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SYMPOSIUM PAPER

Ordnance gelatine as an in vitro tissue simulation scaffold for extracorporeal shock wave lithotripsy

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Abstract In vitro shock wave lithotripsy (SWL) research is typically performed utilizing wet coupling lithotriptors with a mesh basket model. This model does not take into account shock wave energy attenuation through tissue. Models using dry coupling lithotriptors rely on immersion chambers and face similar limitations. Ordnance gelatin (OG) displays strength and viscous properties similar to human tissue and is therefore widely used for ballistic tissue injury research. We present our initial experience using an OG tissue simulating scaffold for dry coupling SWL research. Using 10% OG prepared in a disc-shaped mold (five stone wells/gel), we tested the model using a Modulith SLX-F2 lithotriptor and artificial stone phantoms. Following a test of concept run on an empty gel mold and a material integrity check for leakage, we shocked 60 stones (30 narrow focus [NF], 30 wide focus [WF]) in human pooled urine. Half were shocked using gels containing open-ended wells with the remainder closed-ended wells. Fragmentation coefficients (FC) were calculated across both foci and gel models. All gels successfully completed 5,000 shocks (1,000/well) without loss of gel integrity or fluid leakage. The mean FC using open-ended wells was 77.9 ± 7.6% NF and 74.4 ± 4.8% WF, and for closed wells 75.9 ± 8.0% NF and 67.1 ± 3.5% WF. The total model cost including the preparation of gels and begostones was assessed at approximately $1 per stone (Canadian). Ordnance gel serves as an excellent surrogate tissue shockwave scaffold providing an easily manufactured, reproducible and inexpensive model for dry coupling SWL research.

Keywords Extracorporeal shock wave lithotripsy · Urinary calculi · In vitro lithotripsy research · Ordnance gelatin

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Introduction

Shock wave lithotripsy (SWL) has attained a prominent role in the contemporary management of urinary stone disease [1, 2]. Despite its widespread clinical availability for more than 20 years in much of the world, significant research interest remains, especially regarding the mechanisms of stone fragmentation and tissue injury.

In vitro lithotripsy research has often utilized water bath type devices such as modified Dornier HM-3 machines [3–5]. Although a relevant model when this type of machine was the dominant clinical device, technological modifications with subsequent lithotripsy units now make water bath research less useful. Similarly, utilizing immersion chambers (dry coupling machines) does not take into account the energy attenuation that occurs as the shock wave travels through different tissue densities and interfaces [6]. Artificial interfaces such as mesh baskets also create artifactual conditions not relevant to clinical use. Finally, the inability to capture all stone material during the testing cycle due to

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fragments falling through the pores prevents an accurate assessment of fragmentation efficiency.

Ordnance gelatin (OG) has physical characteristics that accurately simulate the strength and viscosity of human tissues, making it useful in ballistic research as a tissue surrogate [7, 8]. In this study we evaluated the properties of 10% ordnance gelatin as a model for in vitro lithotripsy research, through its provision as a surrogate of soft tissue and as a means of retaining stone fragments produced during lithotripsy application.

Materials and methods

Urinary stone and ordnance gel preparation

Cylindrical artificial urinary calculi (begostones, phantoms) were prepared using Begostone Plus plaster (Bego Canada, Quebec City, QC, Canada) following the manufacturer's supplied instructions. Briefly, 100 g of plaster powder was stirred into 20 mL double-distilled water (ddH_2O) at room temperature (RT) for 1–2 min until well integrated. The mixture was poured into the wells (13.5 mm diameter) of an empty polystyrene carton used to hold 6 mL blood vacutainers (Becton Dickenson, Mississauga, ON, Canada). The carton was repeatedly tapped firmly on a table 5–10 times to settle the material and remove any air bubbles prior to setting. Stones began setting $\sim$10 min after combining the powder and ddH_2O and hardened within $\sim$30 min. The stones were then sanded using a generic table sander until the edges were smooth and the mass was 2.00 g $\pm$ 0.10 g ($\sim$13 mm diameter $\times$ 8 mm thickness). To more closely mimic the clinical situation, all stones were soaked in sterile human pooled urine obtained from healthy donors (pH 6.5 SG 1.01–1.02)(HPU) for 24 h prior to shocking. Following placement into the ordnance gel wells, stones were covered with 3 mL of HPU in the case of the open model and 10 mL in the closed model. For the open model, the top surface of the gel was covered with plastic wrap to prevent loss of stone fragments or fluid during shocking. The shocking procedure was performed at room temperature, always within 80 min of the gels being retrieved from cold storage.

The ordnance gels (models—Figs. 1b, 2, 3) were prepared following the protocol of Fackler and Malinowski [8] with a few modifications. The method we employed is described herein. One hundred thirty-four grams of unflavored gelatine (Wilton, Etobicoke, ON) was added to 1,206 mL pre-chilled (4°C) double-distilled water (ddH_2O) to create a 10% solution (11g/100 mL). The mixture was gently stirred for 2–3 min to wet all of the gelatin particles while avoiding clumping or the introduction of excess air.

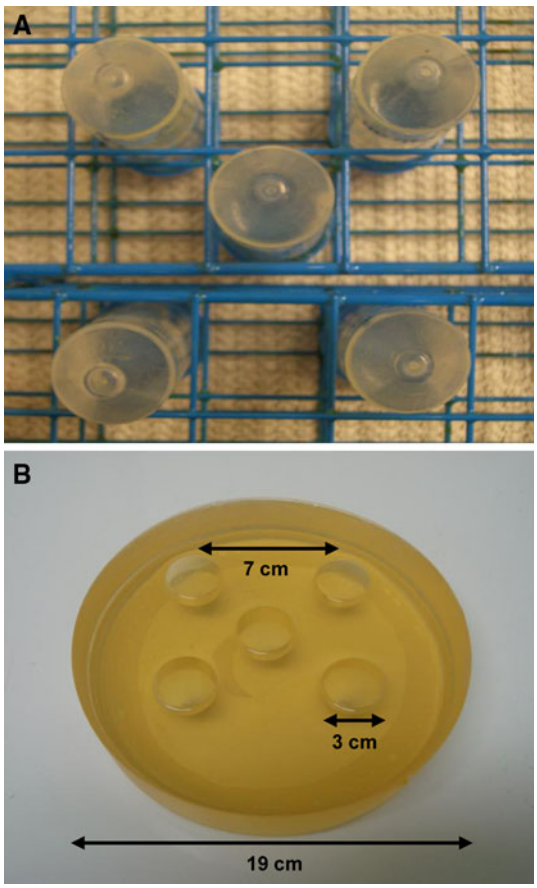

Fig. 1 **a** Conical tube setup showing X pattern arrangement to create stone wells. **b** A completed open-welled model containing five stone wells (well spacing and gel and well diameters displayed in cm)

The solution was placed at 4°C for 2 h to hydrate all of the particles, a process known as blooming. The mixture was then heated in a 40°C water bath with periodic swirling until all of the gelatine was dissolved and 65 μL of cinnamon oil (Bioforce Canada Inc., Montreal, QC) was added to prevent microbial growth. It is important to emphasize that the solution was never heated above 40°C (104°F) as it can alter the gels' final consistency.

In order to create gels that effectively fit the SLX-F2 lithotriptor coupling pad, we utilized 4 L volume cylindrical plastic molds with a 19 cm circular diameter. The molds were first sprayed with a light coating of generic non-stick cooking spray to prevent gel sticking and ease removal. The gelatin mixture was then poured into the containers to a height of 4.5 cm with care taken to minimize air bubble formation. In order to create stone wells in the gels, we first filled five 50 mL polypropylene conical tubes (VWR Canlab, Mississauga, ON) with water and capped them shut. The tubes were coated with cooking spray and arranged in an appropriate tube rack in an "X pattern" such that the centers of each of the outer four tubes were 5 cm from the center of the central tube and

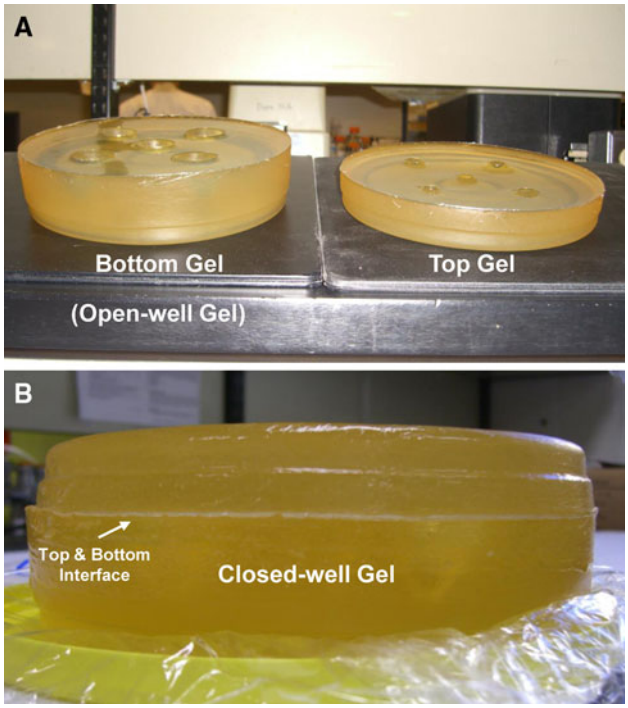

Fig. 2 Closed-welled model. **a** *Top* and *bottom* gels were produced separately, the stones added to the wells of the *bottom* gel (open-welled model is used as bottom gel), the *top* gel inverted and placed on the *bottom* gel and the two gels sealed using liquid gelatine. **b** The resulting closed-welled gel. Using a syringe, the fluid of interest was injected into the wells and any air present removed

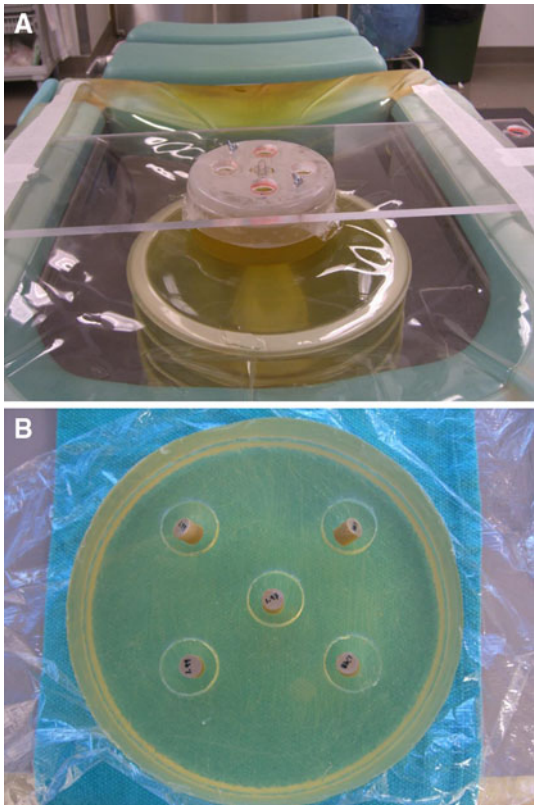

Fig. 3 **a** Model fixed to the SLX F2 lithotriptor using a custom made acrylic support. Note paper clip helps orientation during fluoroscopic target acquisition (*up-down* or *Z* axis). **b** Open-welled gel model containing pre-weighed begostones, just prior to SWL treatment

equally spaced 7 cm apart from each other (Fig. 1a). The tubes were then suspended in the gelatine solution 2.5 cm deep. The molds were placed at 4°C overnight (14–16 h) until completely set. The tubes were then twisted carefully and removed from the gel. The finished gel was removed from the mold, wrapped in plastic wrap and stored at 4°C until use (up to 7 days). The final dimensions of each cylindrical well were 3 cm (diameter) by 2.5 cm (depth) resembling the end of the conical tube. This resulted in one completed open-welled gel (Fig. 1b). To produce a closed-well gel (all stone wells entirely within the gel—Fig. 2b), an additional (top) gel was poured using the same 4 L volume mold to a thickness of 2 cm (Fig. 2a). A similar set of conical tubes arranged in the X pattern was submerged into the gel to a depth of 0.5 cm. This created a small convex-shaped well to trap air in a single central location when the top and bottom gels were sandwiched together, easing air extraction by needle aspiration. Upon setting, the thinner top gel was inverted and bonded to an open-welled (bottom) gel using 2 mL of liquid 10% gelatine prepared similarly and painted on to the gel surfaces. Since there was no access to the closed wells once the two gels were bonded, pre-soaked begostones were numbered and placed in the wells prior to this procedure. The resulting two-gel, closed-well scaffold was placed at 4°C for an additional 4 h to complete the adhesion process. The entire procedure to generate a completed open-welled gel was ~24 h and a closed-welled gel ~28 h.

Lithotripsy procedure

Stone fragmentation was conducted using the Modulith SLX-F2 lithotriptor (Storz Medical AG, Tägerwilen, Switzerland) as follows: To create a thin coupling film, tap water was first applied to the coupling pad and a prepared gel containing stones and urine placed on the pad center. Gels were held in place during shock wave administration initially using kidney-shaped urine pans and masking tape, and later a custom-made (the holder was 19 cm in diameter and 3 cm in depth) acrylic device (Fig. 3a).

In the initial integrity experiments one empty, open model was shocked according to our standard protocol. Following this trial a new gel model loaded with 5 mL of India ink in each well was shocked. In both test runs, the F2 focal point was located just inferior to the lower border of each well, thereby ensuring the gel itself was receiving the brunt of the energy in an attempt to maximize model damage. After shock wave delivery, loss of gel cohesion or leakage was examined by gross visual inspection, looking

for signs of model breakage, stained fractures, or India ink extravasation from the wells.

All stone phantoms were shocked using a standardized protocol consisting of 1,000 shock waves (SW) at an energy level of 8 and a frequency of 2 Hz (120 SW/min) (Fig. 4). The narrow (standard) treatment focus (F2 diameter of 6 mm × 28 mm) was used for half of the phantoms and the wide (large) focus (F2 diameter of 9 mm × 50 mm) for the remaining half. Stone targeting was maintained via fluoroscopy (Fig. 5). The total treatment time to shock one complete model was approximately 1 h; 10 min for gel

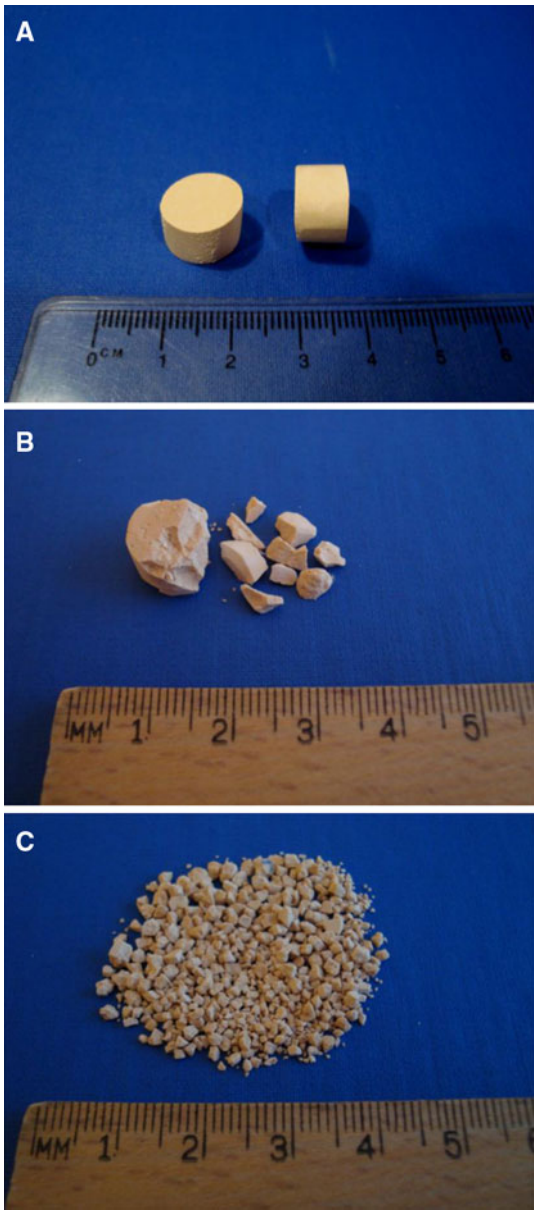

Fig. 4 Begostones prior to (**a**) and following (**b**, **c**) SWL treatment. Stone phantom shown in **b** demonstrates poor fragmentation while that in **c** exhibits complete fragmentation (all fragments <4 mm in diameter)

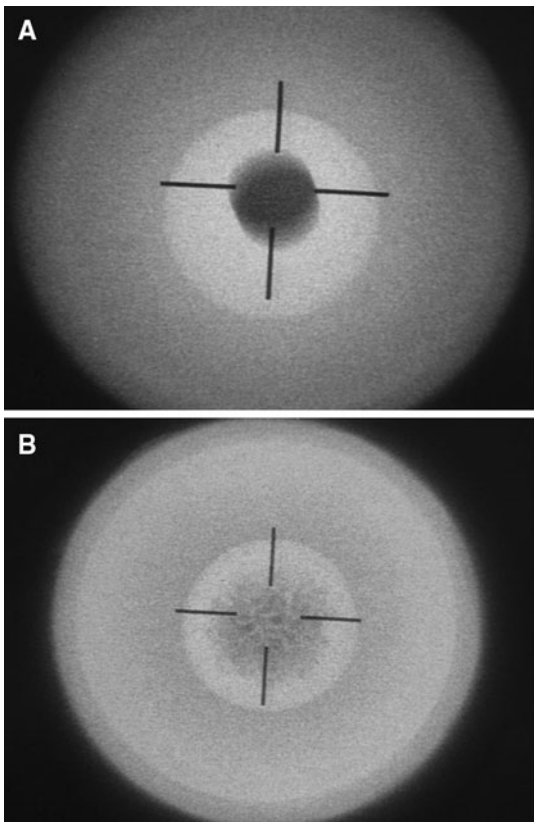

Fig. 5 Fluoroscopic images showing a begstone prior to (**a**) and immediately following (**b**) SWL treatment

positioning and target acquisition, 50 min for SWL application (10 min/stone × 5 stones/model).

After SWL the fragments were collected from each test well and air dried for 24 h in a 37°C incubator (Fig. 4b, c). Fragments were sifted using a pipette tip box (VWR) containing 4 mm diameter openings and those passing through were discarded. The remaining fragments were weighed and the fragmentation coefficient (FC) of each stone calculated using the following formula: (Pre SWL weight − Post SWL weight)/(Pre SWL weight) × 100.

Statistical analysis

The data were analyzed via one-way Analysis of Variance (ANOVA) with Bonferroni's post test (all group comparisons) and unpaired, two-tailed Student *t* tests (open model vs. closed model, narrow focus vs. wide focus) using GraphPad Prism software (GraphPad Software Inc., San Diego, CA). Significance was assessed at $p < 0.05$.

Results

With 5000 SW being applied per model (1,000/well × 5 wells) during the lithotripsy procedure, the potential

existed for gel damage and subsequent loss of integrity. Thus, our first goal was to investigate the effect that the SWL treatment had on the models themselves. Trial runs using an empty model and one with the wells filled with India ink dye were conducted using clinically relevant lithotriptor settings and SW duration. Furthermore, the SW focal point was set within the model itself, just below the inferior well border to ensure that the gel received maximal SW force load. During these initial integrity tests, neither model suffered from gel breakage or well leakage as assessed via visual inspection of the entire gel and complete dye retention within each individual well.

The main study itself involved the shocking of 60 total stones, divided into 4 groups of 15 phantoms each. Two groups involved the use of an open well model, one shocked via narrow focus SWL (Open N) and the other wide focus (Open W). The remaining two groups used closed well gels, again split across the two foci (Closed N and Closed W). This allowed us to investigate the effects that both focus and gel type had on stone fragmentation. The mean overall FC for the four individual groups were Open N-77.9 ± 7.6% (range 17.3–100%), Open W-74.4 ± 4.8% (25.6–100%), Closed N-75.9 ± 8.0% (12.0–100%), and Closed W-67.1 ± 3.5% (53.7–96.1%) with no statistically significant differences observed across all groups ($p = 0.643$; Fig. 6). To assess the effects that gel type alone had on fragmentation, the results for groups Open N and Open W were combined and compared to the combined values for groups Closed N and Closed W (Fig. 7a). No significance was observed during this comparison, with the mean FC using open-welled gels at 76.2 ± 4.5% and closed-welled gels 71.5 ± 4.4% ($p = 0.459$). Finally, the values for groups Open N and Closed N were combined and compared to the combined values of groups Open W and Closed W to assess potential differences across the two clinically used foci (Fig. 7b). The narrow and wide focus FC means were 76.9 ± 5.4 and 70.8 ± 3.0%, respectively, again showing no statistically significant difference ($p = 0.325$).

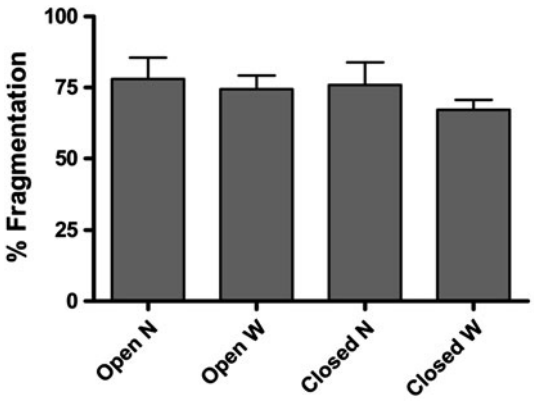

Fig. 6 Mean fragmentation results for all four groups (15 stones/group) tested in this study, open-welled gels using narrow (Open N) and wide (Open W) foci, and close-welled models using the same two foci (Closed N and Closed W)

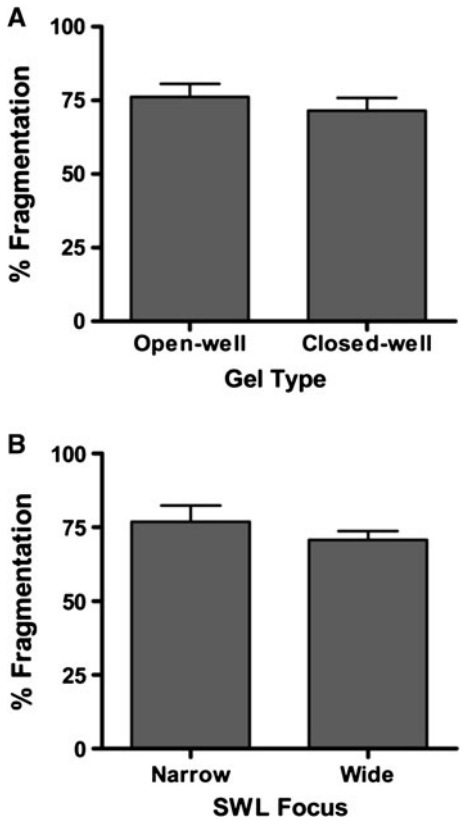

Fig. 7 Mean fragmentation results comparing gel-types (**a**) and foci (**b**) separately (30 stones/group). **a** The results from groups Open N and Open W were combined and compared those of Closed N and Closed W combined. **b** The results from groups Open N and Closed N were combined and compared those of Open W and Closed W combined

Discussion

Despite the widespread clinical availability of SWL, a number of important research questions remain to be answered. Optimal treatment settings to maximize stone fragmentation while reducing the chance of renal injury continue to be debated. Although most lithotriptors produce a similar type of shock wave signature, different shock wave sources and focusing mechanisms make each lithotripsy system unique. Indeed, clinical results measured in stone free, retreatment, auxiliary intervention [9] and complication rates [10] (e.g., perinephric hematomas) from each machine vary widely and Argyropoulos et al. [11] showed that stone-free rates can be improved by providing a second treatment session using a different machine.

Ultimately, the development of a model allowing SWL system evaluation in a reliable, reproducible and inexpensive fashion would be extremely valuable from both basic science and clinical standpoints.

In this work we evaluated a gel commonly used by forensic labs to measure the in vitro effects of armaments on human tissue. The gel itself comprised only three ingredients, double-distilled water, unflavored gelatine (added to 10%), and a trace amount of pure cinnamon oil (50 μL/L—to prevent microbial growth). To ensure that the model was both inexpensive and easily attainable, the gelatine and cinnamon oil used were generic brands purchased from a local supermarket and health food outlet, respectively. During the study we found that gels could be rapidly and reproducibly generated if the method described herein was closely followed. At the same time, the model could be easily adjusted with respect to overall shape (to best fit different coupling pads) and stone well dimensions (enhance focusing, change surrounding fluid volumes) without affecting gel consistency. Thus, the model is both reproducible and modifiable to suit any SWL/laboratory environment.

In the first phase of our experiments, we demonstrated that the model itself is physically able to withstand the rigors involved in repeated SWL cycles in which pressures of up to 107 MPa (narrow focus [44 MPa in wide focus mode]) and energy flux densities of 0.03–3.65 mJ/mm can be reached at F2. Also, within our working time frame (~1 h) any increase in gel temperature (either ambient or SWL induced) did not cause structural failure. In fact, we determined that the gel model can withstand room temperature exposure for at least 2 h before significant loss of structural integrity occurs during SWL (unpublished observations).

Following this initial testing, we evaluated the ability of the model to conduct shock-waves and serve as a stone-retaining tissue scaffold for in vitro SWL research. For this, we utilized both open-welled and closed-well models and applied SW using both narrow and wide foci. While the open-welled gels cost less, are much simpler to make and far easier to utilize, we were concerned that the top air-fluid interface would result in shock wave reverberation and artificial enhancement of FC, thereby decreasing the clinical relevance of the model. Thus, we created a closed-well model in an effort to more accurately replicate in vivo conditions.

Our data showed that the FC of the closed-well model was not significantly different when compared to the open model. Therefore, although the closed model may be a more accurate test platform, the process of creating and priming (fluid instillation, and especially gas bubble elimination) is a relatively cumbersome added technical step that could be avoided without seriously impacting the test results.

Overall, our experiments revealed several key findings: (1) Both ordnance gel models were capable of conducting shock waves; (2) The pressures reached at the F2 focal point were sufficient to cause stone comminution; (3) There were no significant differences in fragmentation rates across either the two gel models or applied foci (Figs. 6, 7).

Begostones, previously shown to possess properties similar to a calcium oxalate monohydrate calculi [3], were chosen for this trial (Fig. 4) because they represent the extreme of the hardness spectrum found in calculi and are more clinically relevant than other available models such as plaster of Paris, which has acoustical properties comparable to struvite [12]. Although difficult to conclusively compare due to different testing protocols and SWL generators used among published studies, our findings of an overall 74% FC is comparable to previously reported (37–88%) in vitro results using the same stone phantom [3, 13].

The use of gelatine in SWL research has been previously described, although it was limited to coupling facilitation [14], tissue simulation for pressure profiling in SWL [10] or cavitation tissue injury [15]. Although we did not directly measure the speed of sound in our model, previously published data on the acoustical properties of a similar gelatine-based tissue mimic (13% gelatine) showed a speed of 1,550 m/s [16], slightly faster than that of degassed water (1,520 m/s) [16]. As our model is comprised of 10% gelatine, it is highly likely that its speed of sound falls between these two values, favorably close to the known velocity for mammalian soft tissues (~1,540 m/s). Furthermore, since shockwave speed may also be influenced by changes in gel temperature during the experimental procedure, we monitored model temperature throughout lithotripsy setup and shockwave delivery. Firstly, models were moved from refrigerated storage (4°C) to the lithotripsy unit (20°C) and coupled to the device pad using 18°C tap water. This resulted in a slow increase in model temperature from 4°C to just under 10°C during setup. This temperature increased to 14.9°C by the conclusion of 5,000 shockwaves, showing that the overall temperature of one model increased 10.9°C throughout the entire process, ~5°C during shockwave treatment. Since previous work involving the aforementioned gelatine-based mimic showed only a ~1.5% increase in shock wave speed upon a 12°C increase above the baseline [16], we conclude that temperature does not significantly affect wave speed within our model. Finally, transverse sectioning of a treated model showed no loss of consistency or bubble formation within the gelatine. Overall, we hypothesize that any temperature change during the SWL test cycle should have a minimal impact on FC.

The model described in our work is novel in that it combines tissue simulation with the ability to serve as a scaffold or physical support for the stone phantom. Its advantages include ease of manufacture, reproducibility

and low cost (approximately $5 CDN per model [$1/stone treated]). Our achieved FC results approximate clinical data obtained for the Storz SLX-F2, suggesting the model is providing clinically relevant information [17]. While the experiments involved test wells with a total capacity of 7.5 mL, most likely mimicking the renal pelvis, the capability to make gels that are size and shape compatible with the coupling pad of any commercially available lithotriptor allows the model to be used with most if not all clinical devices.

Limitations of this research include the fact that being an in vitro model of homogeneous composition, it may not accurately represent the more heterogeneous tissues and stones of real patients, in which the shock wave must traverse multiple tissue interfaces and stone densities and compositions.

Currently, the HM-3 or modifications to water-bath technology are commonly utilized lithotriptors for research. As new HM-3 units are no longer manufactured, it seems obvious that future SWL research should employ features of the latest generation of machines to be clinically relevant. That being said, the technical and physical differences of many of the newer machines are also quite variable, requiring non-standardized, often cumbersome and expensive machine modifications that might not mimic the clinical scenario. The use of our model with its ease, widespread applicability and modification-free use may help simplify this transition and further disseminate SWL research.

Conclusions

The use of the 10% OG model allows the potential conversion of any commercially available dry-coupling (second generation and beyond) lithotriptor into a research unit without modification. The model is also very cost effective and allows for standardized and reproducible testing, achieving FC results comparable to the clinically available literature.

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