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Designing a Process to Reliably Split Extracted Teeth to Access the Dental Root Canal

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ABSTRACT

DESIGNING A PROCESS TO RELIABLY SPLIT EXTRACTED TEETH TO ACCESS THE DENTAL ROOT CANAL

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Introduction

Mesenchymal stem cell (MSC) research is a contemporary topic in modern healthcare. These cells have the ability to divide and generate progeny, which then become committed to specific and distinctive end-stage phenotypes, such as cartilage or bone (Caplan 1991). MSCs have been isolated from a variety of tissues such as the umbilical cord, bone marrow, adipose tissue (Ding, Shyu & Lin 2011) and dental pulp (Takeda et al 2008). Although dental pulp is a viable source for MSCs, peer reviewed research has given limited attention to its retrieval process through intentional tooth splitting of extracted teeth. The purpose of this study is to design a process that will split teeth to access the dental root canal.

Methods

This study incorporated a novel experimental design in order to assess whether scoring a healthy premolar tooth would facilitate both a predictable split, as well as provide access to the dental root canal. The extracted tooth was scored to a guided depth of 1.0mm

on the mesial and distal surfaces along the length of the entire tooth. A tensile force was exerted on the apical surface of the tooth root until the tooth split. The split segments along with any retrievable fixed dental pulp were displayed for a photograph. The scored and split teeth were classified based on specified criteria. These criteria are important as access to the dental pulp will ultimately lead to harvesting mesenchymal stem cells. The teeth were also graded and classified according to whether the root canal was visible in both split segments.

Results

Of the twenty five consecutively scored and split teeth in the experiment, 92% of the total sample split predictably and matched the theoretical control group. In spite of some undesired fragmented pieces, 100% of the data set shows visible access to the root canal.

Conclusion

The evidence shown in this research paper clearly demonstrates that teeth can be scored and predictably split. In addition, splitting teeth can predictably provide access to the dental root canal. These efforts may prove to be a good start to study dental pulp tissue and ultimately MSCs. This research paper provides a minimally invasive technique that can provide a feasible alternative to the current methods of accessing the dental root canal, which usually involve crushing the tooth and drilling or cutting through the tooth to retrieve pieces of the dental pulp tissue.

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CHAPTER 1: INTRODUCTION

Mesenchymal stem cell (MSC) research is a contemporary topic in modern healthcare. These cells have the ability to divide and generate progeny, which then become committed to specific and distinctive end-stage phenotypes, such as cartilage or bone (Caplan 1991). MSCs have been isolated from a variety of tissues such as the umbilical cord, bone marrow, adipose tissue (Ding, Shyu & Lin 2011) and dental pulp (Takeda et al 2008). Although dental pulp is a viable source for MSCs, peer reviewed research has given limited attention to its retrieval through intentional tooth splitting of extracted teeth. The purpose of this study is to design a process that will reliably split teeth to access the dental root canal.

From a dental and engineering perspective, the need for a novel method to split extracted teeth is both well substantiated and feasible. Tooth fracture is a well-researched topic with a vast majority of its published data concerned with preventing teeth from fractures. The same data will also provide information about how extracted teeth can be intentionally split. Similarly, fracture mechanics is a field of engineering that dates as far back as World War II, when tensile fractures sustained by welded ships were being analyzed (Anderson 1995). The study of fracture mechanics, namely tensile strength, which normally prevents fractures, will be explored to create fractures in this case with the purpose of intentionally splitting extracted teeth.

The notion of splitting teeth requires a basic understanding of mechanical properties. It is reported that any material that contains a sharp crack should fail upon the application of a load (Anderson 1995). Mathematical models satisfy the Griffith Energy Balance of equilibrium by demonstrating that a crack propagates along a certain path where

the crack growth occurs (Anderson 1995). The load applied in order to crack a tooth will likewise propagate and release energy (Anderson 1995), obeying the first law of thermodynamics. There are three modes of loading that a crack can experience, Mode I, Mode II, and Mode III. Mode I loading is when the tensile load is applied normal to the crack plane, thereby opening the crack. Mode II corresponds to an in plane shear loading, which corresponds to a sliding of the two surface separated by the crack. Mode III is another shearing load, however, it is out of plane shearing load (Anderson 1995). This study focuses on Mode I loading, where the principal load (tensile stress) is applied normal to the crack plane that will open the crack (Anderson 1995). The propagation of a crack can follow a predetermined path, also known as precracking (Anderson 1995). In this paper, the precracking will be referred to as scoring. While scoring the tooth along its long axis can provide a predetermined path, the site of scoring needs further attention.

Studying the anatomy of premolar teeth shows varying dentin thickness between the root canal and external surface of the premolar tooth root (Bellucci & Perrini 2002). Dentin thickness is greatest in the buccolingual direction as compared to the mesiodistal orientation (Lertchirakarn, Palamara, Messer 2003). Of the factors influencing root fracture, dentin thickness is the most important (Lertchirakarn, Palamara, Messer 2003). To further understand the load distributed within a canal on to the dentin, we must further understand stress loading. Since there is a change in dentin thickness along the length of the tooth, there will be an asymmetric stress distribution. A study was found to conduct a finite-element analysis (FEA) on teeth to analyze the stress distribution. The FEA model showed the highest tensile stresses at the canal wall, with a marked decrease externally toward the apical root surface (Lertchirakarn, Palamara, Messer 2003). When pressure is

applied to a thick walled cylindrical pressure vessel the stress distribution will follow the basic patterns of hoop stresses. The thick walled vessel will experience a tensile stress in a circumferential direction and compressive stress in a radial direction. Since the mesial-distal dentin is the thinnest, the tensile stress provides a circumferential load causing the thin part of the root to expand more readily than the thick part of the root. The asymmetric expansive load on the thin portion of the root creates more tensile stress on the thick root portion, which further loads the thin portion of the root to bend outward. (Engineering Mechanics of Materials 3rd). Stress concentration in a region would predispose a crack to initiate which would also require the tensile strength of the dentin to be exceeded (Materials science and engineering 4th ed).

1.1 Purpose of the Study

The purpose of this research project is to investigate if an alternative and novel method of accessing the dental root canal of extracted teeth exits. Furthermore, this study will investigate the quality of the recovered dental pulp tissue by evaluating viable cells and the quantity of particulate matter collected.

1.2 Research Question and Hypothesis

Research Question

Is it possible to design a novel method of rapidly accessing the dental root canal of extracted premolar teeth?

Null Hypothesis (H_0): The design and fabrication of an instrument will not facilitate the rapid access of the dental root canal from extracted premolar teeth.

Alternate Hypothesis (H_1): The design and fabrication of an instrument will facilitate the rapid access of the dental root canal from extracted premolar teeth.

CHAPTER 2: LITERATURE REIVEW

The purpose of this literature review is to provide support for the importance of stem cells, as one of the primary purposes of splitting teeth is to attain a viable dental pulp, which could permit the harvesting of stem cells. Since this experiment is a novel method, no literature is available on the methodology and traditional methodologies are described later in this paper.

2.1 Dental Pulp Contains Stem Cells

Dental Pulp Stem Cells (DPSCs) are multipotent stem cells that have the potential to differentiate into a variety of cell types. More recently a subpopulation of dental pulp stem cells has been described as human Immature Dental Pulp Stem Cells (IDPSC). There are various studies where the importance of these cells and their regenerative capacity have been demonstrated. Through the addition of tissue-specific cytokines, eventual differentiated cells were obtained in vitro and were found to be of mesenchymal, endodermal and ectodermal lineages.

Several publications have stressed the importance of the expression of pluripotentiality associated markers: the transcription factors Nanog, Sox2, Oct3/4, SSEA4, and CD13. These are indispensable for the stem cells to divide indefinitely without affecting their differentiation potential and for maintaining their self-renovation capacity. The quantification of protein expression levels in these cells is very important in order to determine their cellular fate.

A protocol for isolating and identifying the subpopulations of pluripotent- like stem cells from the dental pulp (DPSC) has been established (Atari, Gil-Recio, Fabregat, García-Fernández, Barajas, Carrasco, Jung, Hernández-Alfaro, Casals, Prosper, Prosper, Ferrés Padró, Giner 2012). The stem cells of interest, specifically, include SSEA4+, OCT3/4+, NANOG+, SOX2+, LIN28+, CD13+, CD105+, CD34-, CD45-, CD90+, CD29+, CD73+, STRO1+ and CD146-, as they show genetic stability in vitro based on genomic analysis with a newly described CGH technique.

2.2 The Dental Pulp Stem Cell's Future Uses

DPSCs were able to form both embryoid bodies-like structures (EBs) in vitro and teratoma-like structures that contained tissues derived from all three embryonic germ layers when injected in nude mice. DPSCs can differentiate in vitro into tissues that have similar characteristics to mesoderm, endoderm and ectoderm layers.

Dental pulp is the soft living tissue inside a tooth. Stem Cells are found inside this soft living tissue. Scientists have identified the mesenchymal type of stem cell inside the dental pulp. This particular type of stem cell has the future potential to differentiate into a variety of other cell types including: myocardiocytes to repair damaged cardiac tissue following a heart attack; neuronal cells to generate nerve and brain tissue; myocytes to repair muscle; osteocytes to generate bone; chondrocytes to generate cartilage; adipocytes to generate fat; and a variety of cells to generate multiple tissues from the oral cavity.

It has also become medically desirable to preserve stem cells for individuals, especially children, for potential future use where the stem cells could be essential in the treatment of certain medical conditions.

2.3 Present Method of Dental Pulp Isolation

The present methods for removing pulp from extracted teeth are fairly primitive, which is surprising in view of the potential value of the stem cells. Extracted teeth are cracked with any available instrument such as tongs or pliers with exposed pulp scraped out, along with fragmented bone. An alternative method involves drilling a hole into the extracted tooth, and using an endodontic broach (barbed wire) to retrieve pieces of dental pulp. This procedure is actually very similar to the root canal therapy procedure often performed in Dentistry. These methods may damage and contaminate significant portions of the cells, however this assumption has not been reported in literature. In addition to damaging cells, debris in the sample may further damage and contaminate the dental pulp. While cutting or drilling through the entire tooth produces unnecessary heat, the full effects of this heat on the health of cells has not been documented.

The proposed method of harvesting the dental pulp has not been introduced into literature and therefore this novel method may offer advantages to traditional methods.

CHAPTER 3: MATERIALS AND METHODS

An experimental model using extracted teeth was used to assess the predictability of splitting teeth into two equal halves and accessing the dental root canal. Freshly extracted teeth will be used to contrast various methods of accessing dental pulp to the method described in this study.

3.1 Study Design

This study incorporated a novel experimental design in order to assess whether scoring a healthy premolar tooth would facilitate both a predictable split, as well as provide access to the dental root canal. An extracted tooth was scored to a guided depth of 1.0mm on the mesial and distal surfaces along the entire length of the tooth. A tensile force was exerted on the apical surface of the tooth root until the tooth split. The split segments along with any retrievable dental pulp were displayed for a photograph. Note the displayed dental pulp was for illustrative purposes and not the goal of this experiment.

The scored and split teeth were classified based on the criteria shown in Table 1. A graphic representation of this is shown in Figure 1. In addition, the split teeth were further classified according to whether the dental root canal was visible in each split segment, as outlined in Table 2.

Table 1. Classification of a Predictable Tooth Split by Visual Inspection

Category	Description
Grade 1	Tooth splits into approximately two halves
Grade 2	Tooth splits into one complete half and the other half has fragmented
Grade 3	Tooth has split into two halves, but both halves are fragmented
Grade 4	Tooth has fragmented into multiple pieces and has not split

Table 2. Access to the Root Canal by Visual Inspection

Yes/No	Access to the Root Canal by Visual Inspection
Yes	The Root Canal is visible in both halves
No	The Root Canal is not visible in both halves

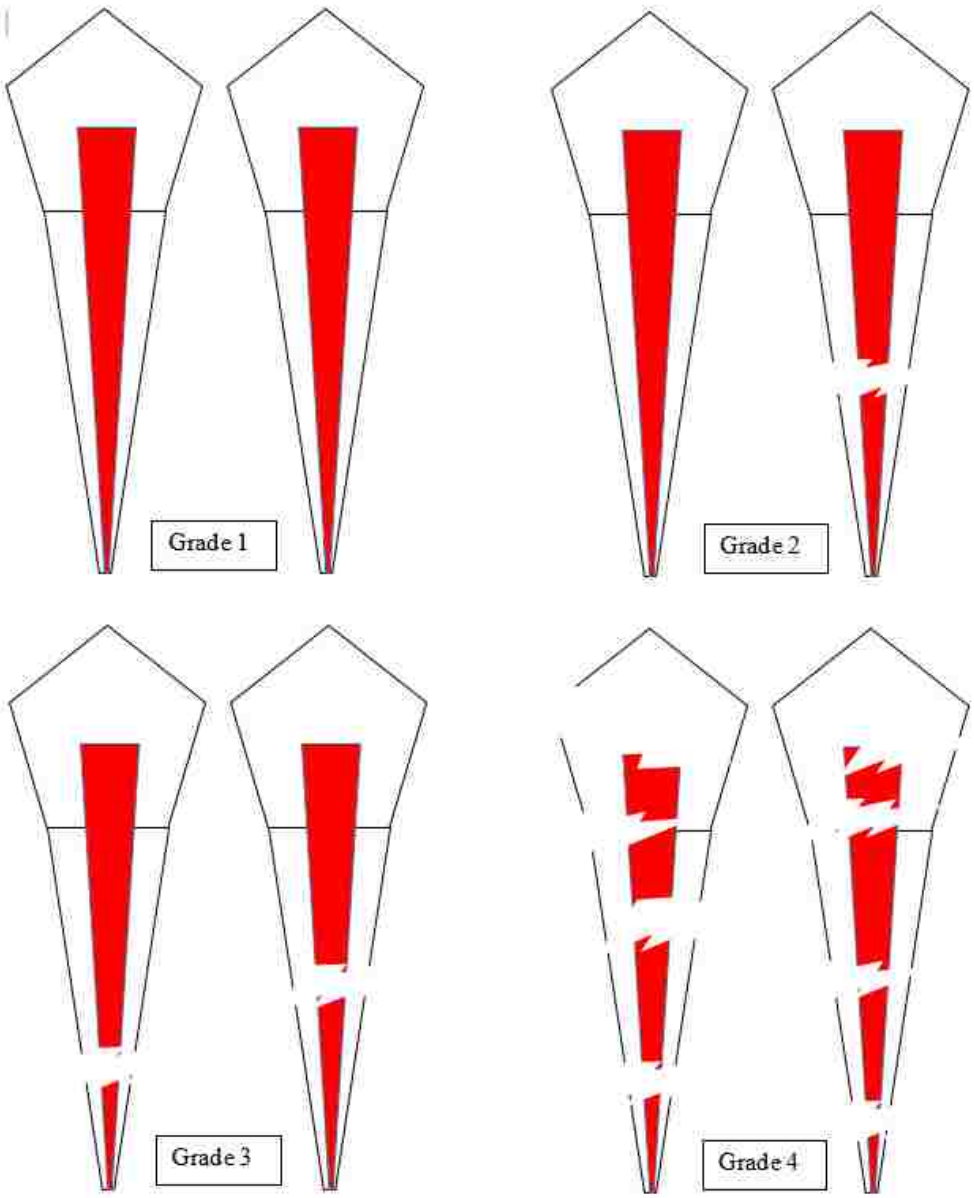


Figure 1. Classification of a Predictable Tooth Fracture (Artistic Rendition)

Particulate Matter and Viability

Three freshly extracted teeth were used to determine the degree of particulate matter contamination and cell viability while harvesting dental pulp tissue. All three teeth were retrieved from the same patient. The three teeth were subjected to three methods of accessing the dental pulp tissue. In the first method, which will be referred to as the CEJ method, the extracted tooth was cut axially across the cementoenamel junction (CEJ) using a round diamond disc with a diameter of 19mm and a thickness of 0.017mm (Ortho Technology Inc, Tampa, Fl, USA). An endodontic broach was then utilized to extirpate the dental pulp tissue, which was then stored in buffered phosphate solution (PBS). The period of time, known as the processing time, including the extirpation and storage of the dental pulp was timed and recorded. The second method involved cutting through the coronal portion of the tooth until the dental pulp chamber was accessed using a dental handpiece and a dental bur number 557 (Brasseler USA Dental, Savannah, GA, USA). As with the first method, an endodontic broach was then utilized to extirpate the dental pulp tissue, which was then stored in PBS. The processing time was again timed and recorded. The second method will be referred to as the Root Canal Therapy (RCT) Method. The third method involved the use of the TC5 and will be referred to as the Tooth Cracker (TC) Method. The exact method involving the TC5 was identical to that described earlier in this paper, with one exception; for this portion of the experiment, a freshly extracted tooth was used. The processing time period was recorded for this method as well. All three teeth with their respective methods were processed within one hour of being extracted.

Experimental specimen for the three methods were evaluated for particulate matter contamination and viability of cells. Cell confluence was measured with a Zeiss Axiovert

40 inverted microscope (Gottingen, Germany). The cells were processed and stained with Trypan blue (Sigma: St. Louis, MO), and the viable cells were counted at 200x total magnification. The Trypan blue negative cells were enumerated by using a VWR Scientific Counting Chamber (Plainfield, NJ) and a Zeiss Axiovert 40 inverted microscope (Gottingen, Germany) allowed photos of the grid hemacytometer. Particulate matter was enumerated with the photomicroscopy slides at 50x total magnification and a data table will illustrate the results.

3.2 Sample Selection and Sample Size

The extracted teeth used in this experiment have been placed through an autoclave and stored in a 10% formalin solution. All the teeth were screened with a periapical radiograph, as well as a visual and tactile inspection. The exclusion criteria included the following: multi-rooted teeth, primary teeth, infection, pathology, restorations, severe dilacerations, fractures and immature roots. Once the teeth were screened a sample size of twenty five teeth was used for the study.

Control

The experiment will utilize a theoretical control. That data set will be compared to the theoretical control in order to determine a specific classification. Ideal scoring and splitting should result in two equal halves, without producing additional tooth fragments as shown in Figure *2*. The theoretical control will also clearly show the root canal is visible to the naked eye. A theoretical control was selected to compare the data set as other studies of its kind have yet to be conducted or published for comparison.

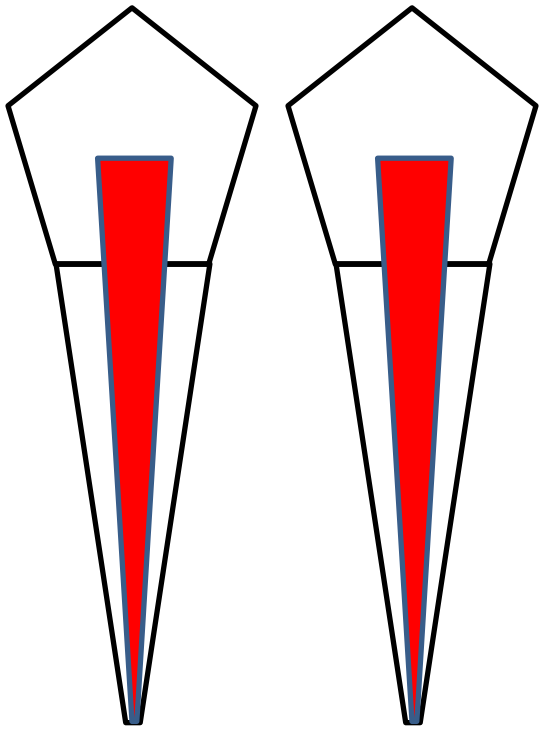


Figure 2. Theoretical Control: Two Equal Halves of a Premolar Tooth, and both halves showing the root canal in red (Artistic Rendition)

3.3 Instrumentation Design

A custom instrument was designed and fabricated specifically for conducting the experiment in this research paper. The instrument is named the Toothcracker 5000 (TC5). The TC5 has the unique ability of safely securing a tooth while both scoring and splitting a tooth. The TC5 is shown in Figure 3.

Figure 3. Toothcracker 5000 instrument

The instrument was drawn on computer aided design software and milled from a block of Aluminum. Aluminum was selected for its machinability properties. The tolerance of the milling machine was calibrated to 0.001" and used throughout the milling process. Figure *4* shows the milling machine used to mill the individual components of the TC5.

Figure 4. Milling Machine

The parts of the TC5 are the Upper Frame, Figure *5*, the Lower Frame as shown in Figure *6*, the Knobs as shown in Figure 7 and the Indexer as shown in Figure *8*. The Upper and Lower Frame telescope into each other to provide an instrument capable of accepting variable lengths of teeth. The indexer was milled from a block of copper and zinc alloy commonly known as brass. The material properties of brass make it a highly malleable material which protects the remainder of the device from excessive wear. The indexer was designed with four flutes milled at ten degrees to provide a converging path for the Tooth Clamping Arms (TCA), Figure *9*. As the Knob on the TCA is tightened, the TCA are drawn through the indexer which provides a progressively tighter hold on the tooth, Figure *9*. The TCA are made from 0.045" stainless steel material that will travel through the brass indexer. The malleable nature of the indexer will allow mechanical wear as the TCA slide through. This part can also be easily replaced to preserve the integrity of the TC5.

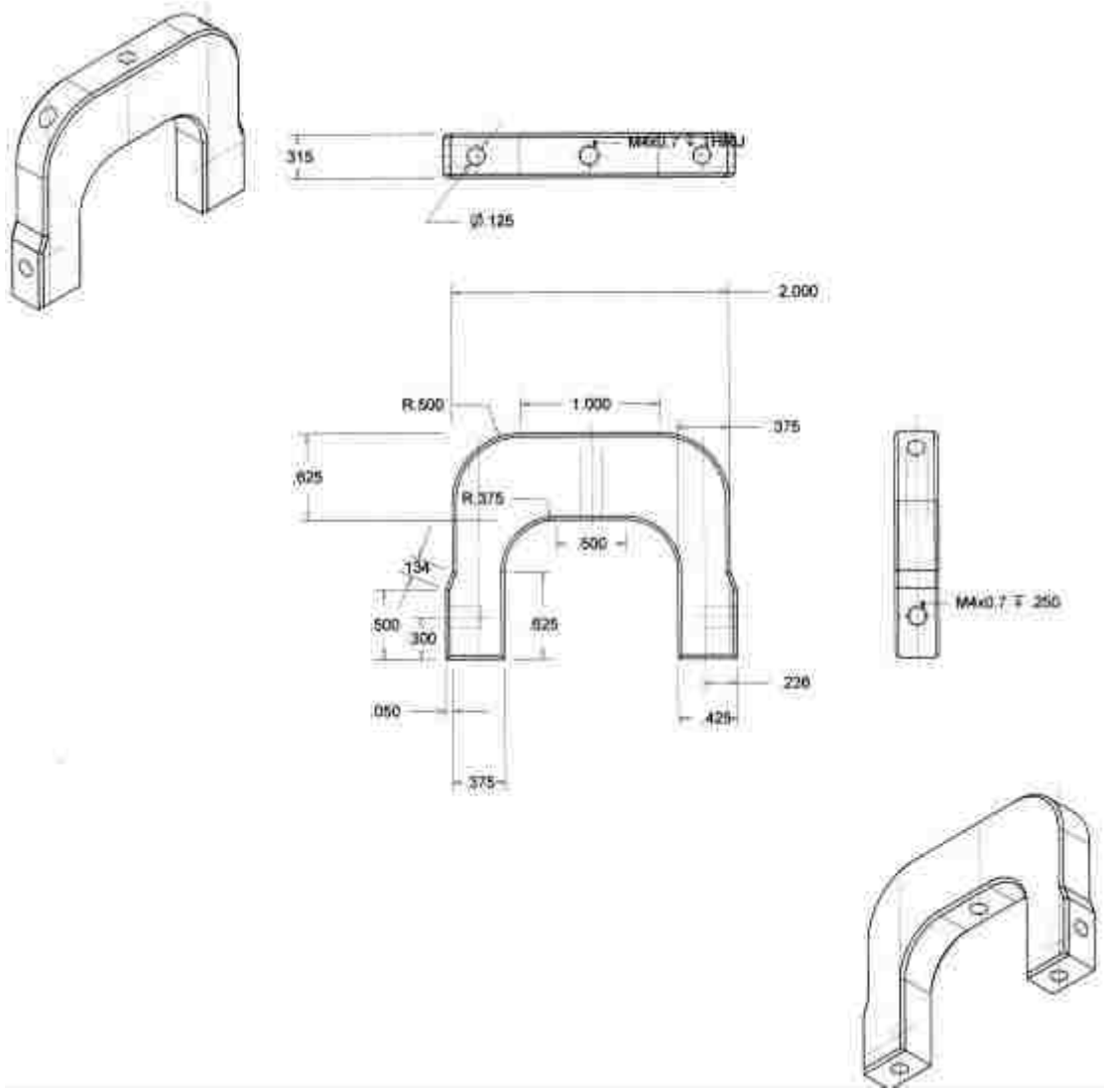


Figure 5. Upper Frame

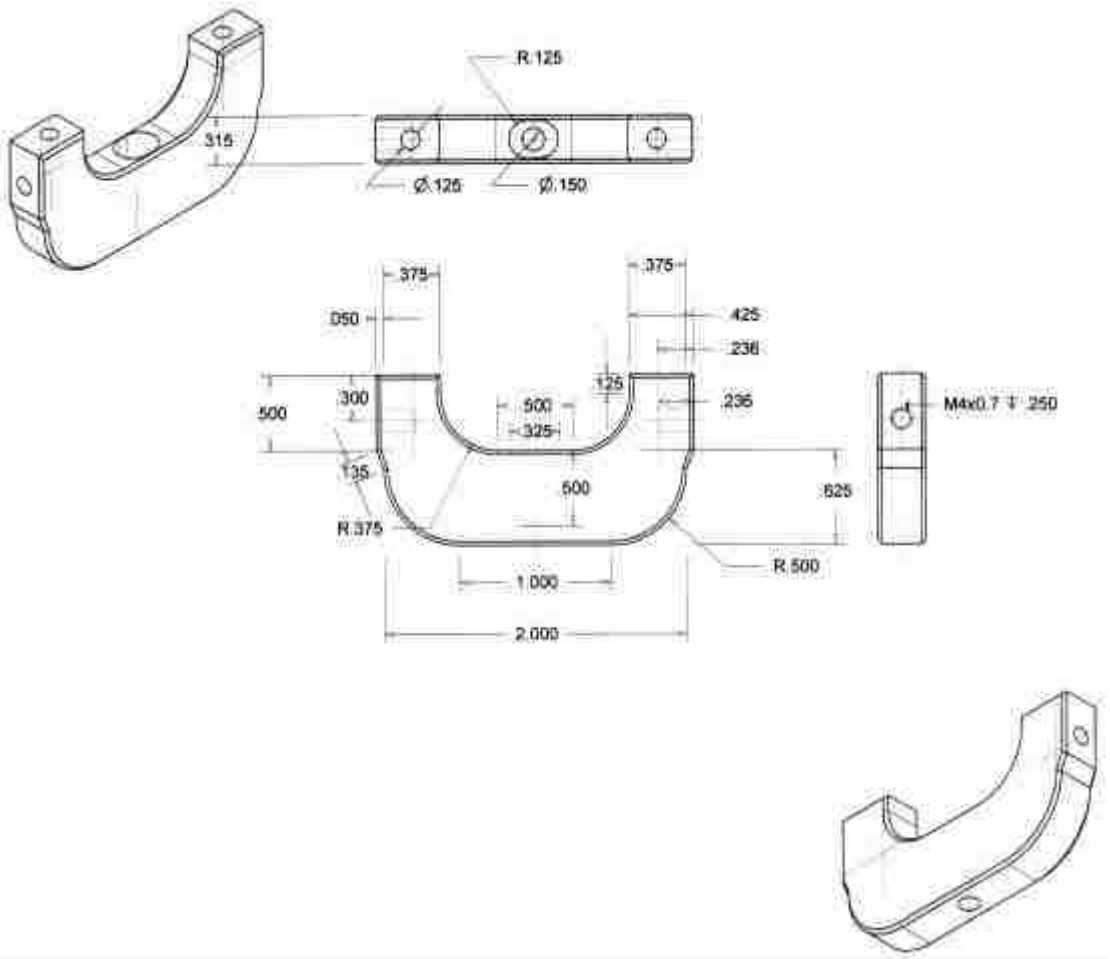


Figure 6. Lower Frame

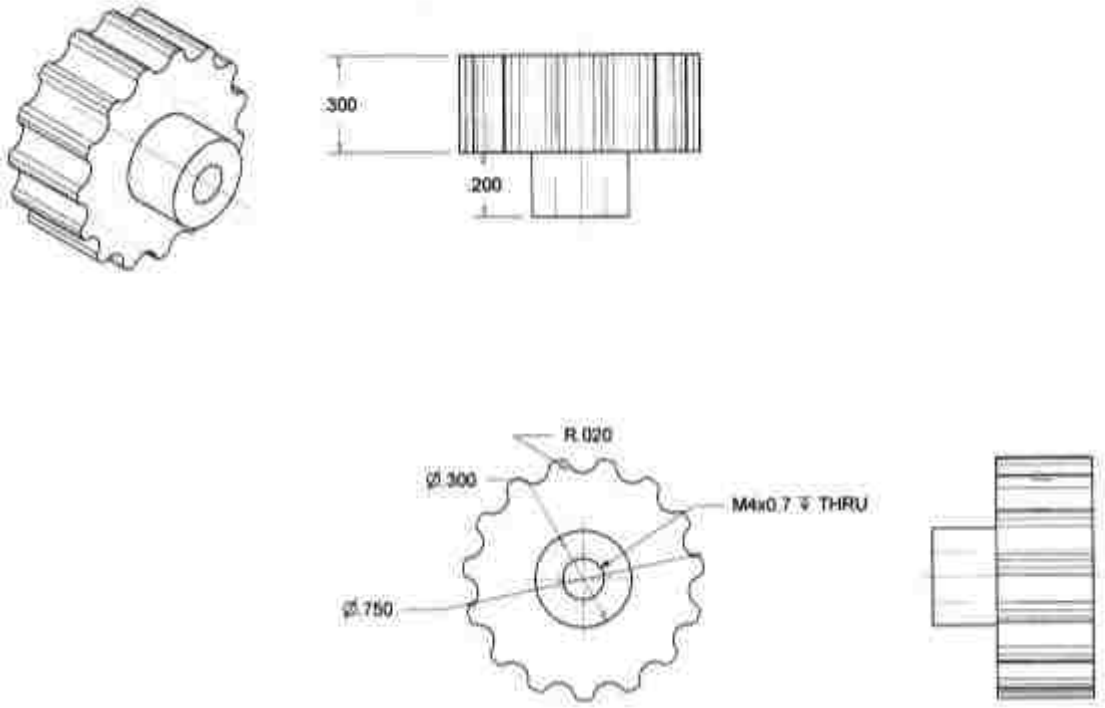


Figure 7. Knobs

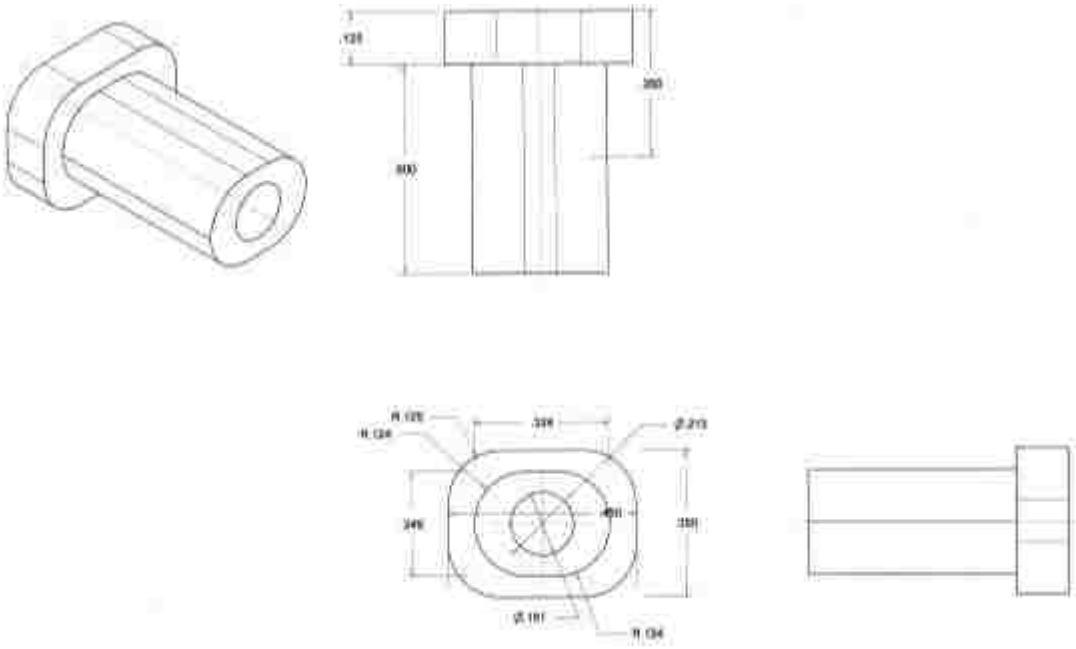


Figure 8. Indexer

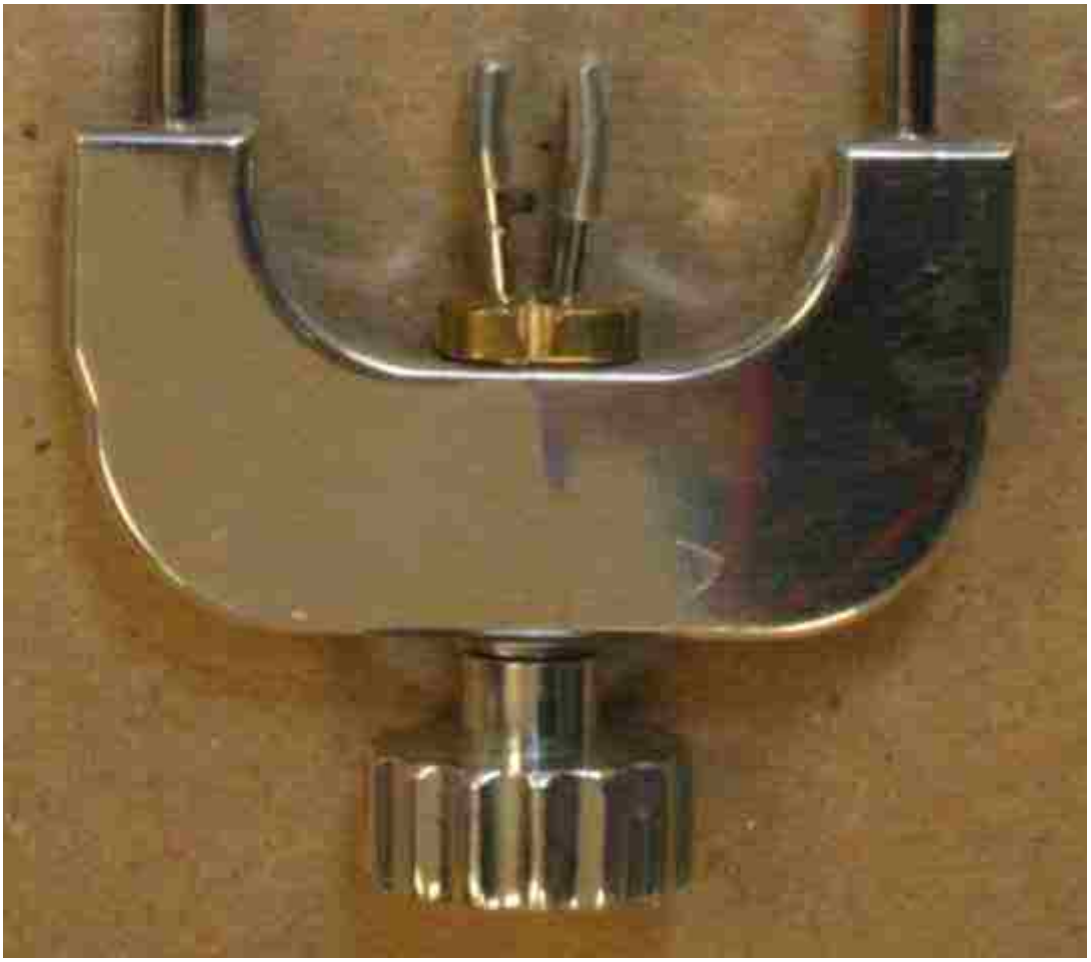


Figure 9. Tooth Clamping Arms (TCA) top middle and Bottom center is Knob

The instrument used to score the teeth was an electric motor (Brasseler USA Dental, Savannah, GA, USA) driven round diamond disc with a diameter of 19mm and a thickness of 0.017mm, (Ortho Technology Inc, Tampa, Fl, USA) shown in Figure *10*. A custom made depth guide has been installed on the disk to provide a 1.0mm depth of cut. The depth guide was secured to the disc, as shown in Figure *11*, and will be referred to as the Custom Cutting Disc (CCD).

Figure 10. Cutting Disc

Figure 11. Customized Cutting Disc (CCD)

3.4 Experiment Procedure

The selected tooth was placed in the TC5 to safely secure the tooth. The crown portion of the tooth is secured in the TCA and the root of the tooth is secured by the threads opposing the TCA as seen in Figure *12*. The tooth is scored along the entire mesial and distal tooth surface. A round carbide rotary disc with a custom depth guide, Figure *11*, provides an accurate depth along the tooth surfaces. The disc is driven by an electric hand piece, maintained at 10,000 revolutions per minute (RPM). The direction of rotation is to force the tooth further into the TC5 TCA to prevent forced ejection of the tooth. The portion of the occlusal surface that was in the TCA needs to be scored by removing the tooth from the device. Once the scoring is complete, the tooth is inserted into a Polyvinylsiloxane Holder (PVSH), shown in Figure *13*. The PVSH is made up of a plastic water bottle cap with self-setting polyvinylsiloxane (PVS) injected around the crown of an ideal

Figure 12. Tooth secure in the TC5

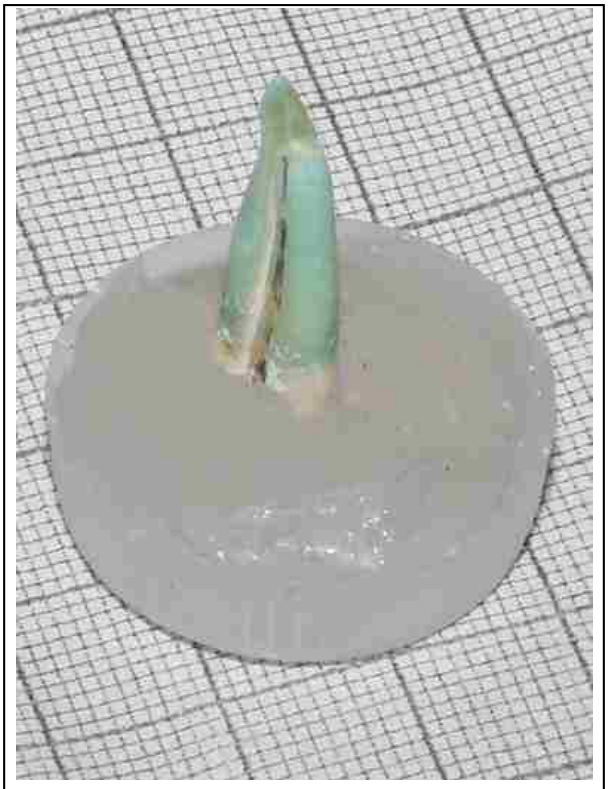


Figure 13. Polyvinylsiloxane Holder (PVSH)

premolar tooth. Once the PVS has setup to its semirigid form, the premolar tooth is removed and the PVSH is ready for the experiment. The scored tooth is inserted into the PVSH and retained by the undercuts naturally occurring on a tooth. The TC5 has an accessory attachment called the Splitting Table (ST) that is inserted, with the appropriate spacers, Figure *14*. The Splitting Blade, has a 3/32" shaft diameter which inserts into a perfectly paired 3/32" diameter bore at the end of the Split Screw, Figure *15*. The Splitting Knob, Figure *15*, is turned to lower the Splitting Blade into the scored portion of the tooth. The Splitting Blade is free to rotate as the Split Screw is advanced toward the scored tooth. An audible 'click' is detected once the tooth has fractured and the depreciation in tension in the Splitting Knob (turning the Split Screw) is easily detected by the experimenter. The tooth is removed from the PVSH and the experimenter can remove the split pieces.

Figure 14. Splitting Table

Figure 15. Splitting Blade (bottom Center) and Splitting Knob attached to threads (Top Center)

Photographs of the results were provided on a common background. The photos were taken with a Canon Rebel T4i with an external TTL flash. The settings of the photographs were consistently taken at shutter speed of 1/200 second and an aperture opening of f/22 and all photographs taken at a fixed distance using a 50mm prime lens.

3.4 Methods of Analysis

The sample will be described as the total number of test samples that match the theoretical control compared to the total sample size. In addition, the total number of teeth that clearly display the root canal will be compared to the total sample size.

CHAPTER 4: FINDINGS OF THE STUDY

TC5 Tests on Previously Extracted Teeth

Of the twenty five consecutively scored and split teeth in the experiment, only two teeth classified as Grade 2 and the remainder of the teeth classified as Grade 1. Test sample 3, Figure *16*, and test sample 10, Figure *17*, were the only two samples with a Grade 2 classifications. The remainder of the split teeth that classified as Grade 1 are shown in a representative sample in Figure *18*. For illustrative purposes the fixed pulp and the attached nerve tissue were removed, when possible, and displayed in the photographs. The results demonstrate in Figure 19 , that 92% of the total sample split predictably and matched the theoretical control group, attaining a Grade 1 classification. Of the total sample, 8% were classified as Grade 2. None of the samples classified as Grade 3 or Grade 4. In spite of some fragmented piece, 100% of the data set shows visible access to the root canal which was seen in both halves, this is illustrated in Figure 20.

Figure 16. Test Sample 3 with a Grade 2 Classification.

Figure 17. Test Sample 10 with a Grade 2 Classification.

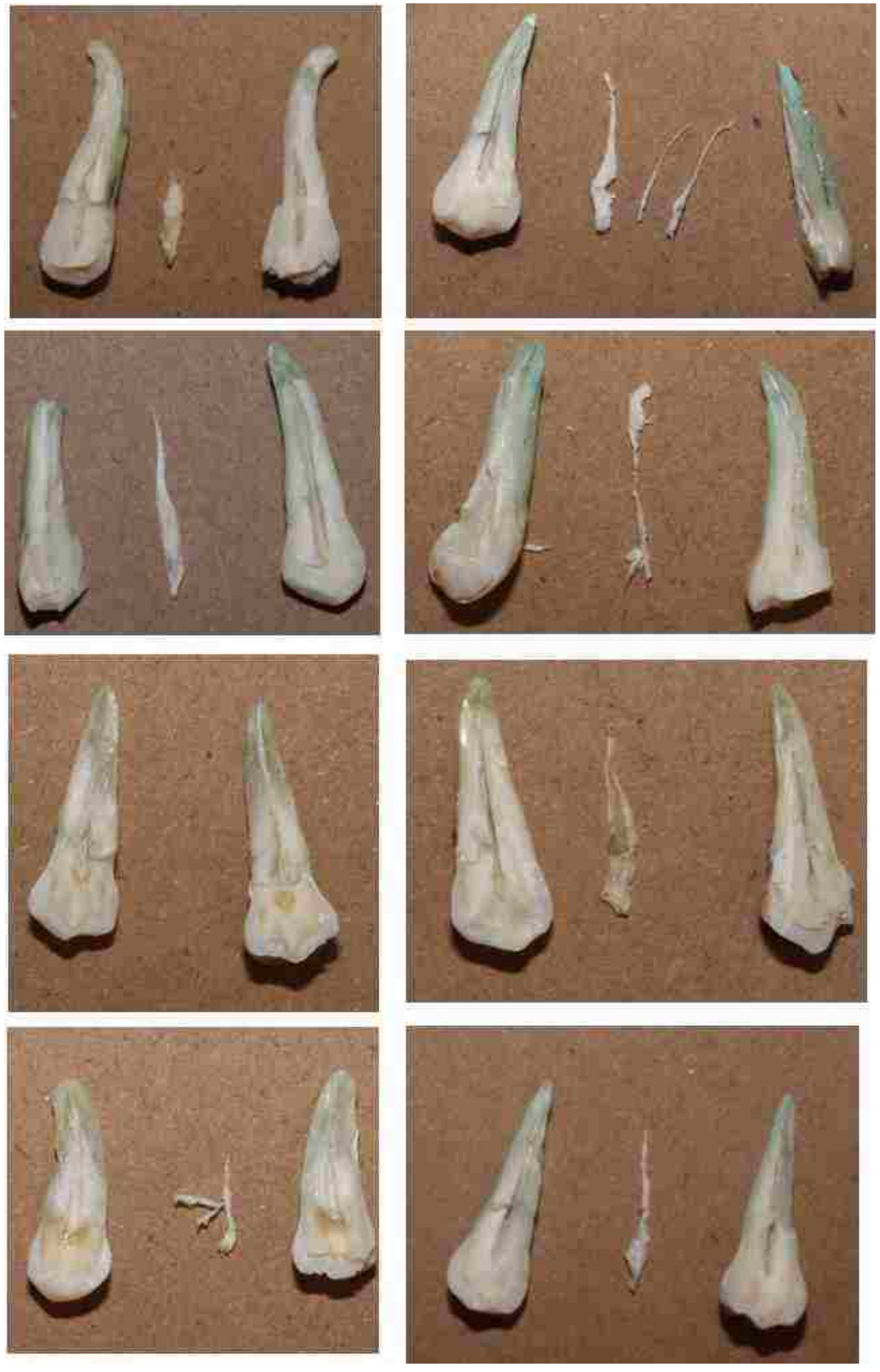


Figure 18. Representative Sample of the Data Set

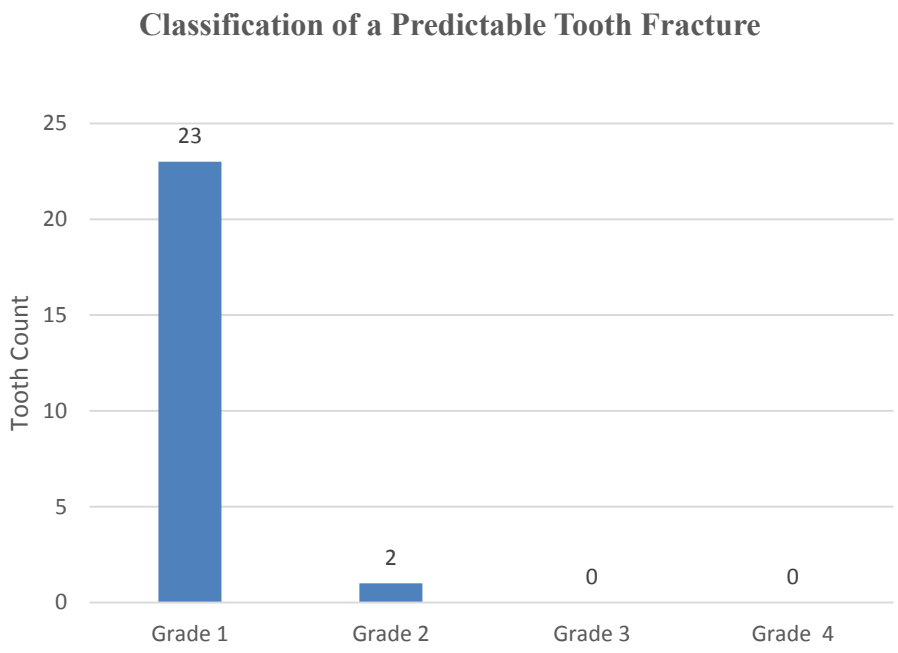


Figure 19. Classification of a Predictable Tooth Fracture

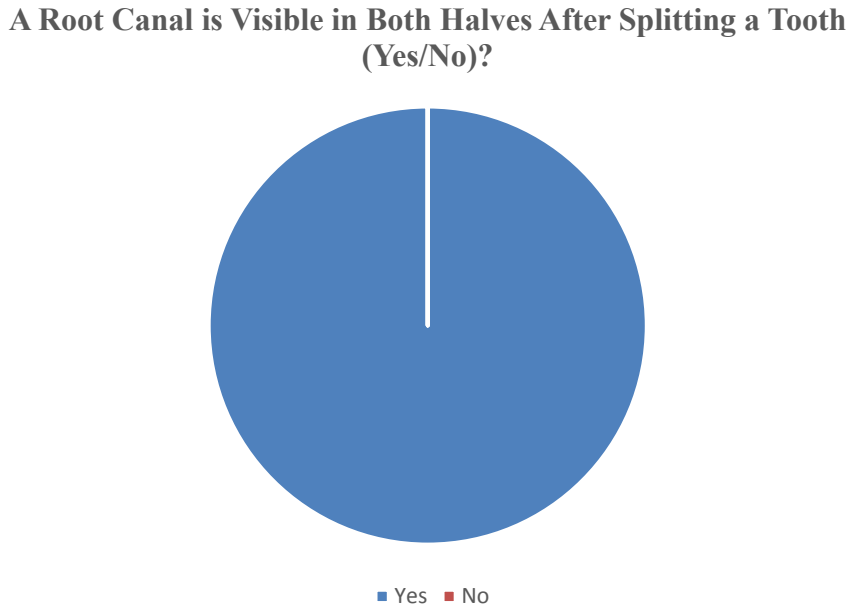


Figure 20. A Root Canal is Visible in Both Halves After Splitting a Tooth (Yes/No)?

Freshly Extracted Teeth

Three freshly extracted teeth were used to evaluate the three methods of accessing the dental pulp tissue. The TC, CEJ and RCT methods were all timed from the initial contact with the tooth specimen through depositing the dental pulp tissue into the Buffered Phosphate Solution (PBS), this is the processing time. Table 3 shows the results from each of the three methods. The TC Method had a processing time of seven minutes and 16 seconds. The CEJ Method had a processing time of five minutes and 50 seconds. The RCT Method had a processing time of eight minutes and 03 seconds. All teeth were processed within one hour of the extraction.

Table 3. Processing Time Utilizing Three Methods of Accessing Dental Pulp Tissue

	Time (Minutes:Seconds)
TC Method	7:16
CEJ Method	5:50
RCT Method	8:03

Note: Processing Time: From initial contact with tooth specimen to placing dental pulp tissue into Phosphate Buffered Solution.

Three freshly extracted teeth were used to quantify the particulate matter produced with the three methods of accessing the dental pulp tissue. The TC, CEJ and RCT methods all produced varying amounts of particulate matter, this is illustrated in Table 4. The size of a visible cell was used as a comparison while evaluating particulate matter. Small

denotes the size of particulate matter which is equal to or twice the size of a visible cell and large denotes any particulate matter that is larger than twice the size of a visible cell.

Table 4. Minimum Quantity of Particulate Matter using Three Methods of Accessing Dental Pulp Tissue

	Small	Large
TC Method	50	10
CEJ Method	100	50
RCT Method	250	250

Note: Small: Particulate Matter between the size of a cell or twice the size. Large: Particulate Matter larger than twice the size of a cell.

Three freshly extracted teeth were used to quantify the viability of cells using the three methods of accessing the dental pulp tissue. The TC, CEJ and RCT methods all yielded varying amounts of viable cells, this is illustrated in Table 5. The TC Method had 54 viable cells. The CEJ Method had one viable cell. The RCT Method had one viable cell.

Table 5. Quantity of Viable Cells using Trypan Blue

	Quantity
TC Method	54
CEJ Method	1
RCT Method	8

Three freshly extracted teeth were used to quantify the total number of cells amongst particulate matter using the three methods of accessing the dental pulp tissue. The TC, CEJ and RCT methods all yielded varying amounts of visible cells amongst particulate matter. The TC Method had 185 visible cells. The CEJ Method had two visible cells. The

RCT Method had four visible cells. These results are illustrated in Table 6 along with the number of viable cells for each of the three methods.

Table 6. Quantity of Viable Cells using Trypan Blue and Quantity of Visible Cells Amongst the Particulate Matter

	Quantity of Viable Cells	Quantity of Visible Cells
TC Method	54	185
CEJ Method	1	2
RCT Method	8	4

Note: Visible Cells: All the visible cells amongst the particulate Matter.

CHAPTER 5: DISCUSSION AND CONCLUSIONS

5.1 Research Question: Hypothesis Assessment

1. Is it possible to design a novel method of rapidly accessing the dental root canal from extracted premolar teeth? To reject the null hypothesis we must consider the following results. It is important to acknowledge that the data set has shown 92% of the predicted splits align with the theoretical control. In addition, 100% of the data set gained access to the root canal. We can reject the null hypothesis and accept the alternative hypothesis and establish that it possible to design a novel method to rapidly access dental pulp from extracted premolar teeth.

5.2 Significance to Health Care

As a newly emerging topic in health care, stem cell research has gained significant traction. In particular, the dental pulp has provided a minimally invasive alternate source of stem cells capable of becoming any type of cell. This ability to differentiate and generate any other cell line has sparked great interest from all fields of health care, warranting advanced research. Although the prospect of stem cells looks promising, further research is required to understand and investigate how the ultimate goal of inducing desired cell lines can be achieved effectively and efficiently. Stem cell research has been identified as one of the most important areas of biomedical research today (Li, Ding, Feng, Wang and Ho 2009).

To facilitate orthodontic treatment the patient may require healthy teeth to be extracted. The extracted teeth provide space to align teeth and provide a functional occlusion. The extracted teeth themselves currently serve no purpose as they are simply

disposed of in a biological waste container. A five year retrospective review of the orthodontic treatment plans at The University of Nevada Las Vegas Department of Orthodontics (UNLVORTHO) revealed an average value of 33% (582/1751) of the treatment plans involved extractions to facilitate treatment, as shown in Figure 21. Figure 22 further illustrates the rate was consistent when comparing all classes for the past five years, this is reported by the graduating year of each class. The rate of extraction reported in the diversity sheets from the UNLVORTHO department does not specify the number of teeth extracted nor does it indicate the specific teeth extracted. It is important to note the data does not include the extraction of third molars. In 1994 a forty year retrospective study showed that 28% of orthodontic treatment plans at a university clinic included extractions (Proffit 1994). Interestingly, this forty year review of extraction frequency nearly parallels the five year retrospective evaluation from UNLVORTHO.

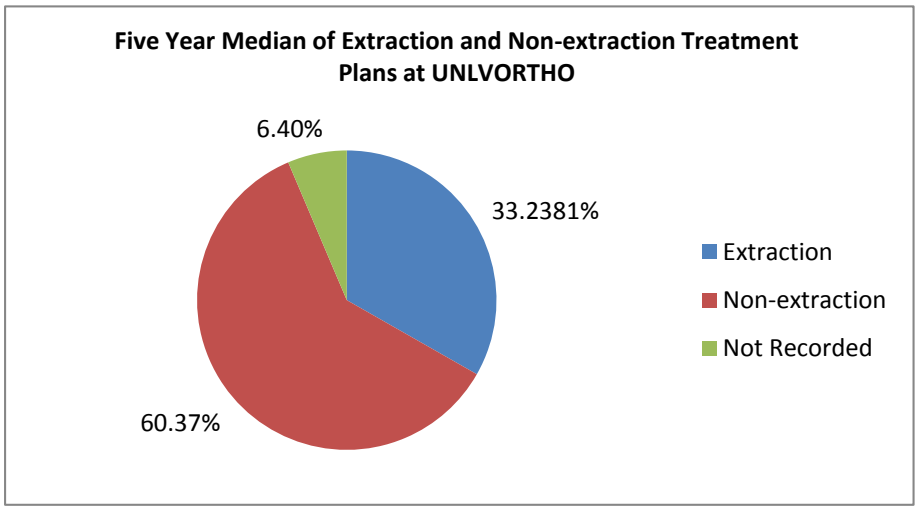
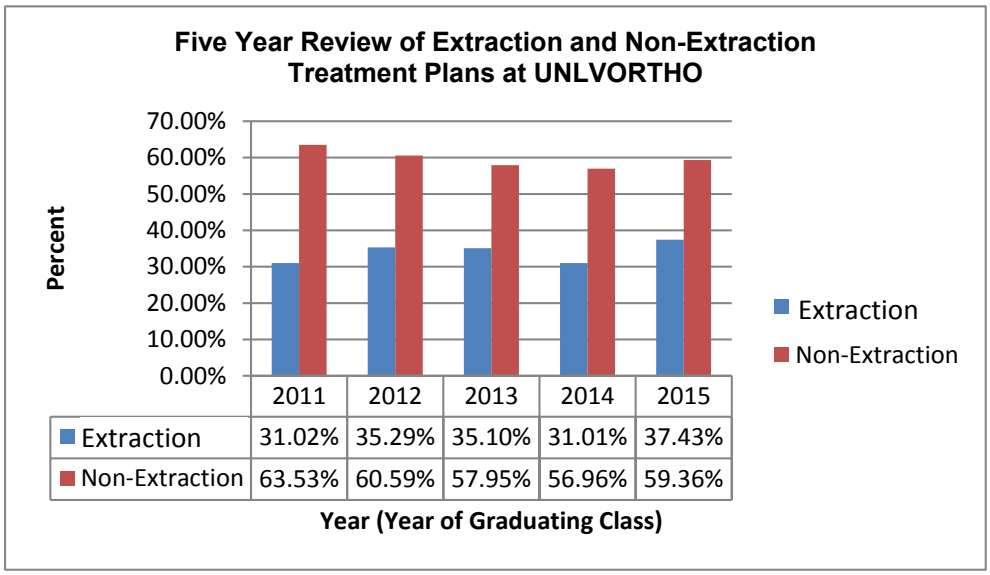


Figure 21. Five Year Median of Extraction and Non-extraction Treatment Plans at UNLVORTHO



	2011	2012	2013	2014	2015
Extraction	31.02%	35.29%	35.10%	31.01%	37.43%
Non-Extraction	63.53%	60.59%	57.95%	56.96%	59.36%

Figure 22. Five Year Review of Extraction and Non-Extraction Treatment Plans at UNLVORTHO

The potentially healthy teeth that are extracted and discarded can provide a potential source of MSCs. Since nearly 1/3rd of the treatment plans for orthodontic treatment provide a source for such cells, this data provides a hope for regenerative medicine. The discarded teeth can be used to harvest MSCs with little ethical concern and a minimally invasive and safer manner; therefore providing a hopeful future. Alternatives such as adipose, bone marrow and embryonic tissue may be viable; however, the safety, ethics and invasive nature may act as a strong deterrent.

The use of dental pulp stem cells has a strong presence and even greater potential in regenerative medicine. Commercial organizations have capitalized on this self-renewal potential and provided dental stem cell storage for use in the future. The results from the UNLVORTHO retrospective analysis indicates a future for potential growth. The potential growth can be highlighted by looking at two fields. The first provides a regenerative potential for participating patients, and this regenerative potential may provide future health benefits. The second is a financial potential in storing dental stem cells for future

use, which may further fund much needed research. Although this is a new field in regenerative medicine, the storage of these cells is not a novel concept. Companies such as Store-A-Tooth, National Dental Pulp Lab and GeneCell are merely a few examples that provide such storage services.

5.3 Evaluation of Study

Instrument

To safely secure and score a tooth, the TC5 proved to be a useful instrument. The alternative would have been to hold the tooth by hand while the scoring process took place. This alternative, however, would have raised a liability issue as the experimenter's safety would have been challenged and possibly compromised. Although many renditions of this instrument were designed, manufactured and tested, the aluminum body TC5 was the most germane solution. Aluminum was selected for its light weight properties, and most importantly for the ease with which its individual parts could be machined. The size of the instrument provided comfort and portability, both properties that would be beneficial in future use of the instrument. The telescoping feature of the device allows various lengths of teeth to be utilized. Although the TC5 can accommodate varying lengths in tooth, the limitations of the TCA restrict the instrument's use to premolar teeth with respect to their shape and anatomy. In order to increase the grasp around the crown of the tooth, plastic sheaths were placed on the terminal portions of the TCA. Their use, however, was more theoretical, as they were immediately subjected to frictional wear when force was applied to secure the tooth, and they quickly deteriorated with repeated use. These shortcomings could benefit from further attention from an engineering perspective to improve the design of future tooth holding components.

The TC5 instrument allows the mesial and distal surfaces of the tooth to be scored, but does not permit access to the occlusal surface. In order to score the occlusal surface, which was held by the TCA, the tooth had to be removed from the TC5 and held manually from the tooth root during the scoring process. This limitation requires further investigation in effort to eliminate it as a shortcoming.

The Splitting Blade received a number of revisions before final testing. This apparatus was initially designed as a sharp point which made contact with the scored surface. However, due to the variance in tooth root anatomy, this design did not accommodate the different teeth and their respective positions within the instrument. The varying root anatomies did not always allow the teeth to be perfectly aligned to have the single sharp point contact the exact location of the score while being held by the TCA. The final design involved a blade with a wider surface as compared to the initially designed sharp point. This blade could accommodate for the variance in root anatomy as well as the position of the tooth within the TCA. The blade made sufficient contact into the scored portion of the tooth, and was able to apply a gradual force to increase the tension and negotiate a fracture.

The CCD was designed to score a thin cross sectional area of the tooth. The shape of the scored portion was an important consideration. The first consideration was a tapering score in cross section. The rationale for this design was to increase the amount of exposed root canal, with the assumption that the wider portion of the taper would be more likely to allow a favorable split ultimately providing access to the dental pulp. Upon further consideration, however, it was discovered that the distribution of tensile loading over a larger scored area would potentially decrease its predictability and would subsequently result in undesired fractured segments. The second design, namely the CCD, ensured that fracture mechanics and predictability were the primary concerns. The cross section of this design was a narrow rectangular shaped score to concentrate the force in effort to propagate a fracture. Although this design proved effective for the purpose of this study, further engineering and investigation into the shape of score, its effects on tensile load, its

predictability, and its subsequent access to the root canal can all be explored at length. Furthermore, scoring and splitting can be evaluated in more detail with an electron microscope.

The PVSH was designed after the instrument was manufactured. Once the tooth was scored, the splitting blade would provide the appropriate load. However, the rigid TCA would restrict the desired crack propagation by holding the segments rigidly and placing undesired force on the segments. This would potentially cause the tooth to fracture in multiple locations, and not exclusively along the desired location marked by the score line. Further design efforts were channeled to provide a functional solution to this problem. The grasp on the tooth during the splitting process requires some rigidity, but sufficient elasticity to allow the scored halves to split and separate from each other. Polyvinylsiloxane (PVS) is a readily available material that exhibits the desired properties to achieve this goal. Although alternative materials could have been tested, and would possibly provide better solutions, the limited resources and funding for this project did not permit such exploration. An ideal shaped premolar tooth was selected from the sample set and used to make an impression using PVS and ultimately forming the PVSH. The elasticity of the material allowed the crack to propagate through the embedded portion of the tooth in the PVSH. The constricted portion of the tooth, known as the undercut, provided sufficient retention within the PVSH during the splitting process. In order to minimize the components required, and attain instrument simplicity, future research can be conducted to design a tooth holding device that would slowly release upon application of a progressively increasing load. This would allow the tooth to split in the anticipated manner without the undesired forces, and would thus eliminate the need for the PVSH.

Teeth

A tooth's response to an external force that is applied for the purpose of scoring and splitting is dependent upon a number of factors; one such factor is the tooth's condition with respect to its level of hydration. Desiccation in teeth causes them to become more brittle and alters their response to applied forces or insults. As crack propagation normally follows the path of least resistance, any factor that alters the composition of the medium will inevitably alter the manner in which the crack will propagate. Although by scoring the tooth we were creating a desired path of least resistance, the tooth's composition still played an indirect role in the ultimate outcome. The teeth used in this experiment were previously extracted, autoclaved and preserved in a formalin solution. Stored teeth can readily desiccate, which as previously discussed could greatly have affected the outcome of the experiment. Despite the anticipated effects of the desiccation, it was astonishing to see that only two of the samples resulted in Grade 2 classifications. These Grade 2 classifications can be partially explained by the fact that the teeth were dehydrated, but can also be attributed to other factors such as microscopic fractures or accessory root canals in the fragmented regions. The focus of this study was the predictable nature of preserved teeth, further research would be required to determine the results of similar testing on freshly extracted teeth. Additional investigation with various imaging techniques could closely monitor changes and provide further insight into the results.

The stringent criteria set by the experiment's outline required both halves to split approximately evenly and display the root canal on both resulting split fragments in order to be considered successful. It is noteworthy that all of the tested samples, including the Grade 2 samples, met this criteria and succeeded in providing the desired access to the root

canal. This access to the root canal is of paramount importance, as it can ultimately be used to harvest mesenchymal stem cells.

Although aligning the score was appraised by the naked eye, more sophisticated alternatives were contemplated. A computer aided scanner, for example, could have evaluated the exact dimension of the tooth, and a computer guided disc could have been used to score a more exact location equidistant from the external surfaces. Theoretically, this would have been an excellent proposition, however, such complex engineering for the study would not have been a practical option.

Comparison of the Three Methods of Accessing Dental Pulp Tissue

Harvesting dental pulp stem cells requires recovering the dental pulp tissue. Most research describes an axial cut through the cementoenamel junction of an extracted tooth using a rotary disc. The result of the axial cut provides two halves of the tooth, namely the root portion and the crown portion. Although two portions remain after the cut, the literature does not indicate which half was used for research. The literature on stem cell research shows variable results obtained while encouraging mesenchymal stem cell growth. While different type of stem cells are found at the apex of the tooth root, at the periodontal ligament and within the dental pulp tissue, further efforts may delineate subtypes of dental stem cells within regions of the dental pulp tissue. This theory may be based on the various results that have been reported in literature, as inconsistent dental pulp tissue is being harvested and cultured for varying results. Therefore, capturing the maximum amount of intact dental pulp tissue is crucial to improving the likelihood of potentially capturing all of the dental pulp stem cells. While this paper will not discuss in detail the various harvesting methods, it will discuss the processing time, the amount of

particulate matter, and the number of viable cells recovered using the three methods of harvesting the dental pulp tissue.

CEJ Method

The CEJ Method cuts through the cementoenamel junction of a tooth and is the most widely described method in the literature for harvesting dental pulp tissue. The small sample in this study demonstrated a relatively short processing time of five minutes and five seconds to harvest dental pulp tissue. The processing time was calculated from the beginning of the initial axial cut to the placement of the dental pulp tissue in the PBS. This short processing time is offset by a significant short coming of accessing the important coronal pulp tissue, which provides the various subtypes of dental pulp stem cells. Figure 23 shows the image from the CEJ Method which clearly shows a very small portion of the root canal area which provides the access point to the large volume of dental pulp. To illustrate the volume of dental pulp a high resolution image using micro computed

Figure 23. CEJ Method with axial cut through the cementoenamel junction of the tooth.

tomography is shown in Figure 24. This image shows the large coronal portion of the dental

pulp, which must be pulled though the small opening shown in Figure 23. Furthermore, since freshly extracted teeth have vital cells, the odontoblastic processes adhere tightly to the walls of the root canal and this provides another challenge to separating the dental pulp tissue from the hard tissue of the tooth (Ring, Murray, Namerow, Kuttler, & Garcia-Godoy

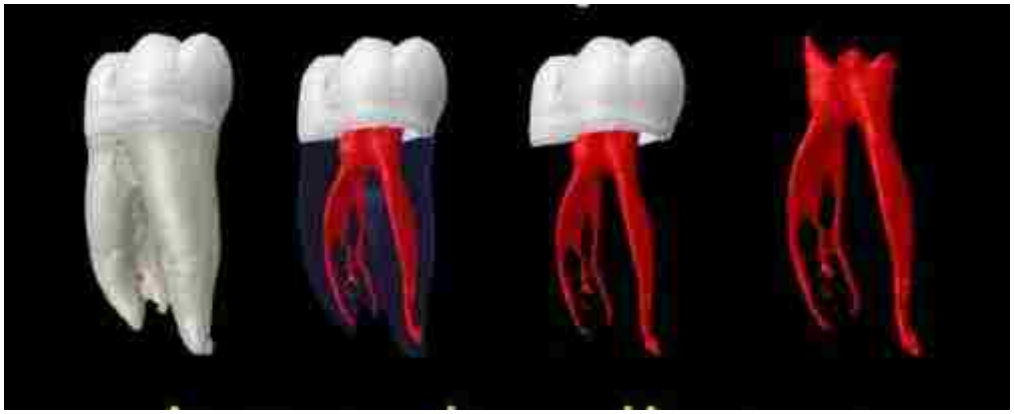


Figure 24. Three dimensional image of the dental pulp shown in red. Source www.google.com

2008). The size of the opening generated by the CEJ Method coupled with the odontoblasts adhering to the tooth creates a challenge to harvest the majority of the coronal dental pulp tissue and the apical dental pulp tissue.

After processing the dental pulp tissue, the tissue was placed in the PBS as shown in Figure 25. The particulate matter collected in the CEJ Method is illustrated using photomicroscopy in Figure 26. The visible cells have been circled in red in Figure 27 and the remaining artifacts seen in the photomicroscopy are labelled the particulate matter. The particulate matter clearly illustrates that it significantly outnumbers the visible cells. As a comparison, the CEJ Method provides less particulate matter than the RCT Method, but the TC Method clearly shows the least amount of particulate matter.

Figure 25. Harvested dental pulp tissue (CEJ Method) in a phosphate buffered solution.

Figure 26. CEJ Method photomicroscopy slide

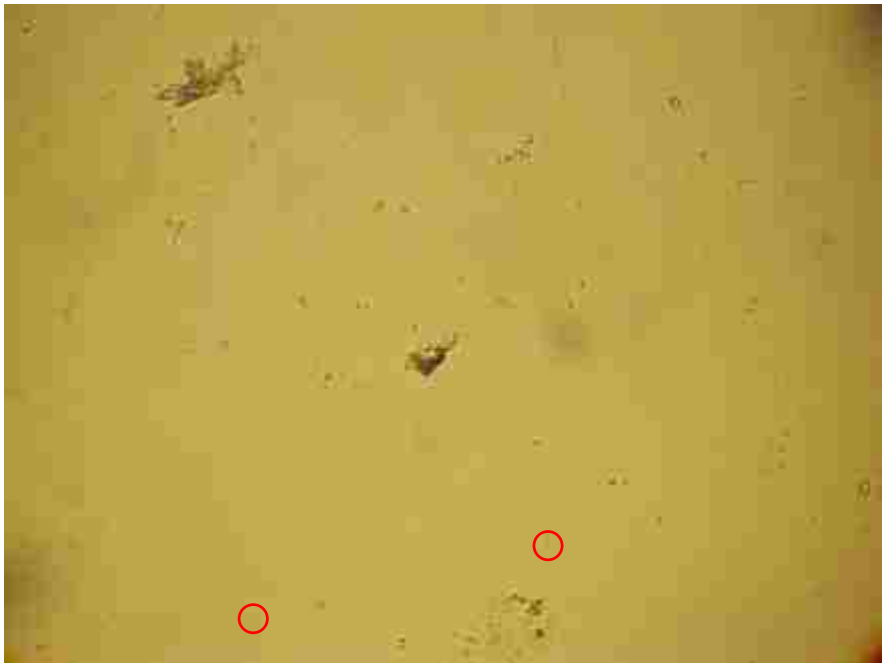


Figure 27. CEJ Method photomicroscopy slide with cells circled in red.

The cell viability is shown using a vital stain, Trypan Blue. This dye exclusion method illustrates absorption of Trypan Blue by disrupted cell membranes and result in a distinctive blue color under a microscope. A sample from the CEJ Method was stained and photomicroscopy was utilized to elucidate cell viability. Figure 28 shows one viable cell. The number of viable cells seen are far less than the particulate matter seen in the field. The scant number of viable cells can be attributed to mechanical and thermal damage. The mechanical damage can be attributed to three sources. The first, the mechanical damage from the dental handpiece as it traverses the cementoenamel junction and passes through the dental pulp tissue. This method directly contacts the tissue, although the contact time with the dental pulp tissue is very brief, the cells adjacent to the cut portion may be affected by mechanical and thermal damage. The second, the particulate matter that is deposited into the pulp chamber may provide an opportunity for further mechanical damage. The particulate matter may be deposited deep into the tissue as it can be driven by the rotary disc that is revolving at 10,000 revolutions per minute. The third is the endodontic broach that is used to remove the dental pulp tissue. The tissue is mechanically extirpated and locks onto the endodontic broach. The difficulty lies in removing the dental pulp tissue from the endodontic broach with smooth forceps prior to placing it into the PBS solution. The amount of thermal energy absorbed by the hard or soft tissue was not quantified, but may potentially affect the viability of cells.

Figure 28. Viable cells (circled in red) seen after using Trypan Blue. (CEJ Method)

RCT Method

While the CEJ Method is widely used amongst published research, it may not provide the vital tissue in the pulp chamber due to the mechanical restraint. Extracting the pulp chamber through the small access opening created from the axial cut is undoubtedly a challenge and thus a limitation. The RCT Method, on the other hand, involves accessing a root canal from the coronal portion of the extracted tooth by drilling an access with a dental handpiece and a cutting bur. This method, as expected, would provide better access to the dental pulp chamber to ultimately harvest the coronal pulp tissue. Figure 29 shows the image of a tooth that was cut using the RCT Method. Figure 29 illustrates a very large opening to the coronal pulp tissue, potentially providing access to the large volume of

Figure 29. RCT Method showing the coronal opening of a tooth after removing the dental pulp tissue with an endodontic broach.

dental pulp tissue. This procedure is very similar to the root canal therapy method, which is usually reserved for non-extracted teeth in living patients. The purpose of the RCT Method was to improve the number of viable cells and total visible cells amongst particulate matter as more of the coronal pulp could potentially be harvested. The small sample in the RCT method illustrates a relatively short processing time of eight minutes and three seconds to harvest dental pulp tissue. The processing time was calculated from the beginning of the initial axial cut to the placement of the dental pulp tissue in the PBS, shown in Figure 30. This processing time was the longest amongst the three methods. Furthermore, like the CEJ Method, it too had a significant shortcoming with the presence of gross particulate matter, as well as the challenge of separating the dental pulp tissue from the hard tissue of the tooth due to freshly extracted teeth having vital cells where the

odontoblastic processes adhere tightly to the walls of the root canal (Ring, Murray, Namerow, Kuttler, & Garcia-Godoy 2008).

Figure 30. Harvested dental pulp tissue (RCT Method) in phosphate buffered solution.

The particulate matter collected in this method is illustrated in figure 31. The particulate matter collected demonstrates its flagrant presence over the number of visible cells. The visible cells have been circled in red in Figure 31 and the remaining artifacts seen in the photomicroscopy reflect the particulate matter. As a comparison, the CEJ Method provides much less particulate matter than the RCT Method, but the TC Method clearly shows the least amount of particulate matter.

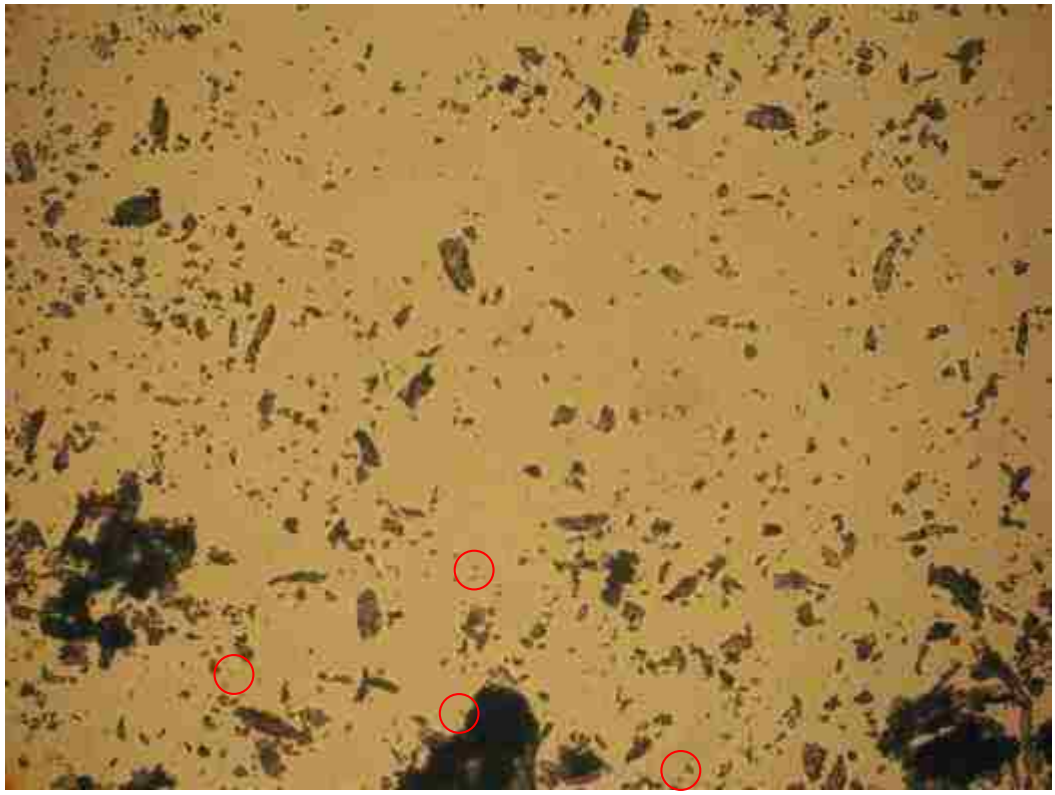


Figure 31. RCT Method Particulate Matter using Photomicroscopy. Visible cells circled in red.

The cell viability is shown using a vital stain, Trypan Blue. A sample from the RCT Method was stained and photomicroscopy was utilized to elucidate cell viability. Figure 32 shows eight viable cells. The number of viable cells seen are far less than the particulate matter seen in the field. The scant number of viable cells can be attributed to mechanical

and thermal damage. This damage can be from four sources. The first, the mechanical damage from the dental handpiece as it enters the roof of the dental pulp chamber. To provide adequate access some dental pulp tissue will be lost while mechanically reducing pulp horns to provide unobstructed access. The second, the particulate matter that is deposited into the dental pulp chamber may provide an opportunity for further mechanical damage. The third is the endodontic broach that is used to mechanically remove the dental pulp tissue, which locks onto the endodontic broach. The difficulty lies in removing the dental pulp tissue from the endodontic broach with smooth forceps prior to depositing into the PBS solution. The fourth is thermal damage that may be sustained from the dental handpiece. The amount of thermal energy absorbed by the hard or soft tissue was not quantified, but may potentially effect the viability of cells.

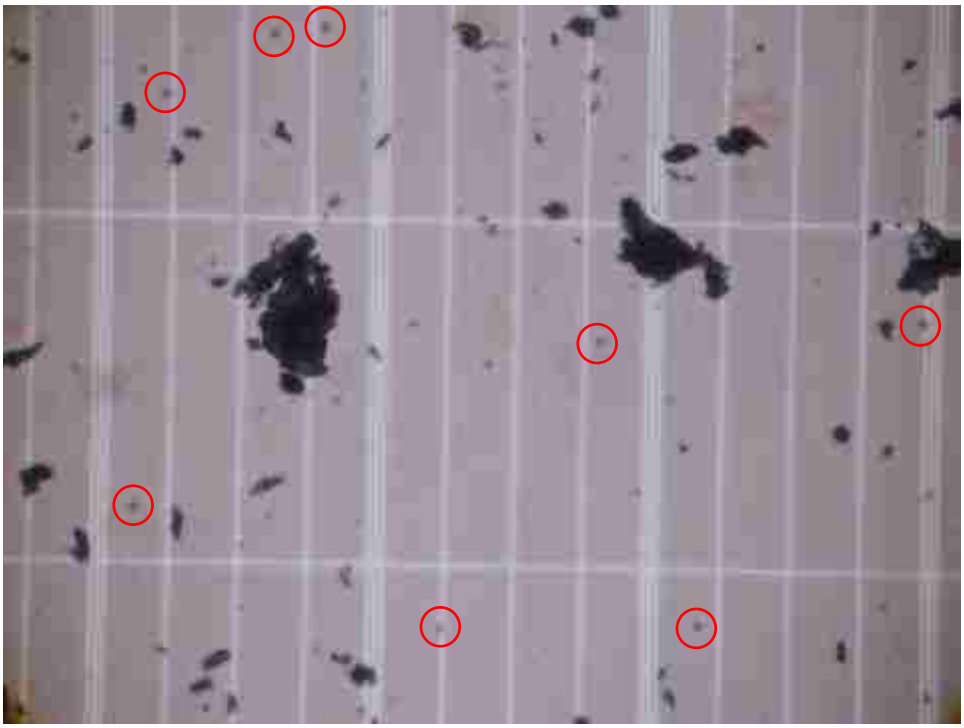


Figure 32. Viable cells (circled in red) seen after using Trypan Blue. (RCT Method)

TC Method

The mechanical damage to the dental pulp tissue that results from both the CEJ Method and the RCT Method suggest the need for an alternative and effective method; this third method is the TC Method. The results from the two previous methods, although not favorable, will provide a basis for comparison to the TC Method, and will further reveal its usefulness. The TC5 was used in the TC Method with a freshly extracted tooth, and the small sample demonstrated a relatively short processing time of seven minutes and 16 seconds to harvest dental pulp tissue. The processing time was calculated from the beginning of the initial scoring cut to the placement of the dental pulp tissue in the PBS, shown in Figure 33. This method provided access to the dental root canal, including the dental chamber, and ultimately provided access to harvest approximately the entire dental

pulp tissue. Figure 34 shows the image of a tooth split with the TC Method, which clearly shows access to the entire root canal which would potentially provide access to the largest volume of dental pulp. As with the other two methods, the TC method also faced the

Figure 33. Harvested dental pulp tissue (TC Method) in phosphate buffered solution.

challenge of separating the dental pulp tissue form the hard tissue of the tooth due to the odontoblastic processes adhering tightly to the walls of the root canal in the vital cells of the freshly extracted teeth (Ring, Murray, Namerow, Kuttler, & Garcia-Godoy 2008).

Figure 34. TC Method to access the dental pulp of a freshly extracted tooth.

The dental pulp tissue collected using the TC Method is illustrated in Figure 35. The particulate matter collected demonstrates its scant presence over the number of visible cells.

The visible cells are clearly seen as small round orange circles in Figure 36 and the remaining artifacts seen in the photomicroscopy are the particulate matter. As a

comparison, the TC Method provides the least quantity of particulate matter. Since the root canal is accessed by a rotary instrument that does not directly contact the dental pulp tissue, minimal debris is expected to discharge into the dental pulp chamber. Some particulate contamination is inevitable and has been identify with photomicroscopy as shown in Figure 35 and 36.

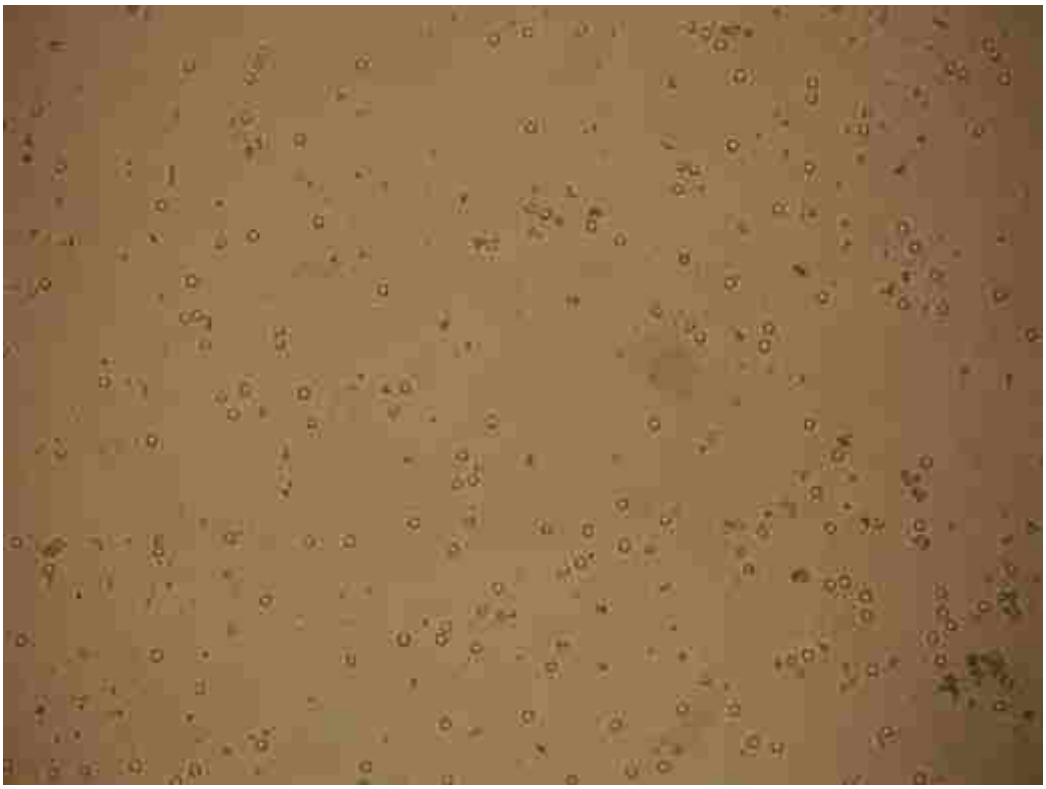


Figure 35. Cells and Particulate Matter observed using the TC Method

The cell viability is shown using a vital stain, Trypan Blue. A sample from the TC Method was stained and photomicroscopy was utilized to elucidate cell viability. Figure 37 shows 54 viable cells and Figure 38 helps identify the viable cells with blue circles. Figure 39 provides a graphic representation of viable cells. The quantity of viable cells is far greater than the particulate matter observed under a microscope. The scant particulate matter as compared to the abundant viable cells can be attributed to minimal mechanical damage. The mechanical damage from the dental handpiece is minimal as direct contact with the dental pulp tissue is avoided and very minimal dental hard tissue is removed with the rotary disc. Moreover, the tissue is not mechanically extirpated using an endodontic broach, instead smooth ended forceps peeled the tissue away from the root canal. Some

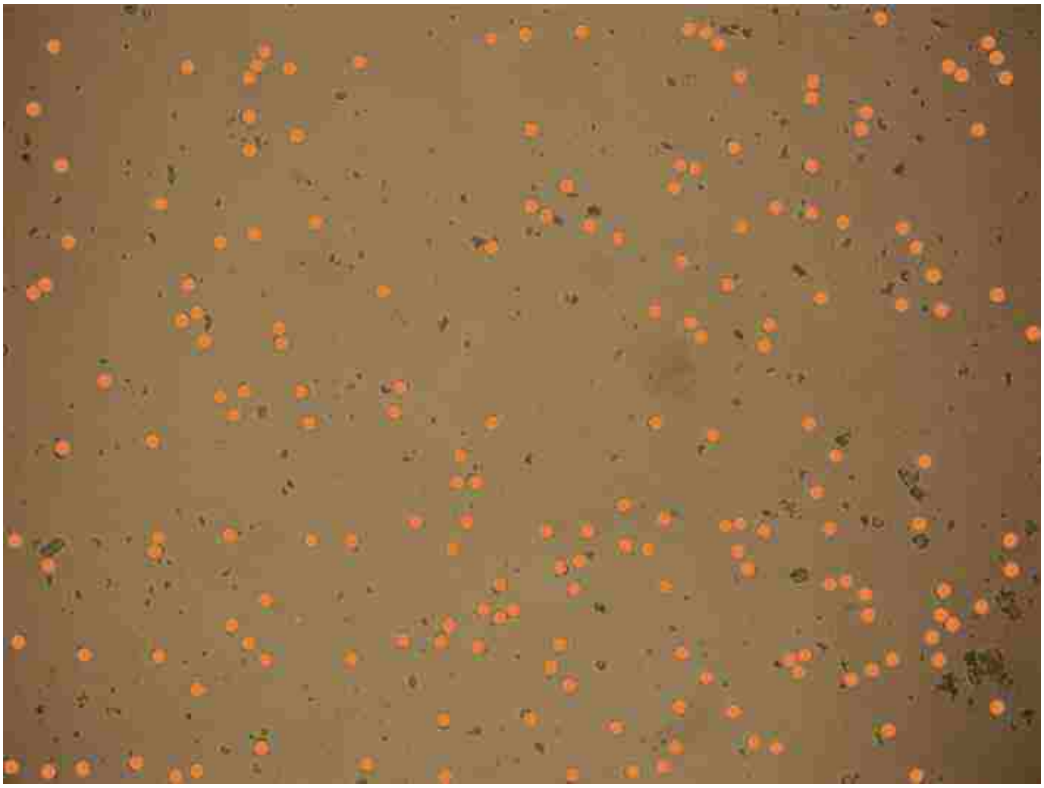


Figure 36. Cells and Particulate Matter observed using the TC Method (Orange circles represent cells).

damage may have been sustained by the smooth end forceps and the mechanical peeling of the tissue away from the root canal dentin wall, but this was not measured. The thermal damage is potentially diminished by the minimal contact time between the rotary disc and the extracted tooth, as well as the absence of direct contact between the rotary disc and the dental pulp tissue. Most importantly, the volume of dental pulp tissue harvested was greater using the TC Method over the other two methods. Although this was not directly quantified, the number of visible cells and the viable cells may provide indirect results for this observation.

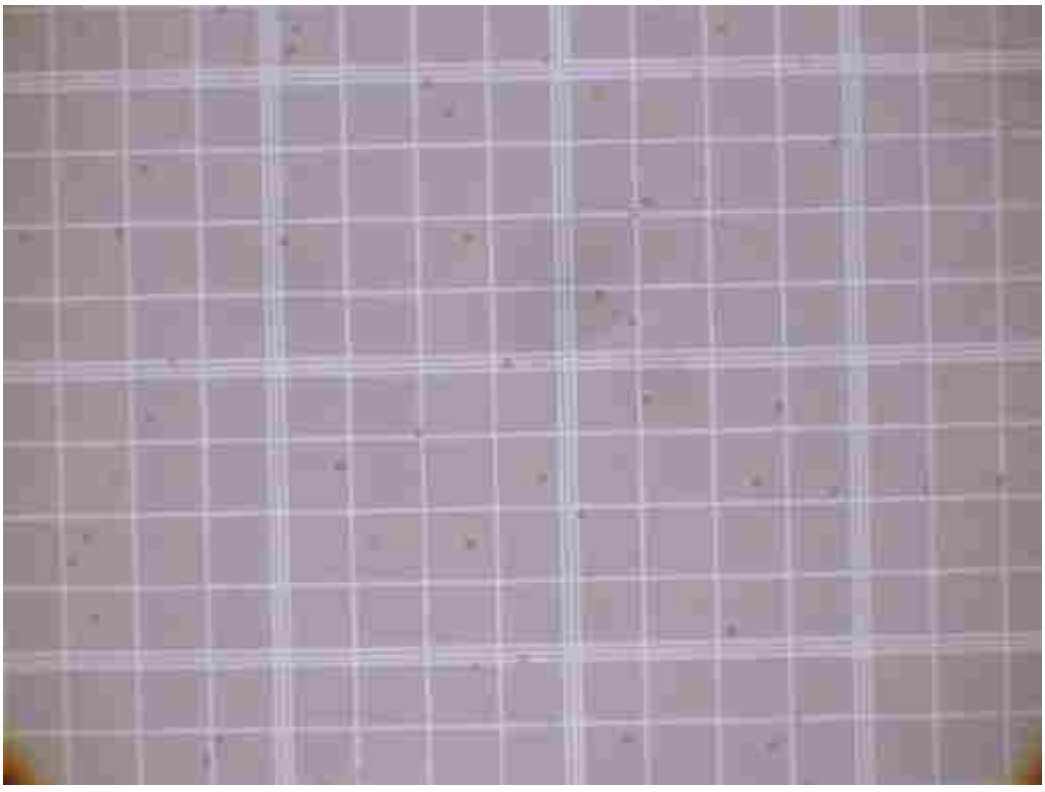


Figure 37. Viable cells seen after using Trypan Blue. (TC Method)

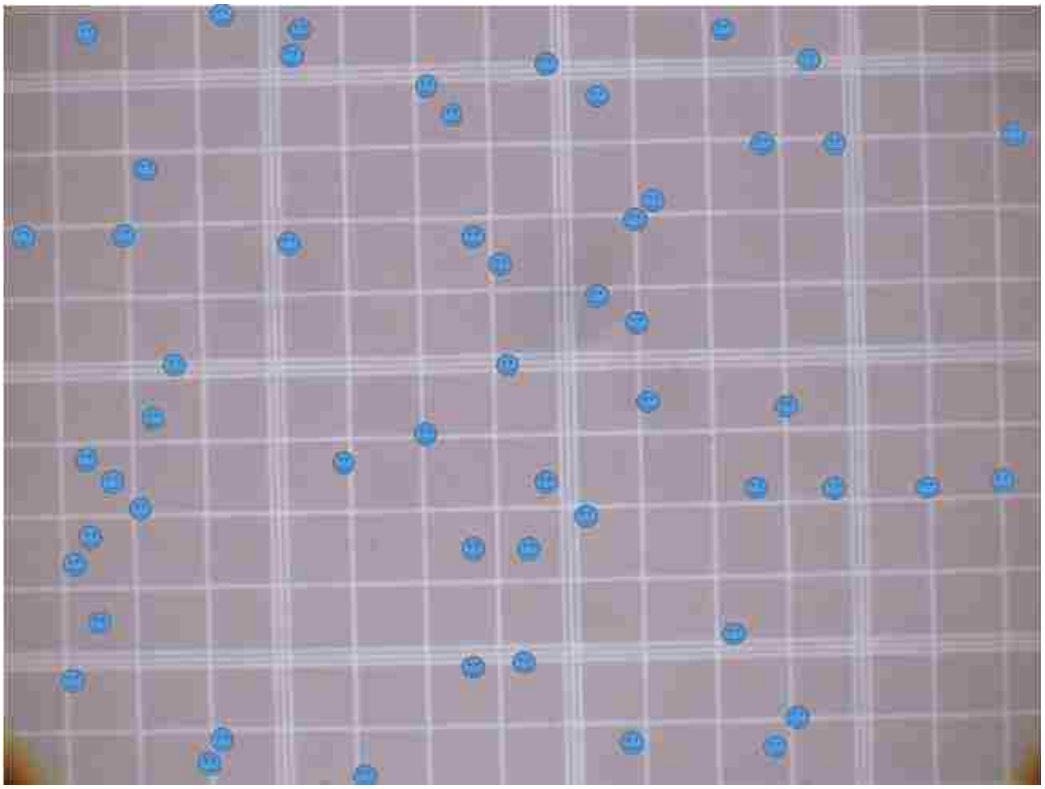


Figure 38. Viable cells (Blue dots) seen after using Trypan Blue. (TC Method)

Summary of Methods of Accessing Dental Pulp Tissue

The three methods for accessing the dental pulp tissue provides a foundation for further investigation. Although preceding literature has been inundated with the CEJ Method, the TC Method clearly provides a significant advantage over both the CEJ Method as well as the RCT Method. Figure 39 illustrates the number of viable cells using each of the three methods. Figure 40 illustrates the number of viable cells and the total number of

visible cells counted amongst the particulate matter. The transcending data produced by the TC Method provides hope for harvesting more dental pulp tissue and ultimately more MSCs; the CEJ Method and the RCT Method provide nearly parallel results, with the RCT Method producing much higher amounts of particulate matter.

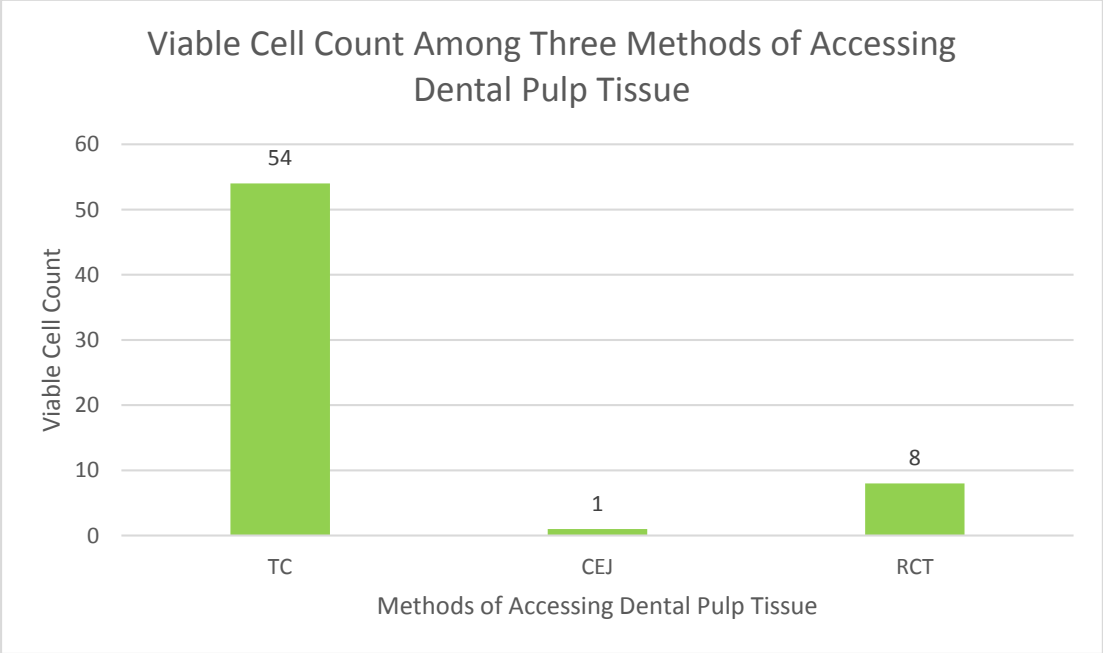


Figure 39. Viable Cell Count Among the Three Methods of Accessing Dental Pulp Tissue

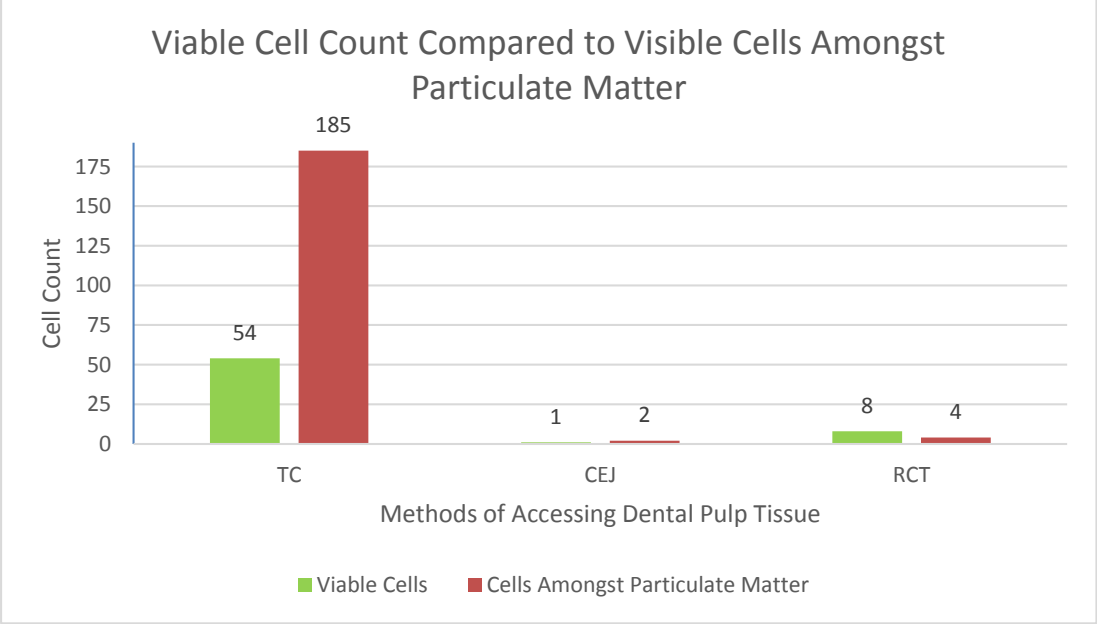


Figure 40. Three Methods of Accessing Dental Pulp Tissue to Provide a Viable Cell Count Compared to the Visible Cells Amongst Particulate Matter

Figure 41 provides a summary of the amount of particulate matter from the three methods of accessing dental pulp tissue. The TC method provides small sized and less quantity of particulate matter in comparison to the other two methods investigated. This graphic representation uses a minimum quantity of particulate matter tallied as an estimation. The RCT Method provides very large quantities of small and large pieces of particulate matter. Although more dental pulp was expected from the RCT Method, the particulate matter may provide an initial deterrent. Further investigation would be required to ascertain if more dental pulp tissue can be extirpated and ultimately provide more MSCs. Knowing the majority of the dental pulp tissue resides in the coronal portion of the tooth, it is important not to discount the RCT Method from the amount of particulate matter alone.

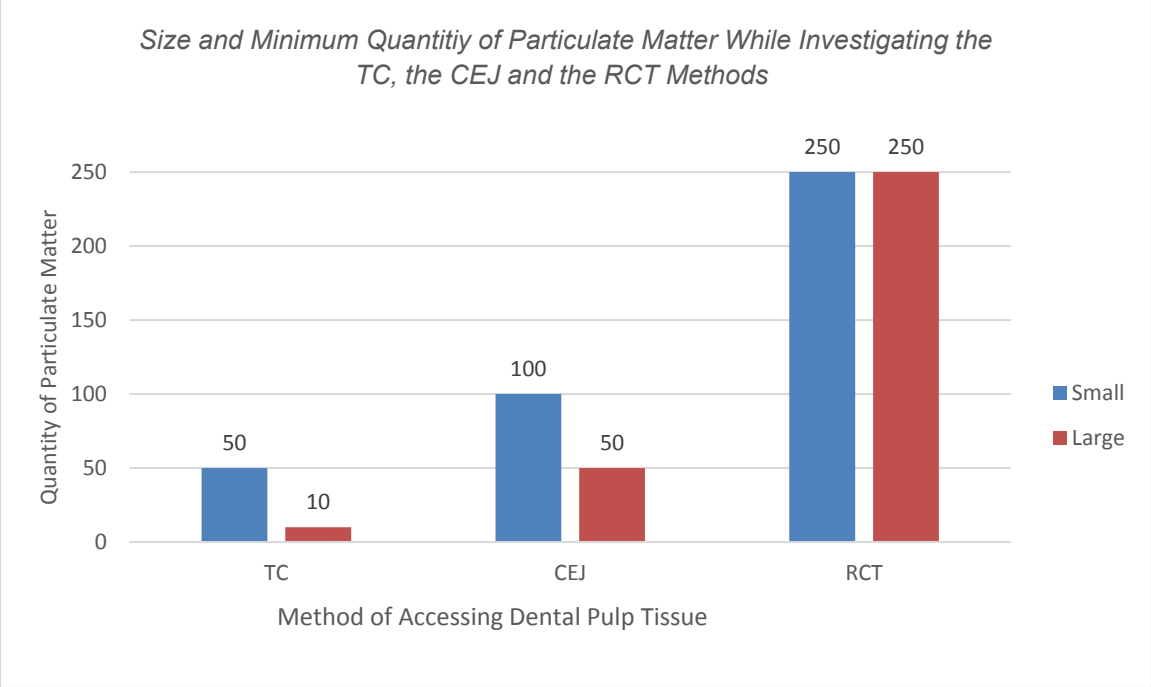


Figure 41. Size and Minimum Quantity of Particulate Matter While Investigating the TC, the CEJ and the RCT Methods

5.4 Study Limitations

Experiment design was a difficult task as studies of its kind have not been published in literature. In order to have a control, this paper discusses the use of a theoretical control to compare the data set. Although this control may not be ideal, this paper provides a benchmark for further studies. Considerable time was invested into designing an instrument to conduct the experiments. These efforts could have proved more useful in the testing phase of the study, but limited resources were a hindrance. Basic engineering principles were used in designing the instrument used in the experiment, however, advanced knowledge in design and machining would have been beneficial. The design and manufacturing of the device is in its infancy and deserves attention to further validate the results. The results of this study have a small sample size of previously extracted teeth. Although these teeth have been stored in an appropriate medium of 10% formalin, they do not model the exact nature of freshly extracted teeth.

Teeth

In lieu of using freshly extracted teeth to evaluate the predicable nature of the process, scarce resources provided previously extracted teeth that were sterilized and stored in 10% formalin. Once the teeth are removed from the oral environment, they can readily desiccate and become brittle. Further research is needed to evaluate the predictable outcome of splitting freshly extracted teeth using the same design, and further test the TC5. Each of the three methods discussed needs further evaluation with a larger sample size. The samples of viable cells collected should be cultured with various cell lines and markers

to illicit specific cell growth. These efforts can further validate the best method of accessing the dental pulp tissue.

Freshly Extracted Teeth Sample

The largest limitation in evaluating freshly extracted teeth was the sample size. The small sample does not provide statistically significant data to support one particular method, however it does provide significant differences among the three methods.

Instrument

The aluminum body of the instrument is appealing, but from a manufacturing perspective the device requires further attention. If the instrument was disposable then the material of choice only requires sufficient rigidity to conduct one test, as it would be discarded thereafter. With the intention of designing an instrument for multiple tests, however, a pragmatic solution may be a stainless steel product with a rigid framework that could also withstand sterilization under high temperature and pressure. Moreover, attention to engineering, ergonomics, and general ease of use is required to provide a commercial solution.

A carbide cutting disc was the first option for scoring the teeth. The carbide cutting disc would have less surface contact to the tooth than a diamond disc. The less surface contact results in less heat transfer to the dental pulp, which has yet to be studied for its significance. This study used a carbide disc as it was the only available disc that allowed ease of disassembly to modify the apparatus with a depth guide. This is not a direct limitation to this study as viable cells were not sought, but it can potentially affect the viability of cells form freshly extracted teeth. This is a topic for future research.

5.5 Recommendations for Further Research

The primary goal of this research study was to determine if it possible to access the dental root canal by scoring and splitting a tooth. Since this has not been published in the past, it is a new process and this study defines a benchmark for future research. The following provides guidance to some future research opportunities.

Instrument

Further engineering expertise would be beneficial in designing an instrument that can be practical, efficient and economical. Various polymers, metals and alloys can be tested to withstand varying loads, sterilization environments, and general usability of the instrument. Incorporation of a scoring device to the TC5 instrument can be an option, although it may not be financially feasible.

Further details of the instrument can be studied, in particular the TCA apparatus. The instrument pivots on this important handle to provide a safe and rigid environment to conduct the scoring process. An ideal tooth holder would secure the tooth rigidly while the tooth is being scored, and then gradually releases upon application of tensile load. This would potentially eliminate the need for the PVSH, and would render a single step instrument for commercial application. A design modification that allows the entire tooth to be scored, including the occlusal surface, would be beneficial from a safety perspective as the experimenter would not be at risk of unintended injury while holding the tooth manually to score the occlusal surface.

The scoring can be varied in depth and thickness to determine changes in the final result. Furthermore, alternative scoring location can be evaluated in addition to the mesial and distal locations as demonstrated in this study. Ease of access to the dental pulp can be

evaluated by varying the score thickness. It should be noted, however, that although this may potentially provide more access to the dental root canal, it could concurrently result in a less predictable fracture. As mentioned earlier, because a material requires a sharp crack to cause failure upon application of a load (Anderson 1995), a wide tapering crack would not provide predictable results.

An electron microscope may provide further insight into the relationship of the scored interface and split location. This examination may provide further explanations for teeth that did not match the theoretical control. It particular, the electron microscope could help explain the role of accessory canals in potentially redirecting crack propagation while splitting teeth. This information could further be applied to determine whether fractures in live patients have any correlation with contribution from accessory canals. Prior to three dimensional imaging, these accessory canals were given little attention, but with the aid of current advanced imaging techniques, their role and importance should not be overlooked. Carbide and diamond discs can be examined to determine the amount of energy absorbed by the tooth, thus potentially harming important cells of interest.

Teeth

This study can be repeated with freshly extracted teeth and the results can be documented and compared to this study. A study conducted in conjunction with dental pulp stem cell research may be beneficial as the freshly extracted teeth may provide cells in addition to providing data for splitting teeth. In addition, teeth with restorations, dilacerations, multi-rooted teeth, and prophylactically extracted third molars can be evaluated for their predictability in splitting scored teeth and access to the root canal.

Freshly Extracted Teeth

While a very small sample size was used to compare the three methods of accessing dental pulp tissue, the results were distinctive. The TC Method provided the most number of visible cells amongst particulate matter and the most number of viable cells. To further support this event, large sample sizes will be required to provide statistically significant results. Moreover, evaluation of the various regions of the dental pulp tissue and how it differentiates can illustrates the importance of harvesting the entire dental pup tissue. The importance of such geographical importance of dental pulp tissue could further validate the importance of selecting the best method of accessing the dental pulp tissue.

Commercial Interest

Currently, there are 4 companies in the USA that will harvest and store dental stem cells, two of which are located in the Las Vegas, Nevada. In addition, universities, colleges and private laboratories are all on the forefront of mesenchymal stem cell research.

A survey of research laboratories and dental offices may provide valuable information about the chair side usefulness of such a device with the ability to extract the dental pulp immediately upon tooth extraction. This would provide insight into the use of such a product and determine if research centers are more appropriate than individual dental offices. Dental offices would be more convenient for a patient and therefore potentially provide a larger sample.

Pathology

Potentially having the ability to isolate the dental pulp and the dental nerve can expose many research opportunities. In particular, the size, shape, number of cells and type of cells present can be evaluated for varying pathological states. This can extend to

systemic and local pathology and a direct evaluation of the dental response can be evaluated. For instance, the histological changes for various pathology can be studied in addition to changes in dental pulp tissue volume.

5.6 Conclusions

This study aimed to score the surface, of an extracted human premolar tooth, and reliably split teeth using tensile stress in order to access the dental root canal. The evidence shown in this research paper clearly demonstrates that teeth can be scored and predictably split, as demonstrated in 92% of the data set. In addition, 100% of the data set showed visible access to the root canal in the split teeth. In addition, the three methods of accessing dental pulp tissue provide a comparison of historical methods to proposed method. The TC Method showed 54 viable cells compared to only one viable cell for the CEJ and eight viable cells with the RCT Methods. These efforts may prove to be a good starting point to further investigate the TC Method for accessing dental pulp tissue to ultimately study MSCs. This research paper provides evidence for using a minimally invasive technique that can provide a feasible alternative to the current methods of accessing dental pulp tissue.

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