versus 1-year Outcomes. J Endod 2015;41(8):1201-1206.

36.   Ricucci D, Loghin S, Siqueira JF, Jr. Correlation between clinical and histologic pulp diagnoses. J Endod 2014;40(12):1932-1939.

37.   Watts A, Paterson RC. The response of the mechanically exposed pulp to prednisolone and triamcinolone acetonide. Int Endod J 1988;21(1):9-16.

38.   Zander HA, Glass RL. The healing of phenolized pulp exposures. Oral Surg Oral Med Oral Pathol 1949;2(6):803-810.

39.   Komabayashi T, Zhu Q, Eberhart R, Imai Y. Current status of direct pulp-capping materials for permanent teeth. Dent Mater J 2016;35(1):1-12.

40.   Rasmussen P, Mjor IA. Calcium hydroxide as an ectopic bone inductor in rats. Scand J Dent Res 1971;79(1):24-30.

41.   Tronstad L. Reaction of the exposed pulp to Dycal treatment. Oral Surg Oral Med Oral Pathol 1974;38(6):945-953.

42.   Cox CF, Subay RK, Ostro E, Suzuki S, Suzuki SH. Tunnel defects in dentin bridges: their formation following direct pulp capping. Oper Dent 1996;21(1):4-11.

43.   Goldberg F, Massone EJ, Spielberg C. Evaluation of the dentinal bridge after pulpotomy and calcium hydroxide dressing. J Endod 1984;10(7):318-320.

44.   Witherspoon DE, Small JC, Harris GZ. Mineral trioxide aggregate pulpotomies: a case series outcomes assessment. J Am Dent Assoc 2006;137(5):610-618.

45.   Schroder U. Effects of calcium hydroxide-containing pulp-capping agents on pulp cell migration, proliferation, and differentiation. J Dent Res 1985;64 Spec No:541-548.

46.   Murray PE, Hafez AA, Smith AJ, Cox CF. Hierarchy of pulp capping and repair activities responsible for dentin bridge formation. Am J Dent 2002;15(4):236-243.

47.   Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root-end filling material. J Endod 1995;21(7):349-353.

48.   Song M, Kang M, Kim HC, Kim E. A randomized controlled study of the use of ProRoot mineral trioxide aggregate and Endocem as direct pulp capping materials. J Endod 2015;41(1):11-15.

49.   Camilleri J. Mineral trioxide aggregate: present and future developments. Endodontic Topics 2015;32(1):31-46.

50.   Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasam SP. Using mineral trioxide aggregate as a pulp-capping material. J Am Dent Assoc 1996;127(10):1491-1494.

51.   Holland R, de Souza V, Nery MJ, Otoboni Filho JA, Bernabe PF, Dezan Junior E. Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. J Endod 1999;25(3):161-166.

52.   Torabinejad M, Rastegar AF, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root-end filling material. J Endod 1995;21(3):109-112.

53.   Martell B, Chandler NP. Electrical and dye leakage comparison of three root-end restorative materials. Quintessence Int 2002;33(1):30-34.

54.   Dammaschke T. The history of direct pulp capping. J Hist Dent 2008;56(1):9-23.

55.   Dummer PM, Hicks R, Huws D. Clinical signs and symptoms in pulp disease. Int Endod J 1980;13(1):27-35.

56.   Garfunkel A, Sela J, Ulmansky M. Dental pulp pathosis. Clinicopathologic correlations based on 109 cases. Oral Surg Oral Med Oral Pathol 1973;35(1):110-117.

57.   Seltzer S. Hypothetic mechanisms for dentine sensitivity. Oral Surg Oral Med Oral Pathol 1971;31(3):388-399.

58.   Brannstrom M. The hydrodynamics of the dental tubule and pulp fluid: its significance in relation to dentinal sensitivity. Annu Meet Am Inst Oral Biol 1966;23:219.

59.   Corpron RE, Avery JK. Ultrastructure of odontoblasts in dentinal tubules of mice. J Dent Res 1971;50(2):511.

60.   Trowbridge HO, Franks M, Korostoff E, Emling R. Sensory response to thermal stimulation in human teeth. J Endod 1980;6(1):405-412.

61.   Narhi MV. Dentin sensitivity: a review. J Biol Buccale 1985;13(2):75-96.

62.   Degering CI. Physiologic evaluation of dental-pulp testing methods. J Dent Res 1962;41:695-700.

63.   Bender IB, Seltzer S. Roentgenographic and direct observation of experimental lesions in bone: II. 1961. J Endod 2003;29(11):707-712; discussion 701.

64.   Bender IB, Seltzer S. Roentgenographic and direct observation of experimental lesions in bone: I. 1961. J Endod 2003;29(11):702-706; discussion 701.

65.   Eversole LR, Stone CE, Strub D. Focal sclerosing osteomyelitis/focal periapical osteopetrosis: radiographic patterns. Oral Surg Oral Med Oral Pathol 1984;58(4):456-460.

66.   McDougal RA, Delano EO, Caplan D, Sigurdsson A, Trope M. Success of an alternative for interim management of irreversible pulpitis. J Am Dent Assoc 2004;135(12):1707-1712.

67.   EW H, Stanley H, J C, H S. Direct pulp capping treatment : a long-term follow-up. JADA 1978;97(4):5.

68.   Barthel CR, Rosenkranz B, Leuenberg A, Roulet JF. Pulp capping of carious exposures: treatment outcome after 5 and 10 years: a retrospective study. J Endod 2000;26(9):525-528.

69.   Maryniuk GA, Haywood VB. Placement of cast restorations over direct pulp capping procedures: a decision analytic approach. J Am Dent Assoc 1990;120(2):183-187.

70.   Pitt Ford TR. Pulpal response to a calcium hydroxide material for capping exposures. Oral Surg Oral Med Oral Pathol 1985;59(2):194-197.

71.   Matsuo T, Nakanishi T, Shimizu H, Ebisu S. A clinical study of direct pulp capping applied to carious-exposed pulps. J Endod 1996;22(10):551-556.

72.   Kang CM, Sun Y, Song JS, Pang NS, Roh BD, Lee CY, et al. A randomized controlled trial of various MTA materials for partial pulpotomy in permanent teeth. J Dent 2016.

73.   Kim S, Liu M, Simchon S, Dorscher-Kim JE. Effects of selected inflammatory mediators on blood flow and vascular permeability in the dental pulp. Proc Finn Dent Soc 1992;88 Suppl 1:387-392.

74.   Dziedzic TA, Kunert P, Marchel A. Clinical Course and Results of Surgery for Chronic Subdural Hematomas in Patients on Drugs Affecting Hemostasis. J Korean Neurosurg Soc 2017;60(2):232-238.

75.   Kim S, Edwall L, Trowbridge H, Chien S. Effects of local anesthetics on pulpal blood flow in dogs. J Dent Res 1984;63(5):650-652.

76.   Bernick S, Nedelman C. Effect of aging on the human pulp. J Endod 1975;1(3):88-94.

77.   Auschill TM, Arweiler NB, Hellwig E, Zamani-Alaei A, Sculean A. [Success rate of direct pulp capping with calcium hydroxide]. Schweiz Monatsschr Zahnmed 2003;113(9):946-952.

78.   Horsted P, Sandergaard B, Thylstrup A, El Attar K, Fejerskov O. A retrospective study of direct pulp capping with calcium hydroxide compounds. Endod Dent Traumatol 1985;1(1):29-34.

79.   Mejare IA, Axelsson S, Davidson T, Frisk F, Hakeberg M, Kvist T, et al. Diagnosis of the condition of the dental pulp: a systematic review. Int Endod J 2012;45(7):597-613.

80.   Caviedes-Bucheli J, Lombana N, Azuero-Holguin MM, Munoz HR. Quantification of neuropeptides (calcitonin gene-related peptide, substance P, neurokinin A, neuropeptide Y and vasoactive intestinal polypeptide) expressed in healthy and inflamed human dental pulp. Int Endod J 2006;39(5):394-400.

81.   Kokkas AB, Goulas A, Varsamidis K, Mirtsou V, Tziafas D. Irreversible but not reversible pulpitis is associated with up-regulation of tumour necrosis factor-alpha gene expression in human pulp. Int Endod J 2007;40(3):198-203.

82.   Rauschenberger CR, Bailey JC, Cootauco CJ. Detection of human IL-2 in normal and inflamed dental pulps. J Endod 1997;23(6):366-370.

83.   Willershausen B, Willershausen I, Ross A, Velikonja S, Kasaj A, Blettner M. Retrospective study on direct pulp capping with calcium hydroxide. Quintessence Int 2011;42(2):165-171.

84.   Taha NA, M BA, Ghanim A. Assessment of Mineral Trioxide Aggregate pulpotomy in mature permanent teeth with carious exposures. Int Endod J 2015.

85.	Srivastava PK, Nagpal A, Setya G, Kumar S, Chaudhary A, Dhanker K. Assessment of Coronal Leakage of Temporary Restorations in Root Canal-treated Teeth: An in vitro Study. J Contemp Dent Pract 2017;18(2):126-130.

86.	Budig CG, Eleazer PD. In vitro comparison of the setting of dry ProRoot MTA by moisture absorbed through the root. J Endod 2008;34(6):712-714.