Ex Vivo Human Chondrocyte Toxicity After Exposure to Tranexamic Acid

Erika Garbrecht, MD[1]; Benjamin Packard, MD, MSc[1]; Nafisa Elghazali, BS[3]; Phuong A.H. Nguyen, PhD[2]; Christina Salas, PhD, MSc[1,2,3]; Deirdre Hill, PhD[4]; Heather E. Canavan, PhD[2,5]; Michael Decker, MD, MSc[1]

[1]Department of Orthopaedics & Rehabilitation

[2]Center for Biomedical Engineering

[3]Post-Baccalaureate Research and Education Program

[4]Department of Internal Medicine

[5]Department of Chemical and Biological Engineering

Corresponding Author Christina Salas, PhD[1,2,3]. Department of Orthopaedics and Rehabilitation MSC10 5600, 1 University of New Mexico, Albuquerque, NM 8713-0001 (email: chrsalas@salud.unm.edu)

Conflict of Interest The authors report no conflicts of interest.

Funding Funding for this study was provided by the Orthopaedic Research and Education Foundation/Western Orthopaedic Association through the Resident Research Project Grant. Funding was used for purchasing of one time use supplies used in the study.

Acknowledgements We acknowledge NIH award number UL1TR001449 to The University of New Mexico, Clinical & Translational Science Center for biostatistician support. We thank the NIH-funded Post-Baccalaureate Research and Education Program (5R25GM075149) for supporting the training of a co-author. We also thank the Bill & Melinda Gates Foundation for providing a Gates Millennium Scholarship to support the training of an additional co-author.

Ethical approval Institutional review board (IRB) approval was obtained prior to this study.

ABSTRACT

Background: Tranexamic acid (TXA) reduces blood loss by inhibiting plasminogen and preventing subsequent fibrinolysis (stabilizing fibrin clots). Topical TXA has been suggested for use in unicompartmental joint arthroplasty and other procedures with residual viable cartilage. However, the effects on human cartilage have not been thoroughly investigated. The purpose of this study is to determine the viability of human chondrocytes in the setting of various concentrations of topical TXA.

Methods: 24 human osteochondral (OC) plugs were harvested from the knee joint of a human decedent within 36 hours of death. OC plugs underwent two rinses with Dulbecco's Phosphate-Buffered Saline (DPBS) and were then exposed to three concentrations of topical TXA (20 mg/mL, 30 mg/mL, 40 mg/mL) and incubated at 37°C for 48 hours. Control OC plugs were exposed to normal saline (NS). Chondrocyte viability was evaluated with a live/dead viability/cytotoxicity assay at days 0, 2, 4, and 6 after incubation.

Results: For days 0 and 2, viability after TXA exposure was 70.0% ± 15.0% for all concentrations. By day 4, viability dropped to averages below 30.0% for all concentrations. Gross structural breakdown of extracellular matrix surrounding the chondrocytes was observed by day 6.

Conclusion: Commonly used concentrations of topical TXA were shown to be chondrotoxic, and topical application in the presence of native cartilage could result in devastating effects on chondrocyte viability leading to patient morbidity. Use of TXA on native cartilage should be used with caution.

Keywords: Chondrocytes, Tranexamic acid, Cartilage, Death

INTRODUCTION

Tranexamic acid (TXA) is a synthetic agent used to reduce blood loss by inhibiting the breakdown of fibrin clots. TXA reversibly binds plasminogen, inhibiting fibrinolysis and stabilizing fibrin clots, and is used in many surgical specialties and for traumatic hemorrhage.[1] Using TXA in orthopaedic procedures has shown efficacy in various subspecialties, including spine and trauma procedures.[2,3] It is well established that TXA has reduced operative blood loss and post-operative transfusion requirements in both primary total knee and hip arthroplasty and in revision hip and knee arthroplasty.[4-6] Additionally, both topical and intravenous (IV) TXA have been shown to improve post-operative range of motion (ROM) following primary total knee arthroplasty.[7]

No increase in the rate of venous thromboembolism (VTE) with the administration of TXA has been identified in patients with average risk for VTE.[8] However, patients with a history of stroke and cardiac stents are generally considered high risk for VTE, and systemic/intravenous (IV) TXA is typically avoided. In this circumstance, topical or intra-articular TXA is a safe and equally effective alternative.[9]

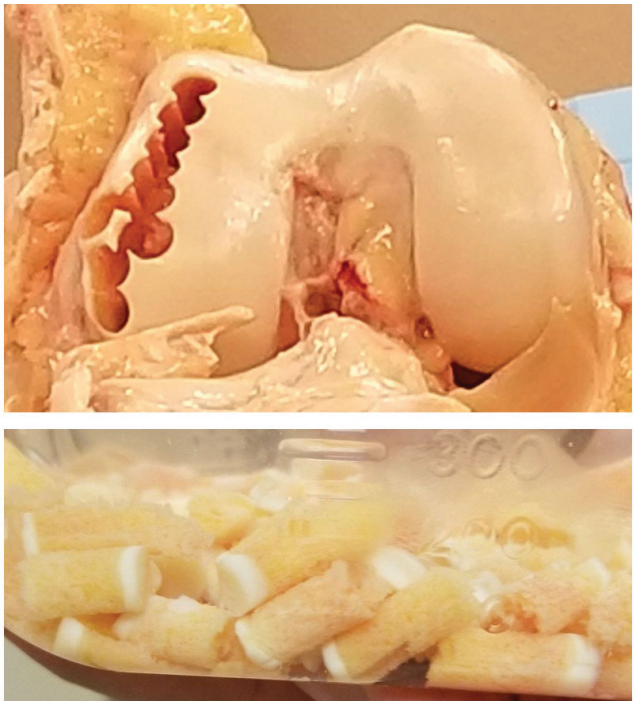

Figure 1. *Osteochondral plugs harvested from the femur and tibia of a 56-year-old, male decedent showing no signs of degenerative arthritis on the articular surface (top) and placement in Fetal Bovine Serum in preparation for washing (bottom).*

Though clear benefits of TXA have been demonstrated in arthroplasty procedures, there remains no consensus on dose or route of administration. Moreover, questions still remain regarding TXA in other surgical procedures involving the hip and knee joints, including hemiarthroplasty, unicompartmental knee arthroplasty, and even the use of topical TXA in anterior cruciate ligament (ACL) reconstruction to reduce post-operative hemarthrosis, promoting earlier and overall increased ROM.[10] In these circumstances, native cartilage would be exposed to TXA.

The impact of TXA on human chondrocytes remains unclear.[11–13] In this study, the authors investigate the effect of a range of commonly used concentrations of topical or intra-articular TXA on human chondrocytes *ex vivo*. It is hypothesized that TXA is chondrotoxic and demonstrates both a concentration and time-dependent effect on chondrocyte viability.

METHODS

Osteochondral Plug Harvest and Storage

Institutional review board (IRB) approval was obtained. Human osteochondral (OC) plugs were harvested from a decedent obtained through a local donor services company. The decedent was a 56-year-old male with no documented surgical history, no history of osteoarthritis, rheumatoid arthritis, or other inflammatory arthropathy that would affect cartilage. OC plugs were harvested

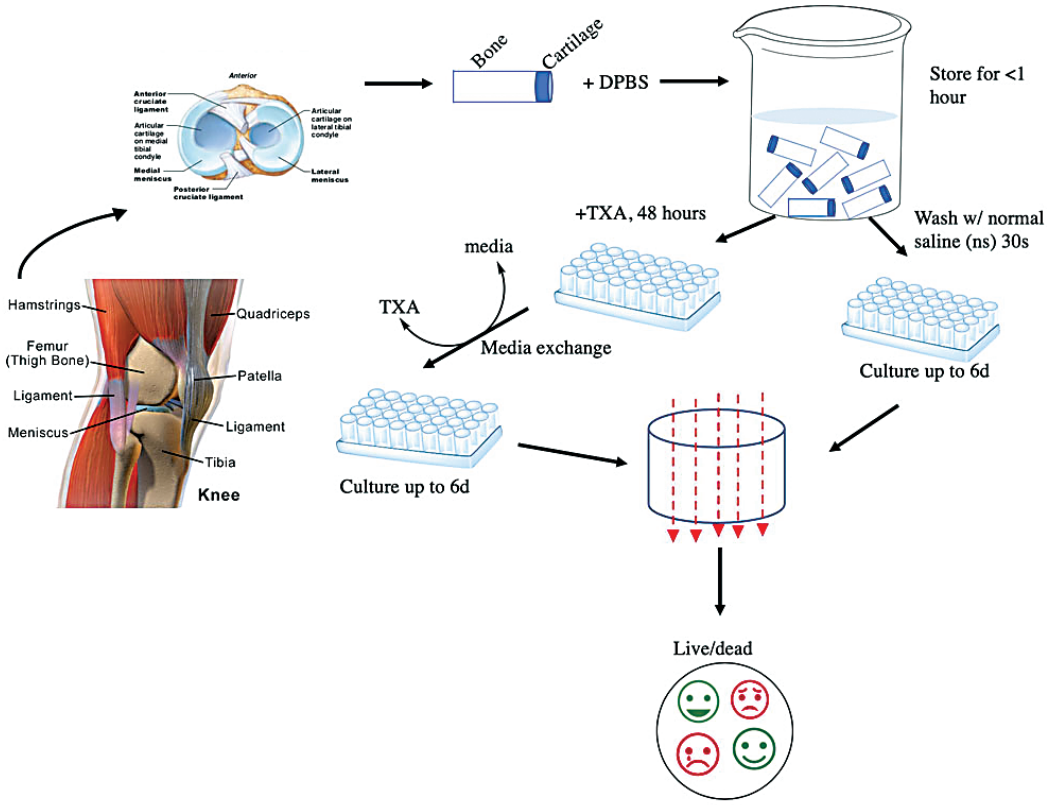

Figure 2. *Visual flow-chart of TXA/NS treatment of osteochondral plugs summarizing the methodology used for this study. Plugs are obtained using OATS, transferred to FBS, washed, placed in cell media for 2 hours, and exposed to normal saline for 1-minute lavage or a 48-hour soak in tranexamic acid dissolved in cell media.*

within 36 hours of death. Harvest and preparation are modeled after Campbell et al.[7] Plugs were obtained from the articular surface of the distal femur and proximal tibia using the osteoarticular allograft transfer system (OATS) (Arthrex, Naples, FL). Harvested knee articular cartilage was visually confirmed to be intact and healthy appearing, with any regions devoid of intact articular cartilage or signs of damage discarded (Figure 1). 48 OC plugs were collected and transferred into fetal bovine serum (FBS) solution followed by a wash and rescue period (Figure 2).

OC plugs were washed with DPBS before being stored in standard nutrient mixture made from Dulbecco's Modified Eagle Medium/F-12 (DMEM/F12) 1:1 media with 10.0% FBS, 1.0% penicillin/streptomycin (P/S), and 1.0% Fungizone at 37° C for 2 hours prior to treatment. All wash and storage materials were purchased from VWR Life Sciences (Sanburn, NY).

Tranexamic Acid Exposure and Control

OC plugs were exposed to either 0.9% normal saline (NS) or to TXA (Pharmaceutical grade obtained from Sigma Aldrich [St. Louis, Missouri]) at 20 mg/mL, 30 mg/mL, and 40 mg/mL (500 mg, 750 mg, and 1000 mg diluted in 25 mL cell media). This was performed by either a 1-minute lavage treatment with NS or a 48-hour soak treatment of TXA. Both groups of OC plugs were kept in cell media for the remainder of treatment. Three OC plugs were used for each group, treatment, and day (NS x 3 plugs x 4 days = 12; TXA x 3 concentrations x 3 plugs x 4 days = 36).

Live/Dead Cell Viability/Toxicity Assay

The protocol for the live/dead assay follows those of Cooperstein et al.[14] Briefly, a combined live/dead solution was made by adding 1µL of calcein AM (1mM stock solution), and 1 µL of ethidium homodimer-1 (2 mM) to 1 mL of DPBS. OC plugs were maintained in cell media, changed every 24 hours, and removed for viability tests at specified times after exposure: days 0, 2, 4, and 6. Plugs were rinsed and all bone removed. The remaining chondral region for each plug was sectioned into approximately nine segments. Cell media was then replaced with a working live/dead solution. The chondral segments were incubated at 37.0°C and 5.0% CO_2 with live/dead solution for 60 minutes and then rinsed with sterile DPBS prior to imaging.

Fluorescent images were obtained with a Nikon Eclipse TS200F inverted microscope at 10X magnification with an epifluorescence attachment (Nikon Instruments, Melville, NY) and a SPOT Insight color mosaic digital camera (Diagnostic Instruments, Sterling Heights, MI). After imaging (~27 images across nine pieces from each plug), cells were analyzed and counted using FIJI (Image J Software), following a previously established protocol.[15]

Statistical Analysis

TXA dose, evaluation time, and the interaction of dose and time were evaluated in relation to the proportion of live chondrocytes (log-transformed to meet normality assumptions) using analysis of variance (ANOVA). Cytotoxicity was defined as the presence of less than 70.0% living chondrocytes.[16] Saline treatment evaluated at the equivalent times served as the normal control. An unstructured variance (allowing heterogeneous variance) provided the best fit to the data. Multiple comparisons were considered using the Tukey-Kramer method. All analyses were conducted using SAS v. 9.4 (Cary, N.C.).

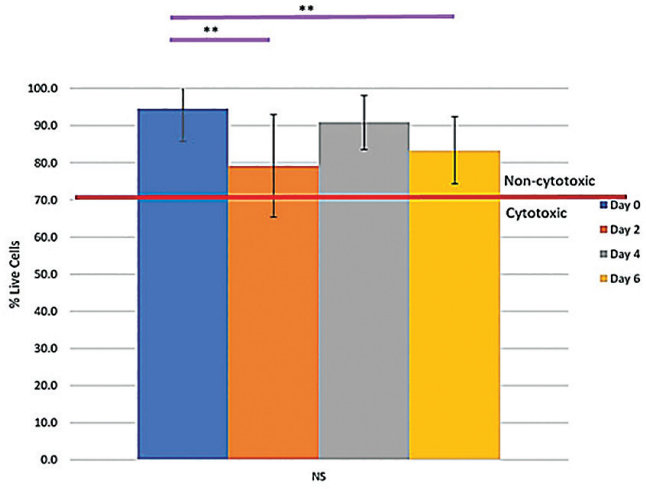

Figure 3. *Percent live cells of 0 days (blue), 2 days (orange), 4 days (grey), and 6 days (yellow) after treatment of NS. The red line is set to 70% viability, below which treatments are considered cytotoxic. ** indicates p-values are <0.05 using Tukey-Kramer test.*

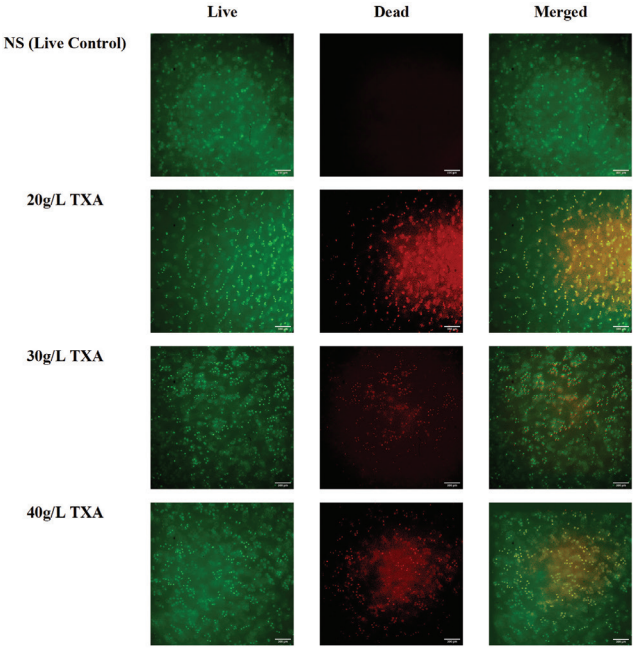

Figure 4. *Live (left), dead (middle), and merged (right) images of live/dead stain of osteochondral plugs at day 0 after treatment of NS, 20 mg/mL TXA, 30 mg/mL TXA, and 40 mg/mL TXA. Scale bar = 300 µm.*

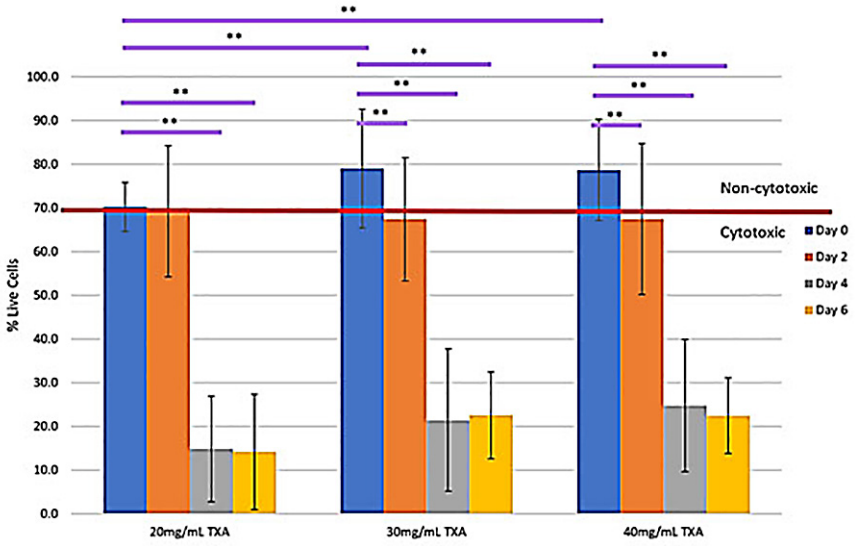

Figure 5. *Percent live cells of 0 days (blue), 2 days (orange), 4 days (grey), and 6 days (yellow) after treatment of 20 mg/mL TXA, 30 mg/mL TXA, and 40 mg/mL TXA. The red line is set to 70% viability, below which treatments are considered cytotoxic. ** indicates p-values are <0.05 using Tukey Kramer test.*

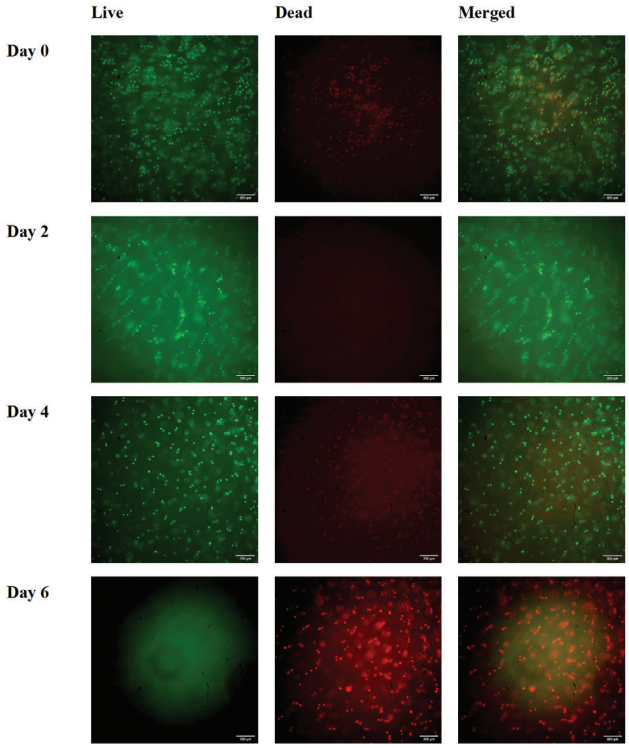

Figure 6. *Live (left), dead (middle), and merged (right) images of live/dead stain of osteochondral plugs at 0 days, 2 days, 4 days, and 6 days after treatment of 30 mg/mL TXA. The indiscriminate green staining is likely a result of calcein AM's interaction with cytosolic esterases that can leak out of cells as they die. Scale bar = 300 µm.*

Results

Chondrocytes exposed to NS used as a control remained viable through day 6 (Figure 3). Immediately, at day 0, fluorescent microscopy showed more viable chondrocytes exposed to NS compared to TXA at 20 mg/mL, 30 mg/L, and 40 mg/ml concentrations (Figure 4). Chondrocytes exposed to all concentrations of TXA demonstrated decreasing viability, ranging between 70.2% to 78.6% on day 0 to 14.2% to 22.5% on day 6 (Figure 5). TXA resulted in near cytotoxic levels of viability at day 0 for all TXA concentrations. At day 2, all levels became cytotoxic. Beginning at day 4, viability decreased drastically, continuing at 6 days after exposure. Figure 6 shows a representative sample from 30 mg/ml concentrations of TXA. Table 1 details percent viability of chondrocytes after exposure to TXA at days 0, 2, 4, and 6 at all concentrations.

Type I and Type III ANOVA analyses indicate that time, dose, and the interaction of treatment and time were significant, suggesting chondrocyte viability is dependent on increasing dose and time (Table 2). The GLM procedure of least squares mean showed that exposure of chondrocytes to 20 mg/mL, 30 mg/mL, and 40 mg/mL of TXA significantly differed from normal saline at all times. Further analysis of TXA concentration effects within individual days showed that chondrocyte viability versus concentration was not significantly different.

Qualitative results during dissection demonstrated degradation of OC plugs over time with exposure to TXA. By day 4 and day 6 after treatment, OC plugs were increasingly difficult to dissect following the same protocols established by previous experiments. Furthermore, initial exposure to TXA rapidly dropped

Table 1. *% viability of chondrocytes (mean & standard deviation [SD]) by days following exposure (0, 2, 4, and 6) and % concentration of TXA.*

Days Following Exposure to TXA	0			2			4			6		
% Concentration of TXA (mg/mL)	20	30	40	20	30	40	20	30	40	20	30	40
% Viability of Chondrocytes (mean)	70.2	79.0	78.6	69.2	67.4	67.5	14.9	21.4	24.8	14.2	22.5	22.4
% Viability of Chondrocytes (SD)	5.6	13.6	11.6	15.0	14.1	17.3	12.1	16.3	15.2	13.2	9.9	8.6

Table 2. *ANOVA type I (top) and III (bottom) results comparing TXA effects of time and dose on chondrocyte viability. All p-values are <0.0001 indicative that exposure to TXA observed over time, increasing concentrations of TXA, and the effects of time and concentration together were significant. Degrees of freedom (DF). Sum of squares (SS)*

Type I ANOVA

Source	*DF*	*SS*	*Mean Square*	*F Value*	*P>F*
TXA effects of time	3	49.72	16.57	61.61	<0.001
TXA effects of dose	3	37.08	12.36	45.95	<0.001
TXA effects of time * dose	9	39.23	4.36	16.20	<0.001

Type III ANOVA

Source	*DF*	*SS*	*Mean Square*	*F Value*	*P>F*
TXA effects of time	3	71.20	23.73	88.21	<0.001
TXA effects of dose	3	37.08	12.36	45.95	<0.001
TXA effects of time * dose	9	39.23	4.36	16.20	<0.001

the cell media's pH, as indicated by a color change in the cell media from red to yellow (due to the presence of phenol red in the cell media). Although OC plugs were rinsed between every cell media change, plugs exposed to TXA required a cell media change every 24 hours due to decreasing pH. These observations were not found in samples exposed to NS treatment.

DISCUSSION

Ex vivo human chondrocytes exposed to common concentrations of topical TXA showed considerable cell death in comparison to controls. Chondrocytes were viable *ex vivo* following NS wash treatment at 6 days after exposure. There was a natural decline in the NS group, demonstrating cell death that naturally occurs to *ex vivo* chondrocytes that remain in cell media. However, a large percentage of the NS OC plugs remained viable. The results of this study contrast with other studies that have also investigated chondrocyte viability after exposure to TXA; however, this is because there were different post-exposure methodologies and TXA concentrations investigated in this study that were not evaluated by others.[12,13] More specifically, this study increased post-exposure evaluation time to account for delayed cell death, increased time of exposure to TXA to simulate the potential effects that plasminogen binding may have on TXA in a surgical setting, and evaluated TXA concentrations above 25 mg/mL, a typical concentration used by surgeons intraoperatively.

Tuttle et al[13] evaluated bovine and murine chondrocyte viability immediately after exposure (day 0) to 25 mg/mL of TXA for 48 hours and found that viability was similar to that of controls. We suggested that there could be a delayed cell death after TXA exposure exceeding 48 hours; therefore, this study extended the observation of cells to 6 days. Other studies have shown the half-life of TXA to be approximately 2.3 hours[17]; however, these findings were the free form of TXA and not bound to plasminogen, which alters the kinematics and half-life of TXA. Ahlberg et al[18] evaluated the diffusion of TXA from the knee joint, showing the pharmacokinetics of TXA in joint fluid

was similar to that of serum. TXA was administered and concentration was measured prior to any surgical insult of the knee joint, inferring the TXA was acting in its solute or unbound form. Proteins in their unbound form demonstrate different pharmacokinetics than their bound counterparts; protein and protein-bound solutes diffuse from a joint at a much slower rate.[19,20] Wallis et al[21] demonstrated that the effusion times of radiolabeled protein (albumin) had an average elimination half-life of ~32 hours. The authors believe that once TXA binds to plasminogen in the joint, it behaves differently from its unbound state. After surgical insult, plasminogen is exposed, readily binding TXA, putting TXA in its bound form and delaying its diffusion from the joint**.** Sangasoongsong et al[22] confirmed this in their study evaluating TXA concentrations in the joint after TKA, measured through a drain intraoperatively placed. Diluted concentrations of TXA were placed in the joint along with a drain, which was clamped. The drains were unclamped at varying time points post-operatively and concentrations of TXA were measured. The concentrations were variable throughout patients, but no concentration change was observed up to 12 hours. In fact, although not statistically significant, TXA levels at 12 hours compared to 2 hours nearly doubled. Given these findings, we believe that protein-bound TXA, which would be expected in the setting of postoperative hemarthrosis, would have a half-life in the joint for at least 12 hours. Therefore, the authors of this study exposed human chondrocytes to TXA for 48 hours to demonstrate a more likely clinical scenario of TXA exposure duration to chondrocytes.

Extended observation demonstrated delayed-cell death when exposed to TXA, demonstrating a likely occurrence if native cartilage were exposed. There was a significant decline in human chondrocyte viability at 4 days and 6 days, suggesting delayed cell death after exposure to TXA. Parker et al[12] investigated the viability of human chondrocytes *in vitro* after exposure to concentrations of 10 mg/mL to 20 mg/mL of TXA for only 3 hours on a 3D human chondrocyte laden gelatin-methacryloyl hydrogel model. They found no significant differences in cell viability compared to controls.[12] The basis for a 3-hour exposure was a study by Ahlberg et al.[18]

This study is comparable to a recent study by Goderecci et al[11], which evaluated *in vitro* human chondrocytes exposed to 20 mg/mL, 50 mg/mL, 70 mg/mL, and 100 mg/mL of TXA for 10 minutes, 24 hours, or 48 hours. Like the study by Parker et al[12], Goderecci et al[11] found that short-term exposure to TXA (10 minutes) was not different in viability from controls, and no appreciable cytotoxicity was seen under 100 mg/mL. Alternatively, with 24- or 48-hour exposure, appreciable cytotoxicity was observed as soon as 1-day post-exposure at concentrations over 20 mg/mL. This study, which uses an *ex vivo* model and concentrations of 30 mg/mL and 40 mg/mL, supports the finding that long exposure times and high concentrations have an adverse effect on viable chondrocytes. Additionally, secondary to decreasing pH, OC plugs exposed to TXA required a cell media change every 24 hours, which was not required in OC plugs exposed to NS. This acidity likely contributed to the decline in cell viability found in OC plugs exposed to TXA. This suggests that native chondrocytes, as seen in hemi-arthroplasty, ligament reconstruction, or arthroscopy would be affected. We were unable to find any similar findings in the literature regarding a lowering of pH with persistent exposure to TXA; we feel that this should be a future area of study.

Topical TXA concentrations also varied widely in studies assessing the impact of TXA. There is no definitive dose of TXA in arthroplasty, and protocols vary greatly for intra-articular or topical administration of TXA, from 250 mg to 3 grams, while actual intra-articular concentrations are also variable and inconsistent, varying from 15 mg/mL to 100 mg/mL. Topical application is also variable with TXA being administered after TXA implants either locally by periarticular injection or injected into a drain after the fascial layer is closed. Picetti et al[23] found that 10 mg/mL to 15 mg/mL of TXA resulted in substantial inhibition of fibrinolysis, but efficacy regarding operative blood loss and postoperative transfusion was not evaluated. The authors feel that obtaining such a specific concentration in an operative setting within the joint would be very difficult, given the differences of joint volume in the setting of post-operative hemarthrosis among patients. Panteli et al[24] found that a dose of more than 2 grams of TXA trended toward decreased post-operative transfusion rates. This suggests that a higher concentration may be needed for clinical effectiveness, although when using topical or intra-articular TXA in the setting of post-operative hemarthrosis, it is difficult to predict final concentration and would be highly variable among patients.

From these results, it can be inferred that doses of intra-articular and topical TXA commonly used may be toxic to native chondrocytes in procedures such as hemiarthroplasty, ligament reconstruction, and arthroscopic procedures. Because it is paramount to preserve and protect cartilage during these procedures, the authors caution against using TXA in the setting of native cartilage. Further investigation of the pharmacokinetics of bound TXA-plasminogen within the joint and the effects of TXA-plasminogen exposure to chondrocytes at various concentrations would be beneficial.

This article is not without some limitations. TXA concentrations were tested on *ex-vivo* cartilage plugs. While conclusions may be drawn for cartilage *in-vivo*, this information needs to be taken with caution. Additionally, all chondrocyte plugs were harvested from one donor. Though there was only one donor, there were numerous healthy OC plugs obtained that had living cartilage for both arms of the experiment. Future studies evaluating numerous donors to obtain more OC

plugs would broaden the understanding of this topic. Nevertheless, this study provides a good framework and baseline for chondrocyte response to TXA, suggesting TXA may be toxic to native cartilage.

Commonly used concentrations of topical TXA were shown to be chondrotoxic to chondrocytes in the *ex vivo* setting, and topical application in the presence of native cartilage could result in devastating effects on chondrocyte viability, leading to patient morbidity. Further studies are still needed to evaluate the true pharmacokinetics of bound TXA within an intra-articular space and the effect of TXA on chondrocytes in an *in vivo* setting, but given the current findings, use of TXA on native cartilage should be taken with caution.

REFERENCES

1. Dunn CJ, Goa KL. Tranexamic acid: a review of its use in surgery and other indications. Drugs. 1999;57(6):1005-1032. doi: 10.2165/00003495-199957060-00017.
2. Amer KM, Rehman S, Amer K, et al. Efficacy and safety of tranexamic acid in orthopaedic fracture surgery: a meta-analysis and systematic literature review. J Orthop Trauma. 2017;31(10):520-525. doi: 10.1097/BOT.0000000000000919.
3. Cheriyan T, Maier SP 2nd, Bianco K, et al. Efficacy of tranexamic acid on surgical bleeding in spine surgery: a meta-analysis. Spine J. 2015;15(4):752-761. doi: 10.1016/j.spinee.2015.01.013.
4. Benoni G, Fredin H. Fibrinolytic inhibition with tranexamic acid reduces blood loss and blood transfusion after knee arthroplasty: a prospective, randomised, double-blind study of 86 patients. J Bone Joint Surg Br. 1996;78(3):434-440.
5. Johansson T, Pettersson LG, Lisander B. Tranexamic acid in total hip arthroplasty saves blood and money: a randomized, double-blind study in 100 patients. Acta Orthop. 2005;76(3):314-319.
6. Noordin S, Waters TS, Garbuz DS, et al. Tranexamic acid reduces allogenic transfusion in revision hip arthroplasty. Clin Orthop Relat Res. 2011;469(2):541-546. doi: 10.1007/s11999-010-1441-2.
7. Campbell J, Filardo G, Bruce B, et al. Salvage of contaminated osteochondral allografts: the effects of chlorhexidine on human articular chondrocyte viability. Am J Sports Med. 2014;42(4):973-978. doi: 10.1177/0363546513519950.
8. Poeran J, Rasul R, Suzuki S, et al. Tranexamic acid use and postoperative outcomes in patients undergoing total hip or knee arthroplasty in the United States: retrospective analysis of effectiveness and safety. BMJ. 2014;349:g4829. doi: 10.1136/bmj.g4829.
9. Wind TC, Barfield WR, Moskal JT. The effect of tranexamic acid on blood loss and transfusion rate in primary total knee arthroplasty. J Arthroplasty. 2013;28(7):1080-1083. doi: 10.1016/j.arth.2012.11.016.
10. Pallante AL, Bae WC, Chen AC, et al. Chondrocyte viability is higher after prolonged storage at 37 degrees C than at 4 degrees C for osteochondral grafts. Am J Sports Med. 2009;37 Suppl 1(Suppl 1):24S-32S. doi: 10.1177/0363546509351496.
11. Goderecci R, Giusti I, Necozione S, et al. Short exposure to tranexamic acid does not affect, in vitro, the viability of human chondrocytes. Eur J Med Res. 2019;24(1):15. doi: 10.1186/s40001-019-0373-x.
12. Parker JD, Lim KS, Kieser DC, et al. Is tranexamic acid toxic to articular cartilage when administered topically? What is the safe dose? Bone Joint J. 2018;100-B(3):404-412. doi:10.1302/0301-620X.100B3.BJJ-2017-1135.R1.
13. Tuttle JR, Feltman PR, Ritterman SA, et al. Effects of tranexamic acid cytotoxicity on in vitro chondrocytes. Am J Orthop (Belle Mead NJ). 2015;44(12):E497-502.
14. Cooperstein MA, Canavan HE. Assessment of cytotoxicity of (N-isopropyl acrylamide) and poly(N-isopropyl acrylamide)-coated surfaces. Biointerphases. 2013;8(1):19. doi: 10.1186/1559-4106-8-19.
15. BTRI Life Sciences. Quantification of live/dead staining using Fiji software. Published 2015. Accessed March 12, 2020. https://brtilifesciences.com/PDF/QuantificationofLiveDeadStainingUsingFijisoftware.pdf
16. Goode J. Use of international standard ISO 10993-1, "Biological evaluation of medical devices - part 1: evaluation and testing within a risk management process." *Dep Heal Hum Serv Food Drug Adm.* 2016;(68).
17. Pabinger I, Fries D, Schöchl H, et al. Tranexamic acid for treatment and prophylaxis of bleeding and hyperfibrinolysis. Wien Klin Wochenschr. 2017;129(9-10):303-316. doi: 10.1007/s00508-017-1194-y.
18. Ahlberg A, Eriksson O, Kjellman H. Diffusion of tranexamic acid to the joint. Acta Orthop Scand. 1976;47(5):486-488. doi: 10.3109/17453677608988725.
19. Luo FJ, Patel KP, Marquez IO, et al. Effect of increasing dialyzer mass transfer area coefficient and dialysate flow on clearance of protein-bound solutes: a pilot crossover trial. Am J Kidney Dis. 2009;53(6):1042-1049. doi: 10.1053/j.ajkd.2009.01.265.
20. Meyer TW, Walther JL, Pagtalunan ME, et al. The clearance of protein-bound solutes by hemofiltration and hemodiafiltration. Kidney Int. 2005;68(2):867-877. doi: 10.1111/j.1523-1755.2005.00469.x.
21. Wallis WJ, Simkin PA, Nelp WB, et al. Intraarticular volume and clearance in human synovial effusions. Arthritis Rheum. 1985;28(4):441-449. doi: 10.1002/art.1780280413.
22. Sa-ngasoongsong P, Chanplakorn P, Wongsak S, et al. An in vivo study of low-dose intra-articular tranexamic acid application with prolonged clamping drain method in total knee replacement: clinical efficacy and safety. Biomed Res Int. 2015;2015:164206. doi: 10.1155/2015/164206.
23. Picetti R, Shakur-Still H, Medcalf RL, et al. What concentration of tranexamic acid is needed to inhibit fibrinolysis? A systematic review of pharmacodynamics studies. Blood Coagul Fibrinolysis. 2019;30(1):1-10. doi: 10.1097/MBC.0000000000000789.
24. Panteli M, Papakostidis C, Dahabreh Z, et al. Topical tranexamic acid in total knee replacement: a systematic review and meta-analysis. Knee. 2013;20(5):300-309. doi: 10.1016/j.knee.2013.05.014.