Research Article   **OMICS International**

A Comparison of Porcine Lens Storage Methods for Optimal Phacoemulsification

Michael D Christensen[1], Sravanthi Vegunta[2], Alex Wright[1], Tyler Boulter[3], Brian Zaugg[1], Brian C Stagg[1], Jeff H Pettey[1] and Randall J Olson[1*]

[1]Department of Ophthalmology and Visual Sciences, John A. Moran Eye Center, University of Utah, Salt Lake City, Utah, USA

[2]University of Arizona College of Medicine-Phoenix, Arizona, USA

[3]Department of Ophthalmology, Texas A & M University, College Station, Texas, USA

[*]**Corresponding author:** Randall J Olson, Department of Ophthalmology and Visual Sciences, John A Moran Eye Center, University of Utah, 65 Mario Capecchi Drive, Salt Lake City, UT, 84132, USA, Tel: (801) 585 6622; E-mail: randallj.olson@hsc.utah.edu

Abstract

Objective: The study purpose was to compare effects of two porcine lens storage methods on phacoemulsification efficiency and chatter.

Methods: This *in vitro* laboratory study was conducted at the John A. Moran Eye Center Laboratories, University of Utah. Porcine nuclei were fixed in 10% formaldehyde and cut into 2.0 mm cubes. An equal number of lens cubes either were stored in a 100% humidity chamber or were partially immersed in balanced salt solution (BSS). Using identical parameters on the phacoemulsification machine, 40 lenses that underwent each storage method were phacoemulsified every two hours for a total of ten hours.

Results: Lenses stored in the 100% humidity chamber experienced a 2.25% increase in efficiency per hour (r^2=0.1922; p=0.3846), while lenses stored partially immersed in BSS experienced a 1.30% increase in efficiency per hour (r^2=0.4084; p=0.1719). Although we found no statistically significant difference between the two groups, the partially immersed lenses were consistently softer than the lenses that were kept in the 100% humidity chamber. Chatter was minimal throughout the testing, but showed an increase over time.

Conclusion: Either the 100% humidity chamber or the partially submerged method can be used for lens storage. Despite some differences between the two storage methodologies, the differences were not statistically significant. We conclude that using either method results in a natural softening of the lenses over time which must be taken into consideration during future experiments. This necessitates the testing time to be as short as possible.

Keywords: Phacoemulsification; Lens storage method; Lens softness; Efficiency; Chatter; Animal *ex vivo* model

Introduction

By 2050, the prevalence of cataracts in the United States is projected to double from 24.4 million to 50 million [1]. Phacoemulsification is a surgical technique which has been the cataract removal procedure of choice since 1967, after it was first described by Charles Kelman [2]. To perform these surgeries, a variety of tips, handpieces, machines and software settings have been developed [3,4]. In order to objectively differentiate between the available options, we developed a porcine lens model. In the laboratory, when prepared properly, porcine lens fragments mimic human cataract lens hardness [5].

While performing porcine lens fragment studies, we observed that when the study duration was approximately six hours, the efficiency (total time required to remove the lens fragment) remained stable during the entire experiment. However, when performing studies that lasted approximately twelve hours, the efficiency improved as the duration of the study increased. Thus, the lenses appeared to be softening over time (Vegunta S, Christensen MD, Boulter T, Jensen JD, Olson RJ; unpublished data). We felt that a better understanding of this unanticipated observation was needed, and that the experiment described in this article could potentially elucidate our findings. Furthermore, we wished to determine if different storage methods would help alleviate this problem.

In previous studies we have used a single lens storage method, known as the partially submerged method. With this method, porcine lens fragments are partially submerged such that the lenses are approximately 50% submerged in balanced salt solution (BSS). In the current study our goal was to compare an alternative lens storage method, known as a 100% humidity chamber or moisture chamber, in an attempt to decrease the apparent lens softening over time. We had also tried storage with no moisture and found the lens fragments would dry out and rapidly harden, so we had already concluded that keeping the lens fragments moist was important in maintaining consistency.

Therefore, a primary aim of this study was to investigate the difference in efficiency and chatter (number of lens-fragment repulsions from the tip), as a potential source for variation in efficiency over time, between the 100% humidity chamber and the partially submerged chamber. We hypothesized that the 100% humidity chamber would have less variation in efficiency and chatter over time, compared with the partially submerged chamber, because we assumed the soaking process was leading to softening of the lens fragments. Thus the major goals of the present study were to validate our lens

storage protocol, determine the rate of lens softening over time, and provide a more robust protocol for future studies.

Methods

Porcine lens preparation

Whole porcine eyes were purchased from Visiontech Inc (Sunnyvale, Texas, USA). We prepared the porcine lens nuclei was conducted as described in a previous publication [5]. Briefly, lens nuclei were dissected from the porcine eyes and then placed in BSS for approximately one hour, until all lenses were removed. Each lens nucleus was fixed for two hours at room temperature, using 10 ml of 10% normal buffered formaldehyde. After fixation, BSS was used to wash each lens three times. The lenses then equilibrated in BSS for up to 48 hours at room temperature.

Porcine lens cube preparation

Porcine lens fragments were also prepared in the manner previously described [5]. The lenses were cut into $2.0 \times 2.0 \times 2.0$ mm cubes. While cutting the lenses, they were partially immersed in a small amount of BSS. After cubing the lenses, they were randomly mixed in a single container. A major difference in methodology with this study is that half of the lenses were then placed in a container with a small amount of BSS, such that these lenses were approximately 50% submerged in BSS. In order to maintain 100% humidity, the other half of the lenses were placed in a container with BSS-soaked cotton balls taped to the lid.

Phacoemulsification

Phacoemulsification of the lens was performed using the WhiteStar Signature Phacoemulsification System from Abbott Medical Optics, Inc. (Santa Ana, California, USA); a 20-gauge, 30 degree-curved LAMINAR Flow phacoemulsification tip; and a 20-gauge infusion sleeve with the Ellips FX handpiece. The vacuum was set at 550 mmHg with the aspiration at 50 ml/min and the power set to 50%. The bottle height was set at 50 cm. Panel mode was used, with all other settings left at default.

We began the experiment by emulsifying 40 lenses from the partially submerged chamber and immediately proceeded to emulsifying 40 lenses from the moisture chamber. For each phacoemulsification trial, we selected a random lens from the center of the container. Afterwards, we immediately covered the container in an effort to maintain constant humidity. In between selection of lenses, we swirled the containers to ensure randomization. We checked the containers every one to two hours for extraneous fluid, and to ensure the cotton balls were properly soaked with BSS. We extracted any extraneous fluid in the 100% humidity container with a syringe, and we swirled the containers every one to two hours to ensure equal distribution of fluid.

We collected six sets of data for each method, for a total of twelve data sets. Each set included 40 individual runs, collected every two hours. The two-hour countdown to collecting an additional data set began at the start of emulsification of the first lens from the 100% humidity chamber. Thus a total experiment time of approximately ten hours was required to complete the collection of data.

Efficiency was measured with a stopwatch. The pedal was depressed to initiate vacuum forces to bring the tip into contact with the lens. The timer was started when the pedal was fully depressed to initiate ultrasound (US). The timer was stopped anytime the lens fragment bounced from the tip, and restarted after bringing the lens back into contact with the tip. Therefore, we measured efficiency as the total time in seconds to remove the lens fragment, excluding the chatter delay time.

Statistical analysis

After comparing the data collection, we averaged efficiency times and calculated a standard deviation (SD). Outliers (data points that were more than two SDs from the mean) were removed from the final analysis. Their removal was based on prior research showing that these lenses have the consistency of very hard human nuclei. Occasionally, some of the harder fragments take an abnormally long time before becoming set on the needle tip, at which point they are promptly emulsified [5]. We recalculated means and SDs after excluding the outliers. We used linear regression analysis to determine significant differences in efficiency with either method over time.

Chatter events were counted and means and SDs of total chatter events were calculated. Linear regression was used to determine significant change in chatter over time. Statistical analyses were performed using GraphPad Prism (GraphPad Software, Inc, La Jolla, California, USA). Significance was set at p<0.05.

Results

Lenses stored in the 100% humidity chamber experienced a 2.25% increase in efficiency per hour (r^2=0.1922; p=0.3846; Figure 1). Similarly, we observed a 1.30% increase in efficiency per hour by the lenses that were partially submerged in BSS (r^2=0.4084; p=0.1719; Figure 2). Although the efficiency times of the two methodologies were slightly different, ultimately there was no statistically significant difference between the two methods (p=0.830). The general trend over time was an increase in efficiency; but both methods resulted in slightly lower efficiency times at both six and eight hours, and then higher efficiency times at ten hours.

Chatter was minimal throughout the testing but did show an increase with time. The increase in chatter events was greater in the partially immersed group than the 100% humidity chamber group (r^2=0.4084, p=0.3728 for the partially immersed group; Figure 3. r^2=2 $\times 10^{-16}$; p=1.0 for the 100% humidity chamber group; Figure 4). The difference between the two groups was not significant (p=0.502).

Discussion

An explosion of phacoemulsification technology has resulted in virtually limitless combinations of handpieces, machines, tips, and software settings. Our group has created original laboratory methods using brunescent human nuclei from Tanzanian patients in order to further investigate and optimize such technology [6]. These lenses were 3+ to 4+ in hardness, and thus are suitable for testing efficiency. As obtaining human nuclei for frequent experiments would be difficult and expensive, we created a porcine model with similar hardness to the Tanzanian nuclei [5]. Since the development of this model, many important clinical questions have been answered. For example, a recent study showed that when combining torsional and longitudinal modalities, longitudinal power is most critical at achieving efficient phacoemulsification with concurrent lower torsional power settings [7]. Another study showed that a 0.9 mm, 30-degree angled tip is most

efficient when compared to similar 1.1 mm and 0.7 mm tips [8]. This is just a sampling of the many papers that have clarified clinical arguments about superiority in instrumentation definitively and for the first time [5-23].

Figure 1: Effect of time on efficiency using the humidity chamber method.

Figure 2: Effect of time on efficiency using the partially submerged method.

While performing a recent study comparing the efficiency of two tips (Vegunta S, Christensen MD, Boulter T, Jensen JD, Olson RJ; unpublished data), our team observed efficiency times that were drastically different from previous work showing that a comparison of two tips typically yields similar efficiency times when the power setting is the same for both tips [12]. We thought the abnormal efficiency times may have been related to the duration of the experiment. We had begun conducting studies of longer duration, which resulted in softer nuclei by allowing the lenses additional time to soak in BSS. As a result, we were concerned that the efficiency of a tip used at the end of a long experiment may have erroneously appeared to be greater due to lenses that were easier to emulsify, rather than the result of a truly superior tip. Furthermore, we came to the conclusion that the tip being tested for the first half of an experiment could result in falsely inferior efficiency measurements, compared to the second tip used at the end of a study, due to a natural softening of the lenses over time. Once we realized that the lens softening problem would need to be addressed, we performed the experiment we have described here.
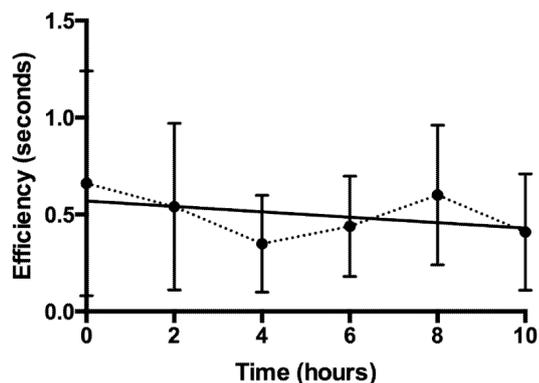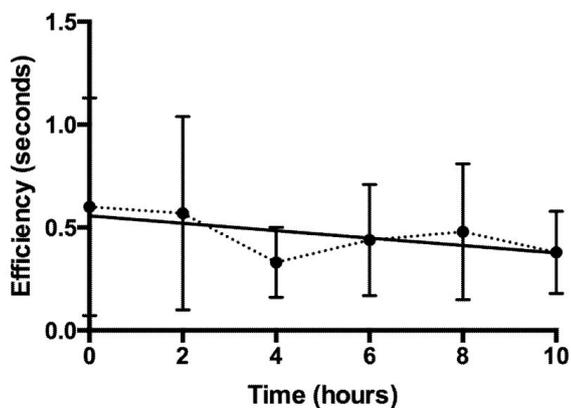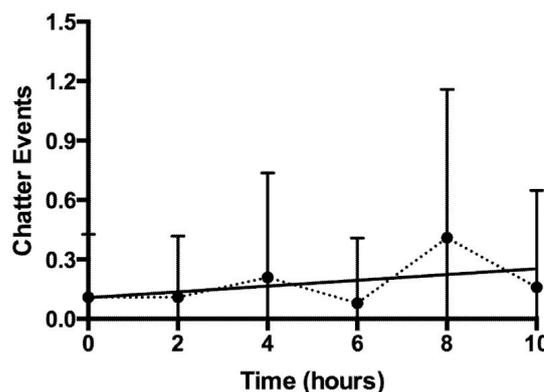

Figure 3: Effect of time on chatter using the partially submerged method.
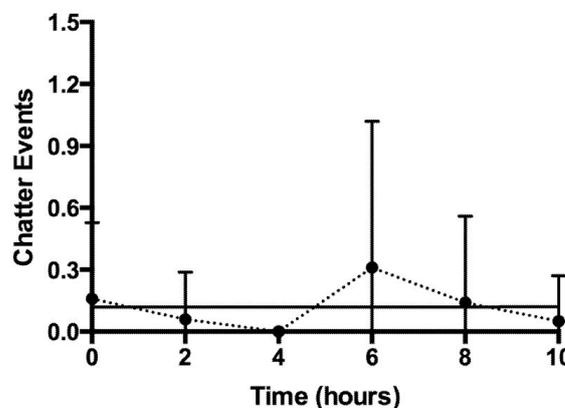

Figure 4: Effect of time on chatter using the humidity chamber method.

It is important to note that in previous studies, all nuclei were prepared and combined in one container in order to control for variation in preparation. This technique has added validity to studies by randomizing lenses which may not be identical in size, shape, and/or hardness. However, at the same time, our methodology may have introduced a form of bias by allowing lenses to soak in a storage container for such long periods of time.

It is also important to note that increased efficiency can be considered a surrogate for lens softness. We come to this conclusion because our methods were held constant throughout each trial. Furthermore, without changing power, vacuum pressure, tip size or

other parameters, we can infer that the properties of the lens fragments themselves are changing, specifically becoming softer over time. Therefore, by using efficiency as a surrogate we can conclude that there is an approximately 1-5% increase in softness per hour.

Chatter was minimal throughout the study but increased with time. Although the reason for slight differences in chatter observed with both methods is not well understood, we believe this must be a result of fundamental differences in the storage environments.

Future experiments comparing the efficiencies of two different tips could alternate between tips after the emulsification of approximately 10 lens fragments. By performing future experiments in this manner, large gaps of time between the two tips will be avoided. Thus, lens softening will be evenly distributed across both variables of interest throughout the entire data set, resulting in an accurate comparison of the variables of interest.

Additionally, we recommend randomizing power settings when comparing the efficiency of two tips, so that collecting data in increments from 10% to 100% power is avoided. For example, in future studies comparing two tips, we suggest emulsifying a small number of fragments with the first tip at a randomly selected power setting and then switching to the other tip. Alternating back and forth as described here has the drawback of adding additional time to the study, but certainly adds more validity and consistency to the data. As a result of this study, we now know that the natural softening of the lenses may falsely improve the efficiency times of power settings used at the end of an experiment, as these lenses have been soaking for the longest time.

Limitations include the *in vitro* nature this study. We did not test lenses using our original storage technique of complete immersion in BSS. We also did not test lenses stored without any fluid. Additionally, we used only one phacoemulsification platform and tip.

We were able to demonstrate that either the 100% humidity chamber or the partially submerged method can be used to store lenses for phacoemulsification experiments. Although there were some differences between the two storage methodologies, the differences were not statistically significant. Furthermore, we conclude that using either method results in a slow natural softening of the lenses over time; this must be taken into consideration when planning experiments.

Grants or Other Financial Supporters of the Study

Acknowledgment

References

1. National Eye Institute (2016) Cataracts. Washingon, DC: National Eye Institute.

2. Kelman C (1967) Phaco-emulsification and aspiration. A new technique of cataract removal. A preliminary report. Am J Ophthalmol 64: 23-35.

3. Devgan U (2006) Phaco fluidics and phaco ultrasound power modulations. Ophthalmol Clin North Am 19: 457-468.

4. Fishkind WJ (2013) The phaco machine: analysing new technology. Curr Opin Ophthalmol 24: 41-46.

5. Oakey ZB, Jensen JD, Zaugg BE, Radmall BR, Pettey JH, et al. (2013) Porcine lens nuclei as a model for comparison of 3 ultrasound modalities regarding efficiency and chatter. J Cataract Refract Surg 39: 1248-1253.

6. DeMill DL, Zaugg BE, Pettey JH, Jensen JD, Jardine GJ, et al. (2012) Objective comparison of 4 nonlongitudinal ultrasound modalities regarding efficiency and chatter. J Cataract Refract Surg 38: 1065-1071.

7. Ronquillo CC, Zaugg B, Stagg B, Kirk KR, Gupta I, et al. (2014) Determining optimal torsional ultrasound power for cataract surgery with automatic longitudinal pulses at maximum vacuum ex vivo. Am J Ophthalmol 158: 1262-1266 e2.

8. Farukhi AM, Stagg BC, Ronquillo C, Barlow WR, Pettey JH, et al. (2014) Effect of phaco tip diameter on efficiency and chatter. J Cataract Refract Surg 40: 811-817.

9. Shi DS, Jensen JD, Kramer GD, Zaugg B, Stagg BC, et al. (2016) Comparison of Vacuum and Aspiration on Phacoemulsification Efficiency and Chatter using a Monitored Forced Infusion System. Am J Ophthalmol 169: 162-167.

10. Jensen JD, Boulter T, Lambert NG, Zaugg B, Stagg BC, et al. (2016) Intraocular pressure study using monitored forced-infusion system phacoemulsification technology. J Cataract Refract Surg 42: 768-771.

11. Jensen JD, Shi DS, Robinson MS, Kramer GD, Zaugg B, et al. (2016) Torsional power study using CENTURION phacoemulsification technology. Clin Experiment Ophthalmol Epub.

12. Boulter T, Jensen JD, Christensen MD, Lambert NG, Zaugg B, et al. (2016) Comparison of a torsional and a standard tip with a monitored forced infusion phacoemulsification system. J Cataract Refract Surg 42: 613-617.

13. Henriksen BS, Gardiner G, Garff K, Gupta I, Stagg BC, et al. (2016) Thermal evaluation of two phacoemulsification systems. Can J Ophthalmol 51: 14-18.

14. Stagg BC, Gupta I, Cahoon J, Ronquillo C, Shi D, et al. (2015) Bent versus straight tips in micropulsed longitudinal phacoemulsification. Can J Ophthalmol 50: 354-359.

15. Gupta I, Cahoon JM, Gardiner G, Garff K, Henriksen BS, et al. (2015) Effect of increased vacuum and aspiration rates on phacoemulsification efficiency. J Cataract Refract Surg 41: 836-841.

16. Jensen JD, Kirk KR, Gupta I, Ronquillo C, Farukhi MA, et al. (2015) Determining optimal ultrasound off time with micropulse longitudinal phacoemulsification. J Cataract Refract Surg 41: 433-436.

17. Garff K, Jensen JD, Cahoon J, Gupta I, Stagg B, et al. (2015) Impact of micropulsed ultrasound power settings on the efficiency and chatter associated with lens-fragment removal. J Cataract Refract Surg 41: 1264-1267.

18. Gardiner GL, Garff K, Gupta I, Kramer GD, Farukhi MA, et al. (2015) Effect of pulsing ultrasound on phacoemulsification efficiency. J Cataract Refract Surg 41: 2560-2564.

19. Cahoon JM, Gupta I, Gardiner G, Shi D, Zaugg B, et al. (2015) Comparison of venturi and peristaltic vacuum in phacoemulsification. J Cataract Refract Surg 41: 428-432.

20. Gilbert M, Zaugg B, Stagg B, Olson RJ (2015) Safety Profile of Venturi Versus Peristaltic Phacoemulsification Pumps in Cataract Surgery Using a Capsular Surrogate for the Human Lens. Am J Ophthalmol 160: 179-184.e1.

21. Kirk KR, Ronquillo C, Jensen JD, Zaugg B, Barlow WR, et al. (2014) Optimum on-time duty cycle for micropulse technology. J Cataract Refract Surg 40: 1545-1548.

22. Gupta I, Zaugg B, Stagg BC, Barlow WR, Pettey JH, et al. (2014) Phacoemulsification efficiency with a radiused phaco tip. J Cataract Refract Surg 40: 818-821.

23. Meyer JJ, Kuo AF, Olson RJ (2010) The risk of capsular breakage from phacoemulsification needle contact with the lens capsule: a laboratory study. Am J Ophthalmol 149: 882-886.e1.