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Can Hyaluronic Acid be used to Stabilize Traumatized Teeth?

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Abstract

Objective: Dental trauma may occur during daily life because of unexpected accidents. These dental traumas may affect facial esthetics and self-image; thus, successful dental trauma treatment is important. The objective of our study is to determine whether hyaluronic acid (HA) may be used as a stabilizer in the periodontal ligament space of traumatized teeth.

Methods: *Ex vivo* study of pig mandibular central incisors will be used as a dental trauma model. Mandible segments containing central incisors will be fixed in acrylic. The incisor will receive luxation trauma using a simple extraction procedure. Subsequently, some samples will receive periodontal injections of HA and other samples will be used as untreated controls, Mineral Oil or Saline. Traumatized samples will be tested for mobility using 1) manual tests, 2) the Periotest, and 3) a materials testing system (MTS).

Keywords: *Hyaluronic Acid; Dental trauma; Facial esthetics; Phagocytes; Juve'derm; Periodontal Ligament*

Background

Dental trauma may occur through accidents and may affect facial esthetics and self-image [1]. Good treatments that reduce the damage in oral tissue and support the healing process are essential. The goal of our study is to determine whether HA can be used in treatment of dental trauma to stabilize tooth movement. As background for this study, we need to understand the HA molecule, the histology and function of the periodontal ligament, and dental trauma.

Hyaluranic Acid

Hyaluronic Acid (HA) is a polysaccharide, found in all tissues and body fluids of vertebrates [2]. HA is a component of the extracellular matrix [3] of mammalian tissues such as skin, cartilage, bone, synovial fluid [3], and periodontal ligament [4]. Most of the HA is circulated in the body by the lymph system. The lymph nodes extract 80-90% of HA from peripheral lymph. In addition, an adult human can have 15g HA content that is mostly it in the skin [5].

HA works as lubrication, shock absorption and provides bulk in human skin [6]. According to Mow., et al. [7], the proteoglycan, which exist in cartilage, is attached to the long hyaluronic acid molecules that contribute to viscoelastic properties of cartilage. Hyaluronic acid viscoelastic properties play a role in cell membrane protection and stabilization. The HA also presents on epithelia, macrophages and other mononuclear phagocytes as well as some type of neurons [6]. The HA interacts with the surface of cells with receptors that bind to HA with high affinity, for examples, the transmembrane glycoprotein receptors in epithelia that mediate cell attachment to the basement membrane and epithelial cell proliferation. Macrophages also have large numbers of the transmembrane glycoprotein receptors for HA that helps macrophages to migrate [6].

HA binds water molecules, which leads to increased skin hydration and turgor [2,8]. According to Laurent., *et al*. [9], if the H.A. increases, then the amount of water will increase. H.A. is a good osmatic buffering substance and a water homeostasis regulator in the body.

HA is a non-immunogenic, non-toxic and non inflammatory molecule [3], and can be used in tissue engineering and treatment in humans and animals. Because it preserves its innate biocompatibility when it is injected, HA is used in facial cosmetic surgery to improve tissue viscosity, and elasticity [8]. There are many types of hyaluronic acid products for cosmetic surgery that are legally used in the U.S. such as Hylaform, Restylane, Juve'derm, and Captique. Each type has similar and different proprieties. The type of hyaluronic acid is chosen according to the patient needs and the location of facial wrinkles. For instance, Hylaform is used to treat moderate to severe facial wrinkles and folds and the effect lasts for about 12 weeks. Restylane, which is derived from bacterial (*Streptococcus*), is used to smooth wrinkles and folds around the nose and mouth and the effects last for about 6 months. Juve'derm is a type of hyaluronic acid that contains the highest concentration of non-animal and cross-linked hyaluronic acid. There are two type of Juve'derm; 24HV, which is used in deeper filling and 30 HV dermal filler, which is used for more shallow and superficial dermal augmentation. Captique is a newer hyaluronic acid that is similar to Hylaform but it differs in that it is from a bacterial source while Hylaform is from an avian source. It is used to treat moderate to severe facial wrinkles [8]. HA has also been used as treatment of equine traumatic arthritis, and a delivery mechanism for implants, preventing postoperative adhesions and scar formation [9].

Furthermore, in *in vitro* and *in vivo* animal studies HA showed a positive effect on bone regeneration and wound healing. In dentistry, the application of 0.8% HA to the periodontal pocket showed positive effects on the reduction of plaque and probing depth and may prevent recolonization by periodontopathogens. Patients also applied 0.2% HA gel onto the gingival margin twice daily over the following 14 days [10]. According to this study, using hyaluronan-containing gels and treatment of periodontal disease shows antibacterial action on *Campylobacter rectus* and controls the amount of *Prevotella intermedia* and *Porphyromonas gingivalis*. HA is a molecule, which has many characteristics that support its potential use as a tooth stabilizer in dental trauma.

Periodontal Ligament (PDL)

PDL is soft, specialized connective tissue which is located between the cementum that covers the root of the tooth and bone that forms the socket wall. The PDL width ranges between 0.15 to 0.38 mm and the PDL width also decreases with age. The PDL supports the teeth in their sockets, and at the same time permits them to withstand masticatory forces. In the PDL are there many types of cells such as fibroblasts, myofibroblasts, osteoblasts, cementoblasts and osteoclasts [11,12]. The fibroblasts are found between collagen fibers and their orientations are related to those fibers. The myofibroblasts have characteristics of both fibroblasts and smooth muscle cells. The myofibroblasts are responsible for the force associated with wound contraction. They are also implicated in generation of the force of tooth eruption. The osteoblasts are found as a layer of cuboidal cells on the surface of the growing bone. They are responsible for matrix deposition and mineralization of the bone. In contrast, the osteoclasts, located on the bone surface, are responsible for osteolysis and are under the influence of osteocytes. The cementoblasts are located adjacent to cementum and are responsible for formation of cellular cemetum [13].

In the PDL, fibroblasts produce fibers that play a role in tooth movement during mastication, accommodation for growth of the jaw, and compensation for occlusal and inter proximal wear. Different fiber types are included in the PDL, such as collagen types III, XII, and I. These fibers are arranged in distinct fiber bundles that are organized into different groups. These groups are: the alveolar crest group which attaches the cementum and the rim of alveolus, the horizontal group which runs at right angles to the long axis of the tooth from cementum to bone, the oblique group which runs from the cementum in an oblique direction to bone coronally, the apical group which is in the apex of the tooth forming the base of the socket and connecting the cementum and the bone, and the inter radicular group which is found between the roots of multi rooted teeth and runs from the cementum to the bone. In addition, the PDL is well vascularized from superior and inferior alveolar arteries. The PDL is innervated with four types of neural termination, including free nerve endings, Ruffini's corpuscles, a coiled form, and spindle like endings surrounded by a fibrous capsule [12,13].

Dental Trauma

Dental trauma affects teeth, oral soft tissue and the periodontium. Tooth fractures may involve enamel fracture, enamel and dentin fracture, or root fracture, and injuries of the periodontium cause subluxation, luxation, intrusion, or avulsion [1,14]. Although the circumstances that produce the dental trauma are diverse (or alternatively, the traumatic load may be different), these traumas can result from direct or indirect dental injuries. Direct trauma can happen when the tooth is struck, such as against the stairs or a table, while indirect dental trauma can happen by having trauma in the chin the impact of the chin on upper dentition and causes crown or crown-root fractures. Furthermore, dental trauma is more common in boys than girls. For example, 31-40% of boys have affected primary teeth and 12-33% permanent teeth, whereas 16-30% of girls experience trauma in primary teeth and 4-19% in permanent teeth. The maxillary central incisor is most commonly involved [1].

Healthy beautiful teeth play an important role in facial esthetics, self-image, self-presentation, and interpersonal confidence; thus recovery from dental trauma is important. As a first step, we need to understand the repair of oral tissue injuries. According to Andreasen, Andreasen and Andersson, 2007, dental trauma is "an interruption in the continuity of tissues and healing is as the reestablishment of that continuity." Tissue healing includes either tissue repair, in which the healed tissue is different than the original tissue in anatomy and function, or tissue regeneration, which has the same anatomy and function as the original tissue. Our goal in using HA in dental trauma treatment is to find out if HA would help to stabilize the tooth so that the injured tissue can regenerate. In addition, there are many factors that could affect the healing process in dental trauma, such as infection [1]. According to Eick., *et al*. [11], HA shows antibacterial action that would reduce infections. As such, HA includes characteristics that may be helpful in treatment of dental trauma.

The objective of our study is to find out if HA can be used to promote tooth stabilization in the treatment of dental trauma. Prior to doing this study in humans, we must determine the risks and benefits of using HA in dental trauma treatment; thus, studying this question in an animal model can represent human dental trauma [15]. Using pig incisors will provide the answers to our questions because the anterior incisor teeth in pigs are similar to humans; however, to take into account ethical consideration experimentation on animals should be limited [15]. Because of this, we will use *ex vivo* pig tissues, to eliminate pain and suffering in a living animal model. Using *ex vivo* pigs' heads will answer our question of whether HA can be used as tooth stabilizer in dental trauma treatment.

Tooth Mobility Measurements

Two different procedures are used in the clinic to measure tooth mobility. In the first method, the tooth is held between the handles of two metallic instruments or with one metallic instrument and one finger, and an effort is made to move the tooth in all directions. Faciolingual mobility is abnormal and is graded according to the extent of tooth movement. The possible outcomes are normal mobility, Grade I, which is < 0.2 mm of horizontal movement, Grade II, which is 1-2 mm of horizontal movement, or Grade III, which is > 2 mm of horizontal or any vertical movement. Manual mobility measurement is subjective, however, because it differs from one person to another.

A second method used to measure tooth mobility is an electronic device, known as the Periotest. The Periotest is preferred clinically, because of its easy application and ability to measure mobility in the horizontal and vertical dimensions with reproducible results and detection of small changes in tooth mobility. The Periotest also has been used to detect tooth mobility in trauma studies [16]. In this study, the manual mobility and Periotest will both be used because they are the available ways to measure tooth mobility in the clinic. In addition, the materials testing system (MTS), which applies compressive load and measures displacement of the tooth, will be used. MTS has been used in a previous ex-vivo study to measure the biomechanical effects of tooth on growing alveolar bone while the tooth erupts [17].

The Objectives:

1. Measure the viscosity of H.A, Mineral Oil and Saline.
2. Compare the H.A with Mineral Oil and Saline in Stabilizing Traumatic teeth.
3. Compare between Periotest and manual test to measure tooth mobility.

Materials and Methods

In order to compare H.A with Mineral Oil and Saline in stabilizing traumatized teeth, the differences in viscosity between these liquids were tested. Then, the Material Testing System (MTS) was used to compare between H.A and saline, and H.A and Mineral Oil in stabilizing traumatized teeth. Finally, the periotest and manual test were used to measure tooth mobility and to compare between normal conditions and gingival injury.

The Viscosity Test

Three graduated cylinders were filled with 50 ml of the different liquids, H.A, saline and mineral oil, as it shown in Figure 1. A marble was dropped in the saline five times and a stopwatch was used to record the time taken for the marble ball to reach the bottom of the graduated cylinder. The same procedure was repeated for H.A and mineral oil.

Figure 1: *Three-Graduated Cylinders.*

Using the Material Testing System (MTS) to compare H.A and saline

Pig heads were collected from an abattoir (n = 6) but 2 specimens were not used because the teeth broke during specimen preparation. Specimens were prepared making a coronal cut, proximal to the mandibular symphysis with a Butcher Boy's band saw. Mandibles were stored in 0.9% saline solution and frozen until testing.

Before the testing, specimens were removed from the freezer, thawed in a water bath, and embedded in Jet Denture Repair Acrylic as it shown in Figure 2.

Figure 2: *Embedded Specimen in Jet Denture Repair Acrylic.*

The incisal edge of the lateral incisor was ground to the gingival level by using a high-speed hand piece. Either the right or left central incisor was ground 2 mm shorter than the other central incisor in order to concentrate the compressive load on a single tooth. Incisor extrusive luxation was modeled in both left and right central incisor teeth in the pig mandible using simple extraction procedures. A dental elevator was used to sever the periodontal ligaments, the fibrous connective tissue that holds teeth in the sockets. The elevator was placed between the tooth and gingiva and a small amount of pressure was applied to reach the root area. The elevator was rotated within the periodontal ligaments space in order to loosen the tooth and expand the bony socket. Dental force ps were used to grasp the tooth as far down the root as possible. The tooth was rotated a quarter turn and then pulled 5mm upward. The tooth was immediately replaced back into the socket. Two ml of HA was injected into the socket in lingual and buccal location of one of the central incisors in order to stabilize the tooth and two ml of normal saline was injected into the other central incisor. The MTS was used to load the highest crown in compression at 0.5 mm/min, and after this test, the tooth was extracted, Figure 3, in order to apply compressive load to the remaining central incisor.

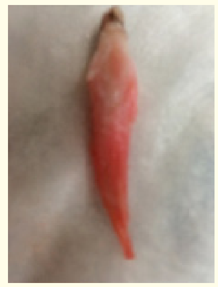

Figure 3: *Extracted Central Incisor.*

Using the Material Testing System (MTS) to compare H.A and mineral oil

Pig heads were collected from an abattoir (n = 8) but one was lost in preparation. Specimens were prepared as described previously, and the MTS was used to load the crown in compression at 0.5 mm/mining the specimen before and after the application of incisor trauma. Simple extraction procedures were used to achieve luxation on the teeth as described previously. After MTS compression testing, the tooth was injected with two ml of HA or mineral oil into the socket in lingual and buccal location of one of the central incisors in order to stabilize the tooth, and left for 30 min. After that, the MTS measurements were made on the tooth that has been injected with H.A or mineral oil. Then, the tooth was extracted and the root length for the extracted tooth was measured. These tests were repeated in the remaining central incisor.

The periotest was measured in the central incisors before they were traumatized. However, the periotest could not measure tooth mobility after trauma was applied because the tooth mobility was higher than it could record. The correlation was preformed between the load deformation and the root length.

The Periotest vs. The manual test

Pig heads were collected from an abattoir (n = 6). Specimens were prepared making a coronal cut, proximal to the mandibular symphysis with a Butcher Boy's band saw. Mandibles were stored in 0.9% saline solution and frozen until testing.

Prior to the experiment, bagged specimens were removed from the freezer and thawed in a water bath. Then, the periotest, Figure 4 and 5, was used on the second premolar.

Figure 4: *Periotest. PERIOTEST M Operating Manual.*

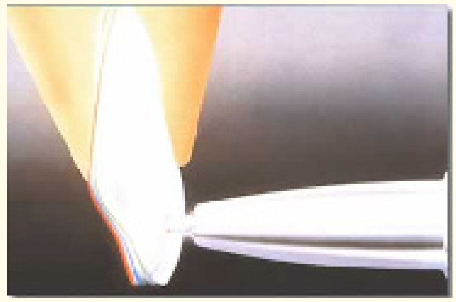

Figure 5: *Periotest while testing the Tooth Mobility. PERIOTEST M Operating Manual.*

The periotest was repeated many times in order to ensure that the specimens and periotest were stable. When the same result was shown three times, that result was recorded, and manual mobility tests were performed. The tooth was held between the handles of two dental probes and an effort was made to move the tooth in all directions. After the periotest and manual test results were recorded for the same tooth, the gingiva was retracted 5 mm with adental elevator; and a probe and a ruler measured the distance. The periotest and manual test were repeated after the gingiva was retracted 5 mm.

Results

The Viscosity Test

Microsoft excel was used to analyze the result of the viscosity test. According to table 1, mineral oil, normal saline and H.A. have different means. The H.A. has the highest viscosity while the normal saline has the lowest viscosity as it shown in figure 6. The mean for mineral oil was 0.338 ml/min ± 0.013 ml/min, while the mean for normal saline was 0.266 ml/min ± 0.011 ml/min and the mean for H.A. was 0.412 ml/min ± 0.019. These means were significantly different.

	Mineral Oil (ml/Minute)	Saline (ml/Minute)	H.A (ml/Minute)
1	0.34	0.27	0.39
2	0.32	0.26	0.4
3	0.35	0.28	0.42
4	0.33	0.25	0.44
5	0.35	0.27	0.41
Mean	0.338	0.266	0.412
SD	0.013	0.011	0.019

Table 1: *The Viscosity Test.*

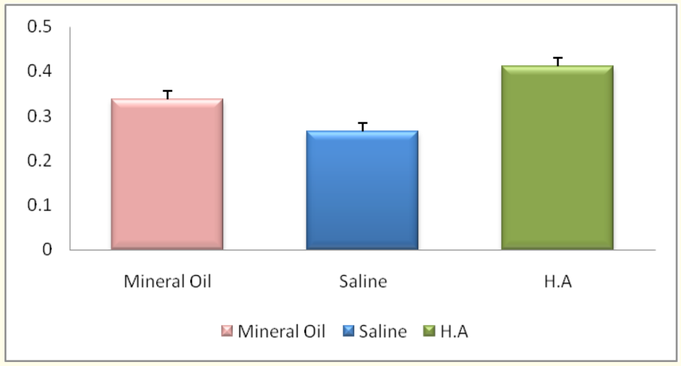

Figure 6: *The Viscosity Test (ml/min).*

Using the Material Testing System (MTS) to compare between H.A and saline

Comparison of mobility values in HA treated vs. saline controls determined whether HA treatment affects the post-traumatic stability of a tooth. Table2 shows the range of the load and extension that was used in the MTS tests in order to calculate the slope. The slope was used to determine the stability of the central incisor. Analysis of variance for time test was used. The F-ratio was 7703.4-119.31 and the probity was less or equal to 0.0001.

FP1		Normal Saline	H.A
	Load	0.25 – 1.82N	0.72 – 4.36N
	Extension	0.52 – 0.7 mm	0.09 – 0.68 mm
	Slope	8.66 N/mm Root Length = 28.65	6.09 N/mm Root length= 33.92
FP3			
	Load	0.17 – 2.07N	0.57 – 5.56N
	Extension	0.03 -0.29 mm	0.04 - 0.55 mm
	Slope	6.95 N/mm Root Length = 35.66	10.31 N/mm Root Length = 39.16
FP4			
	Load	1.24- 3.39N	1.26-1.8N
	Extension	0.021- 0.059mm	0.43-0.49 mm
	Slope	50.94 N/mm Root Length =36.21	10.32 N/mm Root Length = 29.03
FP5			
	Load	1.1-0.16N	NAD
	Extension	1.64-0.25 mm	NAD
	Slope	6.99 N/mm Root Length=29.03	NAD

Table2: *The MTS testing between H.A and saline.*

The slope results of the H.A. and the normal saline are similar. Additional tests were performed using mineral oil instead of normal saline. We also compared between the root length for each group and they weren't significantly different.

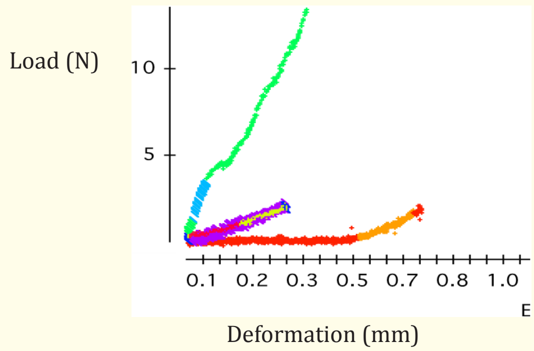

Figure 7-1: *Load Deformation for Normal Saline. FP1 Red/Orange, FP3 blue/Purple, FP4 green/blue, FP5 yellow/red (N/mm)*

Figure 7-1 shows the relationship between the load and deformation for teeth that used normal saline treatment. FP4, which is the green line in figure 7-1, was considered as outlier because it's higher than other specimens. Figure 7-2 shows the relationship between the load and deformation for teeth that used H.A. treatment.

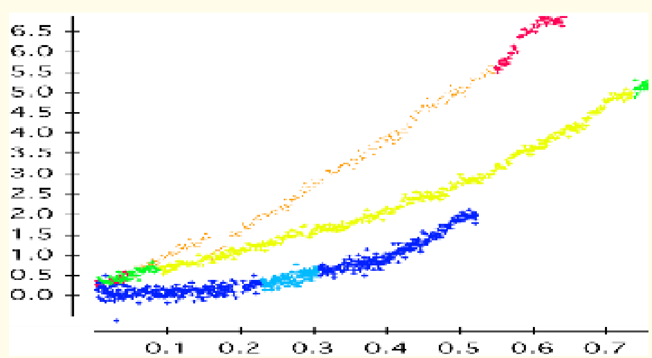

Figure 7-2: *Load Deformation for H.A.Teeth Treatment. FP1 green/yellow, FP3 Red/orange, FP4 blue/light.*

Using the MTS comparison between H.A. and mineral oil

The central incisors that used H.A. as treatment

Comparison of mobility values in the central incisor before and after trauma vs. mobility in the central incisor after H.A. treatment determined whether H.A. treatment affects the post-traumatic stability of the tooth. Table 3 shows the slope of the central incisors before trauma, after trauma and after trauma with the H.A. and the load range that were used. Figure 8-1 shows the relationship between the load and deformation for H.A. teeth before trauma and the load range that were used.

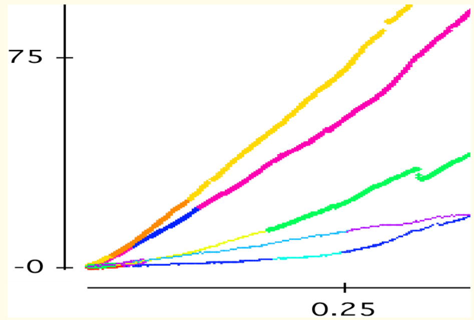

Figure 8-1: *Load Deformation for H.A. teeth before trauma. FP7 Blue/Light Blue, FP8 Purple/ Light blue, FP9 Pink/Blue, FP10 Yellow/ Orange, FP11 Red/Purple, FP13 Green/Yellow (N/mm).*

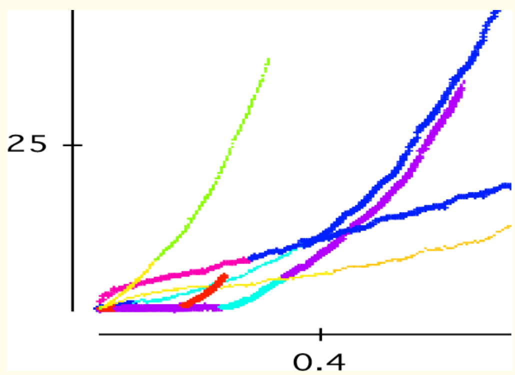

Figure 8-2: *Load Deformation for H.A. Teeth after Trauma. FP7 Blue/Light Blue, FP8 Purple/ Light blue, FP9 Pink/Blue, FP10 Yellow/ Orange, FP11 Red/Purple, FP13 Green/Yellow.*

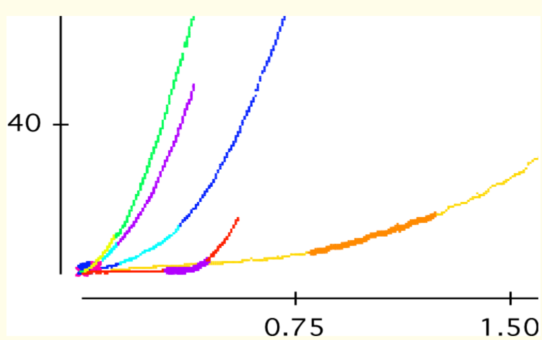

Figure 8-3: *Load Deformation after Trauma with H.A. Treatment. FP7 Blue/Light Blue, FP8 Purple/Light blue, FP9 Pink/Blue, FP10 Yellow/ Orange, FP11 Red/Purple, FP13 Green/Yellow.*

The mean for the slope before trauma was 121.09 N/mm ± 99.56 N/mm. The mean for the slope after trauma was 34.39 N/mm ± 22.46 N/mm. The mean for the slope after trauma with H.A. treatment was 50.26 N/mm ± 26.97 N/mm.

HA Tooth	Slope Before Trauma (N/mm)	Load (N)	Slope After Trauma (N/mm)	Load (N)	Slope After Trauma with H.A (N/mm)	Load (N)	Periotest Before Trauma	Root Length (mm)
FP9 Left C.I	210	7.71-21.43	23.99	7.91-21.05	29.67	0.81-1.94	-	31.63
FP 11 Right C.I	61.9	1.27- 2.11	10.7	0.37- 0.69	37.59	0.85- 3.13	-	41.4
FP7 Left C.I	32.08	3.17-5.51	28.18	1.19- 9.24	44.676	2.61- 12.38	16.7	33.75
FP8 Left C.I	53.93	2.06- 13.26	48.7	0.68- 5.03	75.34	0.97- 7.97	14.1	34.87
FP10 Right C.I	278.46	4.49- 24.11	22.189	5.89- 14.72	23.255	5.6- 15.26	6.3	44.49
FP13 Right C.I	90.17	0.97- 15.21	72.62	0.55- 7.4	91.03	0.78- 10.09	7.1	31.33

Table 3: *The MTS Testing for the Central Incisor before and after Trauma vs. After Trauma with H.A.*

First, the Wilcoxon signed rank test was used to compare between the slopes of central incisors before trauma vs. after trauma in alpha level 0.05. As is shown in table 4, the P value was 0.03. The hypothesis was rejected at Alpha = 0.05; and the slopes of central incisors before trauma are not equal to the slope of central incisors after trauma.

	Rank Totals	Cases	Mean Rank
Positive Ranks	21	6	3.5
Negative Ranks	0	0	-
Ties	-	0	-
Total	21	6	3.5
P value	0.03	-	-

Table 4: *Wilcoxon Signed Rank for Central Incisors before Trauma vs. after trauma.*

Then, the Wilcoxon signed rank was used to compare between the slopes of central incisors after trauma vs. after trauma with H.A. at alpha level of 0.05. As is shown in Table 5, the P value was 0.03; thus hypothesis was rejected at Alpha = 0.05. The slopes of central incisors after trauma are not equal to the slope of central incisors after trauma with H.A.

	Rank Totals	Cases	Mean Rank
Positive Ranks	0	0	-
Negative Ranks	21	6	3.5
Ties	-	0	-
Total	21	6	3.5
P value	0.0312	-	-

Table 5: *Wilcoxon Signed Rank for central incisors after trauma vs. after trauma with H.A.*

The H.A treatment increased the tooth stability after trauma. Figure 8 shows the slopes differences before and after trauma vs. post-traumatic H.A. treatment. When the slopes after trauma and after trauma with H.A. treatment are compared, they showed that after using H.A. treatment the slopes increase.

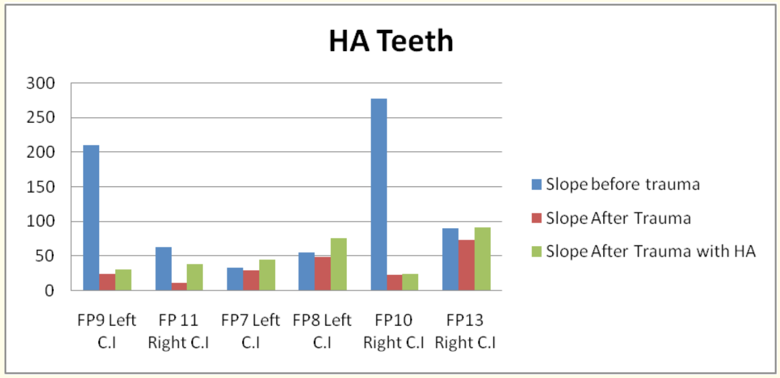

Figure 8: *The Slopes for the Central Incisors before and after Trauma vs. after Post-traumatic H.A. (N/mm).*

The central incisors with mineral oil treatment

Comparison of the mobility values in the central incisor before and after trauma vs. mobility in the central incisor after post-traumatic mineral oil treatment determined whether mineral oil treatment affects the post-traumatic stability of the tooth. Table 4 shows the slope of the central incisors before trauma, after trauma and after with the post-traumatic mineral oil treatment, and the load range that were used. The mean for the slope before trauma was 118.59 N/mm ± 46.79. The mean for the slope after trauma was 17.4N/mm ± 11.53. The mean for the slope after trauma with mineral oil was 21.84 N/mm ± 9.84. Figure 9-1 shows the relationship between the load and deformation for mineral oil teeth before trauma and the load range that were used.

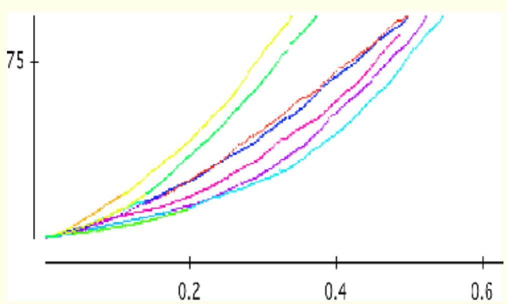

Figure 9-1: *Load Deformation before Trauma for Mineral Oil Teeth. FP7 Blue/Light Blue, FP8 Purple/Light blue, FP9 Pink/Blue, FP10 Yellow/ Orange, FP11 Red/Purple, FP12 Light Blue/Green, FP13 Green/Yellow.*

Figure 9-2 shows the relationship between the load and deformation for mineral oil teeth after trauma and the load range that were used. FP9, which is the pink line in Figure 9-2, was considered as outlier because the load was zero.

Figure 9-3 shows the relationship between the load and deformation after trauma with mineral oil treatment and the load range that were used.

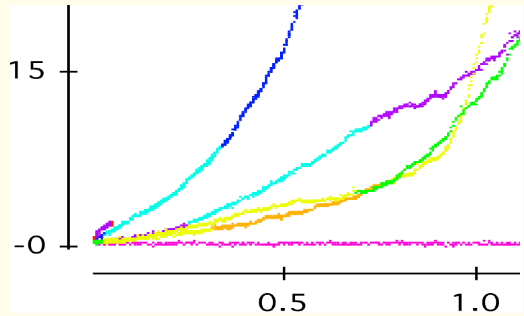

Figure 9-2: *Load Deformation before Trauma for Mineral Oil Teeth. FP7 Blue/Light Blue, FP8 Purple/Light blue, FP9 Pink, FP10 Yellow/ Orange, FP11 Red/Purple, FP13 Green/Yellow.*

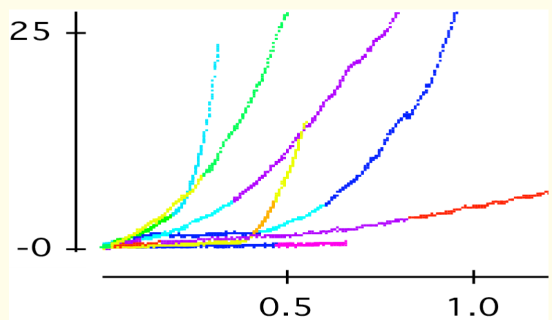

Figure 9-3: *Load Deformation after Trauma with Mineral Oil Treatment. FP7 Blue/Light Blue, FP8 Purple/Light blue, FP9 Pink/Blue, FP10 Yellow/ Orange, FP11 Red/Purple, FP12 Light Blue/Green, FP13 Green/Yellow.*

Mineral Oil Tooth	Slope before trauma (N/mm)	Load (N)	Slope After Trauma (N/mm)	Load (N)	SlopeAfter rauma with Mineral Oil (N/mm)	Load (N)	Periotest Before Trauma	Root Length (mm)
FP9 Right C.I	100	0.8-6.46	N.A	N.A	33.03	0.47- 0.63	-	33.03
FP11 Left C.I	122.9	1.08- 18.26	30.04	0.89- 1.73	3.64	0.68- 3.5	-	38.01
FP7 Right C.I	132.3	6.12- 15.55	22.62	1.6- 6.84	18.98	2.14- 5.27	12.2	29.93
FP8 Right C.I	68.8	3.73- 11.69	18.28	2.03- 10.98	19.27	1.27- 5.79	14.6	33.8
FP10 Left C.I	201.84	5.37- 18.02	9.13	1.6- 5.46	26.03	1.55- 5.83	15.6	40.18
FP12 Left C.I	65.86	1.95- 12.49	N.A	N.A	20.56	0.68-4.22	18	32.34
FP.13 Left C.I	138.48	0.94- 18.19	6.94	0.36- 4.47	31.37	0.46-8.3	13.6	27.8

Table 6: *The MTS testing for the central Incisor before and After Trauma vs. post-traumatic with the Mineral Oil.*

The Wilcoxon signed rank was used to compare between the slopes of central incisors before trauma vs. after trauma at an alpha level of 0.05. As is shown shows in Table 7, the P value was 0.06. The hypothesis was not rejected at Alpha = 0.05; thus the slopes of central incisors before trauma are equal to the slope of central incisors after trauma.

	Rank Totals	Cases	Mean Rank
Positive Ranks	15	5	3
Negative Ranks	0	0	-
Ties	-	0	-
Total	15		3
P value	0.0625	-	-

Table 7: *Wilcoxon Signed Rank for Central Incisors before Trauma vs. after Trauma.*

Then, Wilcoxon signed rank was used to compare between the slopes of central incisors after trauma vs. after post-traumatic mineral oil treatment at an alpha level 0.05. As it shows in table 8, the P value was 0.0625. The hypothesis was not rejected at Alpha = 0.05. The slopes of central incisors after trauma are equal to the slope of central incisors after trauma with mineral oil.

	Rank Totals	Cases	Mean Rank
Positive Ranks	7	2	3.5
Negative Ranks	8	3	2.67
Ties	-	0	-
Total	15	5	3
P value	0.81	-	-

Table 8: *Wilcoxon Signed Rank for Central Incisors after Trauma vs. Posttraumatic with Mineral Oil Treatment.*

The mineral oil treatment did not show significant change in tooth stability after trauma. Figure 9 shows the slopes differences before and after trauma vs. posttraumatic with mineral oil treatment. When the slopes after trauma and the slopes posttraumatic with mineral oil treatment are compared, they did not show significant change.

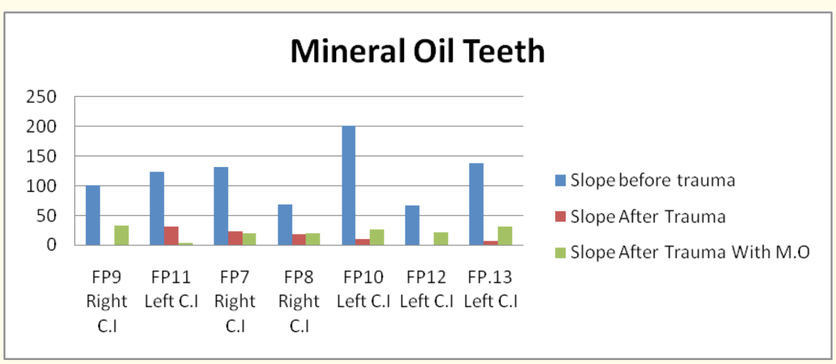

Figure 9: *The Slopes for the Central Incisors before Trauma and after Trauma vs. Posttraumatic Mineral Oil Treatment.*

The result of H.A. treatment vs. mineral oil treatment in the central incisors teeth

Comparison of mobility values in incisors treated with H.A. vs. mineral oil determined whether HA treatment affects the post-traumatic stability of a tooth.

The Mann-Whitney U test was used to compare from the slopes of central incisors before trauma for H.A. teeth vs. before trauma for mineral oil teeth at an alpha level of 0.05. As is shown in table 9, the P value was 0.54. The hypothesis was not rejected at Alpha = 0.05. As resulted, the slopes of central incisors before trauma are equal for both H.A. and mineral oil teeth.

	Rank Totals	Cases	Mean Rank
Slope before trauma (H.A)	37	6	6.16
Slope before trauma (Mineral Oil)	54	7	7.71
Total	91	13	7
Ties between Groups	-	0	-
P value	0.54	-	-

Table 9: *Mann–Whitney U Test for Central Incisors before Trauma (H.A) vs. before Trauma (Mineral Oil).*

The Mann-Whitney U test was used to compare between the slopes of central incisors after trauma for H.A. teeth vs. after trauma for mineral oil teeth in alpha level 0.05. As is shown in Table 10, the P value was 0.18. The hypothesis was not rejected at Alpha = 0.05. As a result, the slopes of the central incisors after trauma are not similar for both H.A. and mineral oil teeth. This statistic might be because the number of cases of the slopes after trauma for mineral oil teeth is very low. But the treatment has not been applied, we expecting no difference.

	Rank Totals	Cases	Mean Rank
Slope after trauma (H.A)	44	6	7.33
Slope after trauma (Mineral Oil)	22	5	4.4
Total	66	11	6
Ties between Groups	-	0	-
P value	0.18	-	-

Table 10: *Mann–Whitney U test for central incisors after trauma (H.A) vs. after trauma (Mineral Oil).*

The Mann-Whitney U test was used to compare between the slopes of central incisors after trauma with H.A. vs. after trauma with mineral oil in alpha level 0.05. As it shows in table 11, the P value was 0.023. The hypothesis was rejected at Alpha = 0.05; thus slopes of central incisors after trauma are not equal for both H.A. and mineral oil teeth.

	Rank Totals	Cases	Mean Rank
Slope after trauma with H.A	58	6	9.67
Slope after trauma with Mineral Oil	33	7	4.71
Total	91	13	7
Ties between Groups	-	0	-
P value	0.023	-	-

Table 11: *Mann–Whitney U test for central incisors after trauma with H.A vs. after trauma with Mineral Oil.*

The H.A has positive effects on the post-traumatic stability of a tooth vs. mineral oil as it shows in Figure 10 and Table 12.

	Slope before Trauma, H.A (N/mm)	Slope before Trauma, MO (N/mm)	Slope After Trauma, before HA (N/mm)	Slope After Trauma, before MO (N/mm)	Slope After Trauma with H.A (N/mm)	Slope After Trauma with M.O (N/mm)
Means	121.09	118.59	34.39	17.40	50.26	21.84
SD	99.56	46.79	22.46	11.53	26.97	9.84

Table 12: *The means and SD of the slopes of H.A. vs. the slopes of Mineral Oil (MO).*

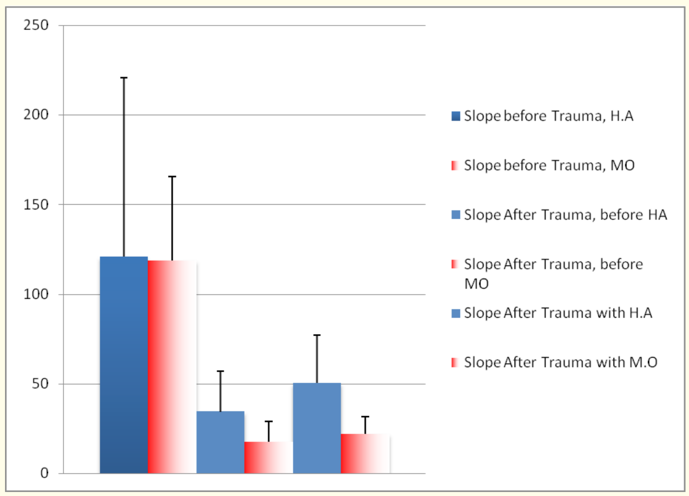

Figure 10: *The means and SD of the slopes of H.A. vs. the slopes of Mineral Oil (MO) (N/mm).*

The Periotest vs. the manual test

The results for the manual test for all the specimens before and after the gingiva was retracted 5 mm were zero, which means that there was no tooth mobility. However, the periotest mean before the gingiva was retracted 5 mm was 5.46 ± 1.86, which is corresponds with a manual measurement of zero as is shown in Table 13, while the mean for the periotest after gingiva was retracted 5 mm was 9.47 ± 2.78, which is corresponds with a manual measurement of 1. Table 14 shows the result of periotest and manual test for each tooth before and after gingiva was retracted.

Clinical Degree of Tooth loosening	Periotest Value Range
0	-8 bis +9
I	+10 bis +19
II	+20 bis +29
III	+30 bis +50

Table 13: *Measuring Natural Teeth by Periotest.*

Tooth	Perio-test	Manual test	Perio-test after retracting the gingiva 5 mm	Manual test after retracting the gingiva 5 mm
1 right 2nd premolar	6.3	0	10.6	0
1 left 2nd premolar	6.3	0	12.4	0
The Mean of Periotest in pig 1	6.3			
2 right 2nd premolar	7.4	0	9.6	0
2 left 1st premolar	7.2	0	16.2	0
The Mean of Periotest in pig 2	7.3			
3 right 2nd premolar	6.4	0	10.8	0
3 left 1st premolar	4.5	0	6	0
The Mean of Periotest in pig 3	5.45			
4 right 2nd premolar	7.8	0	8.4	0
4 left 2nd premolar	5.6	0	8.3	0
The Mean of Periotest in pig 4	6.7			
5 right 2nd premolar	4.8	0	7.9	0
5 left 2nd premolar	4.9	0	7.9	0
The Mean of Periotest in pig 5	4.85			
6 right 2nd premolar	2	0	8.9	0
6 left 2nd premolar	2.3	0	6.6	0
The Mean of Periotest in pig 6	2.15			
Mean	5.46	0	9.47	0
Standard error	1.86	-	2.78	-

Table14: *The Periotest vs. The manual test.*

The average of left and right second premolars for each specimen was taken. Then, Wilcoxon signed Rank test was used to compare between the results of periotest before and after the gingiva was retracted 5 mm at an alpha level of 0.05. As is shown in table 15 and 16, the P value was < 0.0001. The hypothesis was rejected at Alpha = 0.05; thus periotest of first premolar before gingiva was retracted are not equal to periotest after gingiva was retracted.

	Rank Totals	Cases	Mean Rank
Positive Ranks	0	0	-
Negative Ranks	253	22	11.5
Ties	-	0	-
Total	253	22	11.5
Ties differences	2	-	-

Table 15: *Wilcoxon Signed Rank Test for Periotest before and after Retracting the Gingiva 0.5 mm.*

Variance	948.75
Adjustment to variance for ties	-0.25
Expected Value	126.5
Z-Statistic	-4.107
P value	< 0.0001

Table 16: *Wilcoxon Signed Rank Test for Periotest before and after Retracting the Gingiva 0.5 mm.*

Discussion

This study objective was to find out if H.A. could provide tooth stabilization in treatment of dental trauma. Different methods were used to find the affect of H.A. in the stabilization of a traumatized tooth. The MTS comparison was used to compare between H.A. and normal saline or mineral oil. The comparison between normal saline and H.A. did not show different effects on tooth stability. This result might be because H.A. wasn't left long enough after application to show an effect to address the possible effects of treatment time. Because of that, the method was changed and mineral oil was used instead of normal saline because mineral oil is more similar in viscosity to H.A. than normal saline and the time for treatment was increased to 30 minutes. The MTS comparison was performed in different stages, i.e. before the trauma was preformed, after trauma and after trauma with treatment. The comparison between these stages shows the changes of tooth stability and the affect of the treatment on traumatized tooth. When the H.A. was used to stabilize traumatized teeth, the result showed that H.A. treatment increased tooth stability. That might be because the H.A. is osmatic buffering substance that when the H.A. increases, then the amount of water will increase. HA [9]. In the teeth that used the mineral oil to stabilization-traumatized tooth, the data analysis showed that the slopes of central incisors before trauma are equal to the slope of central incisors after trauma. But Figure 4 showed that they weren't equal that might be because the sample size of teeth after trauma was small. In addition, the mineral oil treatment did not show significant change in tooth stability after trauma when it was compared before treatment and after treatment. However, there was some limitation that might affect the result of using the MTS comparison such as the tooth might be stabilized by hitting alveolar bone.

Conclusion

The periotest could not be used as method of comparison for traumatized tooth before and after trauma because the tooth mobility after trauma was higher than the periotest could record. Instead of that a comparison between periotest and manual test was applied. The result showed that periotest is more specific and sensitive than manual test. However, the periotest is more challenging to apply than the manual test because it needs steady hand. In the clinic, some patients might find periotest is uncomfortable measurement tool because the periotest has tapping head that tap on the tooth for 4 seconds and the measurement depend in the change of the distance between the tapping head and the tooth. The periotest is more specific and sensitive measurement tool but manual test is easier to apply in the clinic setting.

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