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Developing an *in vitro* model of post-extraction bleeding and evaluating the feasibility of XSTAT for dental hemorrhage control

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Short Running Title: Repurposing XSTAT for Dental Hemorrhage Control

Summary: XSTAT, a combat casualty care treatment for penetrating wounds, can successfully be repurposed for treatment of dental extraction sockets.

Abstract

Background: Bleeding after a dental extraction is a common complication that is usually easily controlled; however, if the bleeding continues beyond 8 hours or is excessive it is often referred to as post-extraction bleeding (PEB). The current standard of care for tooth extraction and other dental injuries relies on messy and often ineffective hemorrhage control gels or applying pressure with gauze. XSTAT is a Food and Drug Administration (FDA) approved device that treats non-compressible injuries through placing a collection of sponges within the injury. This same

design can be scaled down (i.e., single sponge of smaller diameters) to treat a hemorrhaging extraction socket or other oral injury in a much quicker and cleaner way.

Methods and Materials: An *in vitro* dental extraction model simulating PEB was developed. In order to simulate PEB, an arterial blood flow circuit was generated using an infusion withdrawal pump and polyethylene (PE) tubing along with simulated blood. XSTAT sponges were trimmed in diameter (via cutting die) to dimensions more appropriate to the socket. Following extraction of the various tooth sites, the socket was measured and the appropriate size of XSTAT sponges inserted into the extraction site for five minutes. The contralateral side received control gauze treatment for five minutes. Post-treatment simulated blood loss, pre-/post-treatment weight difference, treatment site hemorrhage control, simulated blood treatment displacement (in centimeters) between the XSTAT vs GAUZE, and post treatment simulated tissue damage were all recorded. All data management and analyses were completed using SPSS or SAS software.

Results: The XSTAT group had an average weight gain of 4849.4±1476.5 mg and the gauze had an average weight gain of 4311.1±532.8 mg. No statistically significant difference was found in weight gain between the two treatments ($p=0.1602$). The XSTAT group had a median difference in weight of 291.7mg (160.4-884.3) and the gauze group had a median difference in weight of 2130.7mg (2005.05-2432.6). This difference was found to be statistically significant ($p<.0001$). In the XSTAT group, 17 out of 18 (94.4%) of treated teeth controlled the hemorrhaging socket. In the gauze group, 11 out of 18 (61.1%) of treated teeth controlled the hemorrhaging socket. The Fisher's Exact Test found this difference to be statistically significant ($p=.0408$). The Gauze group traveled an average length of 2.79±1.26 cm and the XSTAT group

traveled an average length of 15.66±10.56 cm. This difference was found to be significantly different between the 2 treatments (p=0.0001).

Conclusion: In this *in vitro* model, XSTAT sponges cut to size was shown to be a predictable alternative for controlling post extraction bleeding.

Key Words

Extraction, post extraction bleeding, hemorrhage, hemostasis, XSTAT

Introduction

Tooth removal/extraction is one of the most common invasive oral surgical procedures carried out in routine dental practice[2] and post-extraction bleeding (PEB) is a recognized, frequently encountered complication in dental practice[4]. Usually, immediately following extraction of a tooth, bleeding or oozing occurs. Such bleeding is controlled in most cases and almost completely stops within 8 hours of extraction; however, bleeding can continue resulting in adverse patient outcomes. It has been reported that post-operative prolonged bleeding from the mandibular molars is more common (80%) than bleeding from the maxillary molars (20%), likely due to the highly vascular floor of the mouth[6]. Consequences of prolonged bleeding include: (1) patient return to the dental practitioner, or the emergency department; (2) development of a large hematoma or ecchymosis within the oral soft tissues; and (3) in severe cases require blood transfusion and/or hospitalization[5].

Tooth extractions are also one of the most common procedures carried out in military dentistry with mild bleeding being a recognized common occurrence. Though less than 2% of deployment dental emergencies are due to post-operative surgical complications[1], a study has found that 71% of patients (with no coagulation deficiencies) experienced PEB[1]. Also, 12% of deployed personnel will experience some form of oral facial injury while deployed[11].

Simple tooth extraction or surgical removal of broken/impacted teeth creates a penetrating-type injury into the jaw that is not well treated by standard gauze packing. There are several other methods available to achieve hemostasis; however, many are not easy to work with, do not stay in place, or require skill sets and instruments that medics or corpsmen are not equipped with in field settings. As such, a product that improves in areas where current dental hemostatic methods fall short would be highly beneficial to troops, both in-garrison and in deployed environments. A similar issue exists in battlefield penetrating injuries in the truncal and junctional (e.g., axillary or groin) regions. With support from the US Army Medical Research and Materiel Command and US Special Operations Command (Contract #W911NF-10-1-0038), XSTAT was developed to address this issue by stopping high-flow arterial bleeding within seconds without external compression, and has been successfully used in both military and civilian settings. XSTAT is a Food and Drug Administration (FDA) approved device that incorporates the innovative use of small compressed medical sponges coated in chitosan. It treats non-compressible injuries through placing a collection of sponges within the injury. The sponges rapidly absorb blood and within approximately 20 seconds expand 10 to 15 times their pre-compressed dimension. The ability to expand quickly allows the XSTAT sponges to rapidly fill the wound cavity and provide a nearly immediate hemostatic effect without application of any external compression. It is believed that this same design can be scaled down (i.e., single sponge of smaller diameter) to treat a hemorrhaging socket or other oral injury.

The goal of this study is to evaluate repurposing XSTAT, a combat casualty care treatment for penetrating wounds to treat tooth extraction sites. The novelty and value of this research project is utilizing an already FDA-approved medical product by repurposing it into a form that could better treat dental hemorrhage (Figure 1). This is in line with the Uniformed

Services University Postgraduate Dental College Operation Gap Analysis to improve dental materials, devices, and techniques (Gap IV, Sphere A,C) and Simulation (Gap IV, Sphere C). We believe that tooth extractions and oral hemorrhage can be controlled better and more sanitarily with XSTAT sponge, versus the current standard of care. The long-term goal of this research is improving oral hemorrhage control and improving training for tooth extraction and PEB by repurposing XSTAT, a combat casualty care treatment for penetrating wounds, to treat tooth extraction sites.

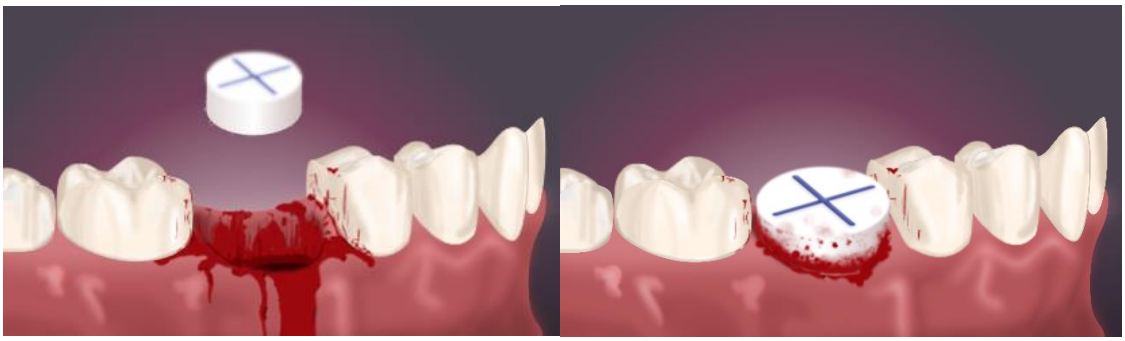

Figure 1. Graphic representation of proposed XSTAT repurposing to treat dental hemorrhage.

To our knowledge there is no laboratory model for PEB, thus producing one will provide realistic training in order to prepare medical staff to take appropriate action with minimal delay in the line of duty. Also, there are currently no randomized controlled trials that evaluated the effects of different interventions for PEB. The current investigation offers a pre-clinical evaluation of a materiel solution to this problem. We expect to produce a product that is easily and cleanly applied, quick to remove after dental procedures, and readily deployed worldwide in sterile packages containing various sizes for predetermined treatments.

Materials & Methods

An *in vitro* dental extraction model simulating PEB was developed using the Kilgore International Oral Surgery Model (Figure 2.). This specific typodont model was selected due to the fact that it allows multiple procedures to be performed including anesthesia (basic infiltration), apicoectomy, torus removal, basic, complicated, and impacted tooth extraction. The gingiva is also designed to allow incisions and sutures. All of these aspects combined are able to provide an exceptional pre-clinical learning experience. In order to simulate PEB, arterial blood flow was generated using a KD Scientific infusion withdrawal pump and PE tubing (1/8”/Fischer Scientific) along with simulated blood (VATA, Canby, OR). The infusion withdrawal pump has the ability to deliver fluids in very small volumes, and at precisely programmed rates or automated intervals. Polyethylene tubing allows us to simulate the physiological diameters and strains mimicking the inferior alveolar artery such that physiologic conditions can be approximated.

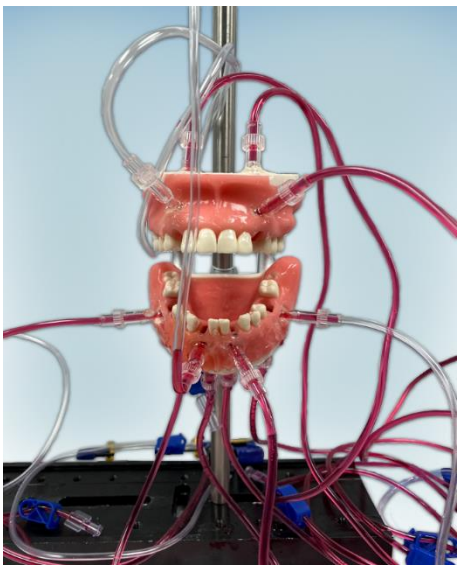

Figure 2. Post extraction bleeding (PEB) model composed of KD Scientific infusion withdrawal pump, Kilgore International oral surgery model, and VATA simulated blood.

Utilization of simulated blood allows modeling of the constant bleeding seen in PEB and represent the possible worst-case scenario for hemorrhage control. VATA Simulated Blood allows production of large quantities for simulation at relatively low cost when compared to human blood. The value of simulated blood has been proved in the past with its use in military and first responder training[15]. Various training models have previously been developed using simulated blood to replicate hemorrhagic trauma of multiple areas such as the pelvis, abdomen, and extremities. These models provide a realistic simulation of arterial bleeding in order to provide a real-life training scenario. Simulated blood has also proved invaluable for use during *in vitro* studies where it has been utilized to model various things such as atrial flow dynamics during atrial fibrillation[16].

Prior to data collection, assembly of the PEB model took place at the Tri Service Research Lab (TSRL). Under the technical expertise of those participating in the study, the optimization of the polyethylene tube placement, fluid measurement, and *in vitro* model action was completed and finalized. Technical refinement of the PEB model yielded a functional product capable of comparing our test and control groups. Teeth #'s 6, 11, 19, 23, 26, and 30 were extracted from 6 models using dental instruments and standard surgical technique. Access points were created at the apical portion of the extraction socket as well as the lateral wall of the socket. PE tubing was then secured in place in these areas. The models were then mounted on a metal laboratory support stand to be used for testing. Tubing attached to the apical access point was then connected to the infusion withdrawal pump and served as the delivery route for the simulated blood. The tubing connected to the lateral access was then secured above the model for testing.

The XSTAT test product was also refined during this stage of the experiment. After initial testing, it was determined that a square sponge with an approximate 3:1 compression ratio was best suited for the dental extraction socket. After discussion with RevMedX, a prototype product was produced and provided for this study. Three different sizes of XSTAT were produced to test molar, canine and incisor extraction sockets (Table 1). The appropriate flow rate for the model was also calculated at this time. Studies have shown the average max velocity flow of the inferior alveolar artery in adults aged 20-39 to be 5.8 cm/s.[12-13]. After careful calculation, it was determined that the appropriate flow rate of the withdrawal pump to simulate these conditions was 1.3ml/min and was delivered over the course of 5 min.

Typodont model groups				
n		Socket Dimensions	XSTAT Dimension	XSTAT Expansion
6	Maxillary Canine #6, 11	8mm x6mm	7x7x7mm	7x7x21mm
6	Mandibular Incisor #23, 26	7mm x4mm	5x5x5mm	5x5x21mm
6	Mandibular 1st Molar #19, 30	8mm x10mm	8.5x10x10mm	10x10x30mm

Table 1: XSTAT Groups

After assembly of the model, data collection was then performed. Two models were placed side by side and the specific extraction site to be tested was chosen and the corresponding XSTAT product was selected and weighed. Standard surgical 2x2 gauze was then selected and weighed. The XSTAT was then placed into the extraction site and gauze placed in the same site on the adjacent model. The model was then secured with IRWIN quick grip clamps to ensure even occlusal force distribution throughout the model. Weigh boats were then placed at the appropriate location underneath the model to collect excess liquid leaving the extraction socket. Simulated blood flow was initiated via the withdrawal pump at 1.3ml/min and monitored over the course of

5 minutes. After the 5 min period the simulated blood flow was stopped and multiple parameters were recorded.

The ability of the treatment to stop simulated PEB via squelching simulated blood flow was measured via two distinct outcomes. First, "hemorrhage control" demonstrated that treatment occluded the cavity and produced enough occlusal pressure to divert simulated blood up the escape tube against gravity (Y/N). Second, the distance traveled in the escape tubing was also measured from the entry into the model to the top of the tubing (cm). Post Treatment Blood Loss was defined as weight(mg) of any blood collected in pre-weighed weigh boats during the observation period (5 minutes). Pretreatment and Post-treatment weight difference was defined as the weight difference of the XSTAT and Gauze before and after treatment(mg). Synthetic tissue damage was also recorded after removal of treatment (i.e., XSTAT vs gauze) by determining if any of the synthetic tissues had been visually damaged. More specifically had any vertical or horizontal tear of the tissue occurred by means other than tooth extraction? Was the synthetic tissue stretched, deformed, compressed, or expanded to the point of being unable to return to its original length and shape?

Sample size was determined by power calculations to obtain 80% statistical power by referring to a published study in which tooth extraction were performed [7,9,10.] Comparisons between XSTAT and GAUZE treatment groups were made. Depending on normality, either a two-sample t-test or a Mann Whitney U test was used to evaluate any differences in the variables Weight Gain, and the Pre/Post treatment difference in weight between XSTAT and GAUZE. Means and standard deviations were used to describe normally distributed variables, and medians with upper and lower quartiles were used for non-normally distributed data. A fisher's Exact test was used to determine if the proportion of teeth that able to control the hemorrhage

differed between XSTAT and GAUZE. Statistical significance was set at alpha=.05 and analysis was done using SAS software version 9.4

Results

Weight gain from XSTAT vs GAUZE were compared using a t-test. The XSTAT group had an average weight gain of 4849.4±1476.5 mg and the gauze had an average weight gain of 4311.1±532.8 mg. No statistically significant difference was found in weight gain between the two treatments (p=0.1602).

Pre treatment and Post treatment differences in weight were compared using a Mann Whitney U test. The XSTAT group had a median difference in weight of 291.7mg (160.4-884.3) and the gauze group had a median difference in weight of 2130.7mg (2005.05-2432.6). This difference was found to be statistically significant (p<.0001).

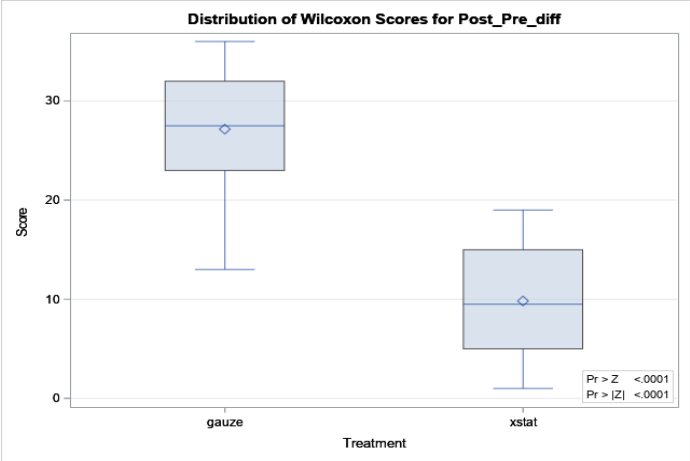

Figure 3. – Distribution of Wilcoxon Scores for difference between pre and post weight gain.

In the XSTAT group, 17 out of 18 (94.4%) of treated teeth were able to seal the socket and adequately control the active hemorrhage by forcing the blood to travel in a different direction against gravity instead of out of the extraction site itself. (Figure 4.) In the gauze group, 11 out of 18 (61.1%) of treated teeth were able to adequately control the active

hemorrhage. The Fisher's Exact Test found this difference to be statistically significant (p=.0408).

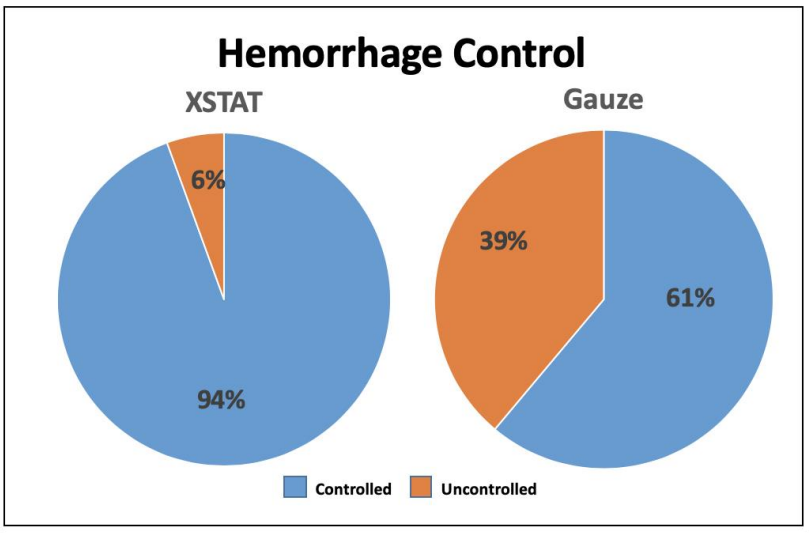

Figure 4. – Distribution of XSTAT and Gauze that were able to control the hemorrhaging socket

Length in CM that the liquid traveled for XSTAT vs GAUZE were compared using a t-test. The Gauze group traveled an average length of 2.79±1.26 cm and the XSTAT group traveled an average length of 15.66±10.56 cm. This difference was found to be significantly different between the 2 treatments (p=0.0001).

Discussion

The main objectives of this randomized controlled trial were to evaluate repurposing XSTAT to treat tooth extraction sites as well as develop a PEB model. To the best of our knowledge, this is the first in vitro PEB model that has been created for the clinical training of dental professionals. The model was shown to be advantageous for the future training and development of students learning how to perform dental extractions as well as many other dental

surgical procedures. Each individual model displayed a certain degree of subtle difference after construction that accounts for the heterogeneity of each individual oral cavity. We believe it could continue to be utilized to enhance the educational experience of 1st and 2nd year dental students prior to actual clinical experience. In many dental schools around the world, different training methods such as using virtual reality is gaining recognition as a valuable tool for pre-clinical training of dental students, but does not afford the student technical hands on experience.[15] Performing surgical procedures on this model will allow students to develop and fine tune the technical skills needed prior to performing these procedures on an actual human patient.

There are very few dental procedures that could potentially result in a life-threatening situation. However, dental extractions are one of those procedures that could become serious very quickly and even result in a preventable death.[16] The results of this in-vitro experiment with the newly created PEB model demonstrated that XSTAT could potentially be used as a viable alternative to gauze for the treatment of PEB. We did not prove that the XSTAT treatment is superior at controlling PEB than the gold standard gauze treatment, but we did show that the XSTAT is very effective in treating PEB in this model and potentially stopping a life-threatening situation. The most useful metric that was recorded was whether or not the treatment could create enough physical pressure inside the extraction socket to control the hemorrhage and divert the simulated blood up the escape tubing against gravity. This was shown time and time again and proved that the XSTAT is capable by itself of creating a significant amount of occlusal pressure inside the extraction socket. We believe, that in a normal clinical environment this would significantly reduce post extraction bleeding time. Currently, the large majority of products available for post extraction bleeding (i.e., collaplug, gel foam, surgicel) merely act as a

scaffolding for the accumulation of clotting factors and stabilization of the clot. None of these products are able to combine the physiologic effects of chitosan and the direct application of pressure to the wound. XSTAT is able to put direct pressure on the wound which is essential for clotting and result in achieving hemostasis more quickly with less blood loss.

During development and technical refinement there were various discoveries that led to what was eventually the final XSTAT product. It was discovered that placing the XSTAT to expand in the direction of the greatest socket diameter was key to the success of the treatment. If placed in the opposite direction the sponge would not have enough room to expand and either work its way out of the socket or not seal the socket completely. As a result of this discovery, a directional marker was then placed on the side of sponge to indicate which direction the sponge would expand when coming into contact with blood. This allows the provider to place XSTAT with ease in the correct orientation. The 3:1 compression ratio was also shown to be appropriate for the extraction sockets. A larger compression ratio would cause the XSTAT to become extruded from the socket and any smaller would once again fail to seal the socket completely.

This initial testing did contain limitations for when comparing both XSTAT and gauze in our modelled PEB. First of all, the lack of clotting factors in the VATA simulated blood did not allow for complete resolution of the PEB. Therefore, bleeding time was not able to be measured. XSTAT and gauze treatments have been compared in other studies[17]; however, *in-vivo* product testing will be necessary for the next technology readiness level. It should also be noted a current negative characteristic of the XSTAT product compared to other hemostatic agents is that it is non-resorbable. This will require removal by either the provider or the patient at some point after placement in the extraction socket. This could be problematic in certain situations if the patient is unable or forgets to remove the XSTAT after placement.

Overall, in this *in vitro* model, XSTAT sponges cut to size was shown to be a predictable alternative for controlling post extraction bleeding. The product developed in this experiment will be beneficial in the continuing research of XSTAT. The next step in this project will be *in-vivo* animal testing where the dimensions used in our project can be extrapolated for use. Eventually, human testing will be required for final product production.

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