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Ultracal-30 gypsum artificial stones for research on the mechanisms of stone breakage in shock wave lithotripsy

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Abstract Artificial stones are used in research on the mechanisms of stone breakage in shock wave lithotripsy (SWL) and in assessing lithotripter performance. We have adopted Ultracal-30 gypsum as a model, finding it suitable for SWL studies in vitro, acute animal experiments in which stones are implanted in the kidney, and as a target to compare the in vitro performance of intracorporeal lithotripters. Here we describe the preparation of U-30 stones, their material properties, shock wave (SW) breakage characteristics, and methods used for quantitation of stone fragmentation with this model. Ultracal-30 gypsum cement was mixed 1:1 with water, cast in plastic multi-well plates, then, the stones were liberated by dissolving the plastic with chloroform and stored under water. Stone breakage in SWL was assessed by several methods including measures of the increase in projected surface area of SW-treated stones. Breakage of hydrated stones showed a linear increase in fragment area with increased SW-number and SW-voltage. Stones stored in water for an extended time showed reduced fragility. Dried stones could be rehydrated so that breakage was not different from stones that had never been dry, but stones rehydrated for less than 96 h showed increased fragility to SWs. The physical properties of U-30 stones place them in the range reported for natural stones. U-30 stones in vitro and in vivo showed equivalent response to SW-rate, with ~200% greater fragmentation at 30 SW/min compared to 120 SW/min, suggesting that the mechanisms of SW action are similar under both conditions. U-30 stones provide a convenient, reproducible model for SWL research.

Keywords Lithotripsy · Kidney calculi · Urinary calculi · Biological models

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Introduction

Artificial stones provide an important tool for research on the mechanisms of shock wave (SW) action in shock wave lithotripsy (SWL), and for quality control testing of lithotripters. Much of what is known about the mechanisms involved in stone breakage comes from work with model stones [1, 2]. Natural urinary calculi are heterogeneous in size and shape, internal structure, mineral composition, material properties and fragility to SWs [3]. Because they are so unpredictable, natural stones prove to be poor test subjects for experiments to determine how best to break them. For similar reasons, natural stones are rarely used to assess the performance of lithotripters [4]. Instead, investigators have developed a variety of artificial, model or phantom stones. The list of stone models is extensive and runs the gamut from crude pebbles to sophisticated dental polymers [5, 6]. Perhaps the most elegant artificial stones are those described by Heimbach and coworkers [7, 8] in which multi-lamellar stones are prepared from minerals found in natural stones. Sophisticated or crude, artificial stones have been useful, if even for very specific applications.

We have adopted a gypsum-based (calcium sulfate dihydrate) cement, Ultracal-30, first reported as a model stone by Dahake and Gracewski [9]. U-30 gypsum is simple to handle, easy to replicate and can be cast to desired size and shape. We have used U-30 stones in vitro to study the role of cavitation in stone breakage [10]; to assess pulse timing in dual-pulse SWL [11]; to test theoretical predictions of SW-stone interactions, such as the influence of stone shape [12, 13] and the role of shear waves in stone breakage [14]; and to model the effect of respiratory motion on stone breakage in SWL [15]. We developed a percutaneous procedure to implant U-30 stones in the kidney and found with this in vivo model that stones break better at slow SW rate than at fast rate [16]. U-30 gypsum has also proven to be an excellent model to compare the effectiveness of intracorporeal lithotripters [17].

This report provides details for preparation of U-30 stones, describes their physical characteristics, and gives methods for the quantitation of stone fragmentation using this model.

Materials and methods

Preparation of U-30 gypsum stones

Ultracal-30 gypsum (United States Gypsum, Chicago, Ill.) is a low-expansion, high surface-hardness cement used by artists for casting accurate surface molds. Dry powder was mixed 1:1 (g:ml) with tap water, first mixing by hand, then with a magnetic stir bar for 5 min. Aliquots (400 µl) of the slurry were delivered to 96-well polystyrene plates (Corning 3595). The height of the stones was influenced by mixing time, such that overall mixing for 9–12 min gave stones ~7.2–7.6 mm high. Lengthy mixing, up to 30 min, yielded stones significantly less fragile than stones from slurry mixed for 5 min. Thus, for studies of stone fragility, mixing time was held constant. Plates were allowed to sit at room temperature for 1 h, during which time particulates settled and the gypsum solidified leaving water at the top of each well. Plates were immersed in water to avoid dehydration and trapping of gas within the gypsum. The next day, with minimum exposure of the stones to air, the plates were cut into segments using a hot wire, and the pieces transferred to chloroform in glass dishes atop an orbital shaker. The plastic was dissolved over a period of 2 days with multiple changes of chloroform. Stones were thoroughly rinsed, then stored submerged in a minimum volume of water in a capped container. Stones stored in a large volume tended to dissolve over time.

Breakage of stones in the lithotripter

Studies were performed using a research electrohydraulic lithotripter that had acoustic output comparable to an unmodified Dornier HM3 [18]. Several methods were used to hold stones at the geometrical focus (F2) of the lithotripter. When the study called for the delivery of a pre-determined number of SWs, stones were held in latex finger cots or placed in flat-bottom, polypropylene vials (4 ml, 15×35 mm: Cole Parmer EW-98814–30) with the flat side facing the shock source. When the desired end point was a count of the number of SWs to complete comminution, stones were placed in a basket fashioned from 2 mm nylon mesh. The holders were filled with water from the lithotripter water bath; thus, stones were bathed in filtered, degassed, deionized water to which $NaHCO_3$ was added to regulate conductivity (~620 µS).

Quantitation of stone breakage

SWs to complete comminution

SWs were delivered until all stone fragments passed through the 2 mm mesh of the basket. Occasionally, fragments smaller than 2 mm in one dimension would fail to fall through the mesh for many SWs beyond what might have been expected. No correction or estimate was made for these "stubborn" fragments, and SW administration was not stopped until all particles passed through the mesh.

Measuring stone breakage using projected fragment area

This method allows the quantitation of breakage following a fixed dose of SWs. Before SW exposure, each stone was imaged by placing it in a plastic dish and scanning it on a flat-bed scanner (at 300 dpi). Similarly, after exposure to SWs, all pieces were placed in a dish and scanned. Images were processed to erase the edge of the dish (using Adobe Photoshop), then thresholded in NIH Image or ImageJ (http://rsb.info.nih.gov/ij/) for measurement of total image area of stone before SW treatment and of fragments following treatment. Breakage index was defined as area of fragments after SW exposure, normalized to area of stone before treatment minus one. Thus, a breakage index of 200% means that the broken pieces occupied a surface area on the dish bottom three times that of the unbroken stone. We have found that the breakage index correlates linearly with the energy applied to break a stone (Fig. 1). Breakage index allows quantitation without extensive handling of the fragments, and is especially useful when the amount of breakage is minimal to moderate. In cases when much of the stone has been reduced to sand, this method is not as useful, and a sieving method is superior.

Measuring stone breakage by sieving fragments

This method also allows quantitation of breakage following a fixed dose of SWs. We take the fragments remaining after exposure to SWs, shake them gently

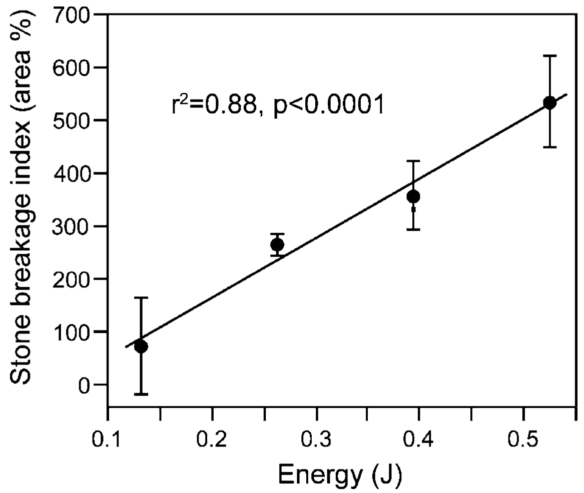

Fig. 1 Demonstration that quantitation of stone fragment area (projected area measured using flat-bed scanner, normalized to unbroken stone) correlates well with energy applied to U-30 stones. Stones (12 in all) were broken using a drop impact method; mean ± SD are shown

over a 2 mm mesh and weigh the fragments retained on the mesh. This weight is then normalized to stone weight before treatment. This is straightforward, but in handling fragments it is possible to cause additional breakage. However, this method may be the only way to quantitate breakage when much of the stone has been reduced to small particles.

Determination of the physical properties of U-30 stones

The density of U-30 stones was measured using the Archimedes principle and porosity was calculated by the difference in mass of wet and dry stones. Longitudinal and transverse wave velocities in U-30 stones were determined using a time-of-flight technique employing paired 10 MHz longitudinal wave transducers or 5 MHz shear wave transducers (V129 and V157 Panametrics-NDT, Waltham, Mass.) in contact with the stones under water [19].

Micro-computed tomography

Fractures within stones were observed using a μCT 20 laboratory CT scanner (Scanco, Switzerland) [20]. Stones were scanned dry at settings giving resolution of ~25–34 μm.

Statistical analysis

Data were analyzed using JMP (SAS Institute, Cary, N.C.). Means were compared using Student's *t*-test, least-squares linear regression analysis, and Tukey-Kramer HSD test as appropriate.

Results

Fragility of U-30 stones in the lithotripter was assessed for three parameters of SW delivery including voltage, number of SWs and rate of SW administration. Fragmentation of fully hydrated U-30 stones showed an appropriate response to voltage and to SW number (Fig. 2). Also, rate of SW delivery significantly affected stone breakage (Fig. 3). U-30 stones showed a similar effect of rate when breakage was measured by counting the number of SWs to complete comminution (data presented below). At the typical clinical rate of 120 SW/min these relatively small stones (~0.48 g wet weight, ~7 mm in greatest dimension) required ~1,400 SWs for complete comminution, demonstrating that U-30 stones show considerable resistance to lithotripter SWs,

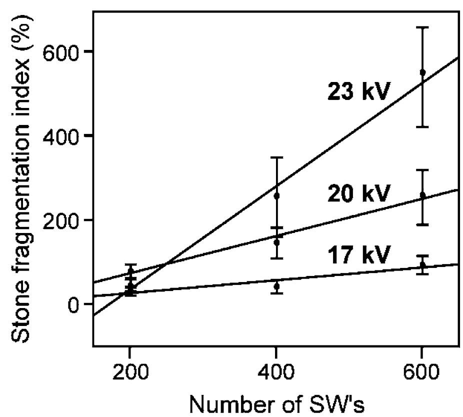

Fig. 2 Dose response data for U-30 stones, exposed to SWs in an electrohydraulic lithotripter. Note significant increase in stone fragmentation with increasing numbers of SWs applied, and increasing slope of dose-response with increasing electrode voltage

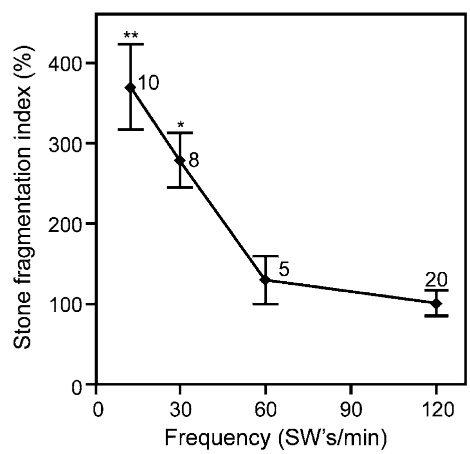

Fig. 3 Effect of SW administration rate on breakage of U-30 stones. Stones were exposed to 400 SWs at 20 kV; number of stones shown beside each point. ** Different from 60 and 120 SW/min, $P < 0.001$; * different from 120 SW/min, $P < 0.05$ (both by Tukey-Kramer HSD test)

comparable to that required to break cystine stones in vitro [3].

Gypsum softens with time in water and we observed that the fragility of U-30 stones changed as they aged. In order to determine the window of time in which stones exhibited stable fragility, an experiment was designed to compare stones of advancing age with freshly prepared stones (Fig. 4). Stones stored in water for 32 days and longer exhibited reduced fragility compared to new stones.

Typically, stones were stored in water until needed. However, when it was necessary to ship stones between laboratories, the stones were dried before shipping. In this case it was necessary to rehydrate the stones before use. We estimate that U-30 stones have a porosity of approximately 60%. Although this value is relatively high, we observed that dry stones placed in water for a matter of hours broke more readily than stones that were never allowed to dry. In order to determine the rehydration time necessary for U-30 stones to exhibit breakage comparable to fully hydrated stones, an experiment was run in which breakage of stones rehydrated for various lengths of time was compared to stones from the same batch that were never dried (Fig. 5). Dry stones required ~96 h in water to show breakage equivalent to stones that were never dried. The idea that increased fragility was due to the retention of air is supported by measures showing decreased sound speed in under-hydrated stones (Fig. 6).

We measured U-30 stones to have a density of 1,700 kg/m^3, longitudinal wave speed of 2,840 m/s and shear wave (transverse wave) speed of 1,430 m/s. These values fell near mid-range for data that have been reported [7] for natural urinary stones and are closest to uric acid and struvite (Fig. 7). Unlike most natural calculi, U-30 stones were observed to be highly homogeneous in internal structure (Fig. 8), with no apparent organization as is typically seen for natural stones [21]. We also observed that damage to U-30 stones was consistent with model predictions [13, 14] of internal stress due to spall, and surface erosion due to cavitation (Fig. 8).

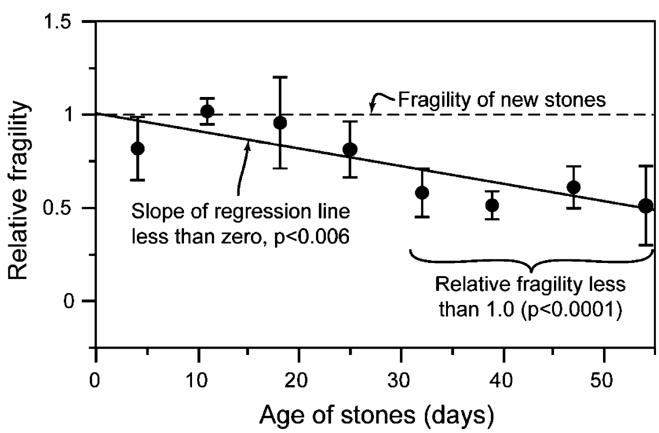

Fig. 4 Decrease in fragility of U-30 stones with age. U-30 stones from a single production batch were stored in water at room temperature and each week ten were exposed to SWs (400 SWs each, 20 kV, 1 Hz). A fragility value of 1.0 indicates fragment area equal to that of freshly made stones. Means ± SD are shown. The 40 stones from days 32–54 as a group showed reduced fragility (by *t*-test)

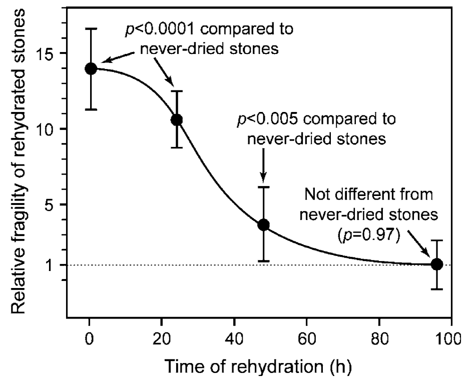

Fig. 5 Effect of rehydration time on fragility of U-30 stones. Fragility (calculated from fragment area) of stones rehydrated for times from 20 min to 4 days was compared to stones that were never dried. All stones received 400 SWs at 20 kV, 1 Hz, with ten stones in each group. Curve determined by eye

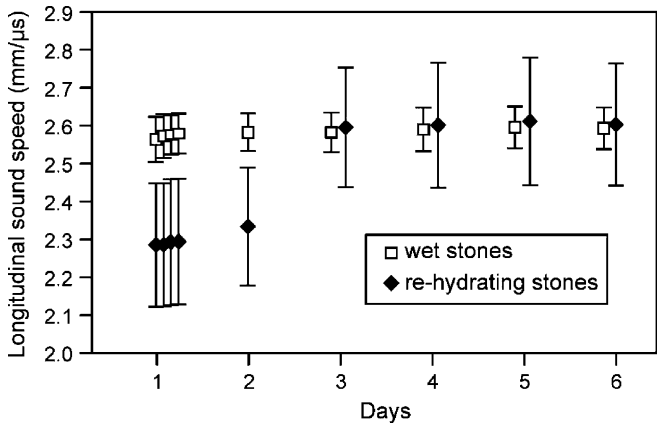

Fig. 6 Measures of longitudinal sound speed for U-30 stones showing effect of rehydration. Dry stones were placed in water and measures of longitudinal sound speed were collected at various times. Stones returned to water for less than 3 days showed a reduction in sound speed compared to stones that were never allowed to dry. This is evidence that air is retained within U-30 stones rehydrated for less than ~72 h

Discussion

Having a reliable stone model is important in lithotripsy research. In our work, in which we are trying to understand the multiple mechanisms of SW action, determine the role of SW rate and voltage/power in stone breakage, and compare the performance of current lithotripters and assess new and emerging technologies, we are keenly aware of sources of variability in the test

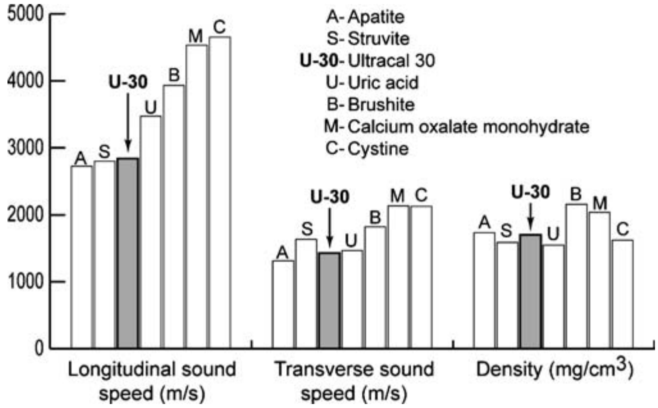

Fig. 7 Acoustic properties of U-30 stones compared with natural stones. Data for natural stones from [7]

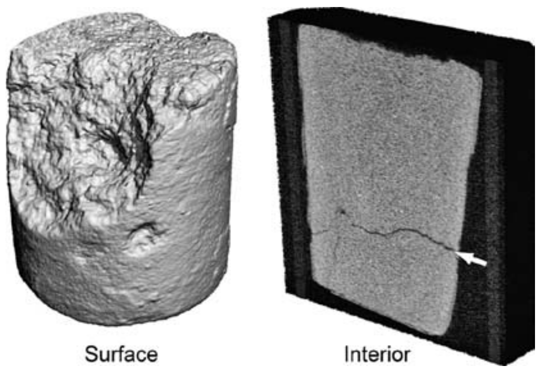

Fig. 8 SW-treated U-30 stone shown by micro-CT. 3-D surface reconstruction (*left*) shows damage typical of erosion due to cavitation, and image slice (*right*) shows a transverse fracture (*arrow*) characteristic of stress within the stone (e.g. spallation). Orientation is with SWs delivered from above. The image slice demonstrates a homogeneous internal structure of the U-30 material

systems we use. For example, there can be substantial shot-to-shot variability in the acoustic output of lithotripters; electrode wear in electrohydraulic machines reduces the effectiveness of pulses, and even with electromagnetic lithotripters the condition of the water in the test tank can change during an experiment, influencing cavitation. A stone model should not be a source of additional variability. This requires an understanding of the pitfalls or limitations of the stone model, and is why we have stressed the importance of controlling factors such as mixing time, age of the stones and the time necessary for complete rehydration of dried stones when working with U-30 gypsum.

An unexpected observation in our characterization of U-30 stones was the great length of time (~4 days) it took for dry stones to become fully rehydrated. It is understandable why incompletely hydrated stones break more readily. Gas retained within a stone will present a SW-reflective boundary that both disrupts SW propagation and increases tensile stress within the stone [13]. Also, fracture of an incompletely hydrated stone will likely release gas, seeding cavitation that contributes to breakage. It may be valuable to keep in mind that natural stones develop within a fluid environment and, as such, remain fully hydrated. Thus, for studies of the mechanisms of SW action in stone comminution, the presence of gas within a model stone is an artifact, and the degree of hydration appears to have a dramatic effect on fragmentation. This is one advantage of working with stones that are made under water and never allowed to dry. Investigators rarely note the time of rehydration of stones, and we do not know of any published report in which the degree of rehydration of natural or model stones has been rigorously assessed. We believe that the hydration state of stones is one aspect of methodology that should be reported in work with artificial (and natural) stones.

We found that selected material properties of U-30 gypsum stones place them in the vicinity of values for natural uric acid and struvite stones. This should ensure that the state of stress induced by SWs in these artificial stones is equivalent to that in natural stones. However, because the fracture mechanisms of stones are likely to be dependent on other factors as well, such as overall shape, or internal structure and the arrangement of mineral components [3], we do not consider U-30 stones to be a model of any specific type of urinary stone. Still, the damage patterns seen in U-30 stones are consistent with spall and cavitation, which are likely the dominant processes involved in the fragmentation of natural stones [1, 10, 12, 13, 14].

Our work with U-30 stones suggests that in vitro studies can be highly relevant. Figure 9 shows data from experiments in which we implanted U-30 stones into pig kidneys via percutaneous access to assess the role of SW rate in stone comminution [16]. The data showed that SWs delivered at a rate of 30 SW/min broke stones

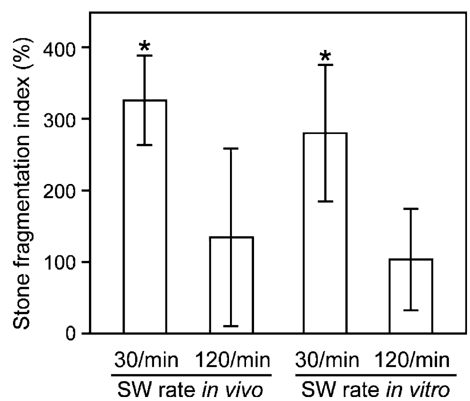

Fig. 9 Fragmentation data measured for U-30 stones implanted in live pig kidneys (in vivo, on left) compared with fragmentation measured in vitro (all stones 400 SWs, 20 kV). Averages for six in vivo stones at 30 SW/min, seven at 120 SW/min, and eight and 20 stones in vitro at the two rates, respectively. *Error bars* show SDs; * 30 SW/min showed significantly greater stone fragmentation than 120 SW/min, and in vivo and in vitro were not different, $P < 0.02$ by Tukey-Kramer HSD test

significantly better than the typical clinical rate of 120 SW/min. Data from that study are shown alongside values for U-30 stones broken in vitro. The in vitro stones were treated with the same dose of SWs (400 SWs, 20 kV) and at the same SW rates as the in vivo stones. The magnitude of the rate effect was virtually identical for both and there is no significant difference between the in vivo results and the in vitro results. These results deal specifically with the effects of SW rate, but the close correlation between the data argues that the mechanisms of SW action in vitro are very similar to those in vivo. This finding is encouraging and provides additional validation for the use of in vitro models in SWL research.

Conclusions

Artificial stones made from Ultracal-30 gypsum provide a reproducible model that has been used for a variety of in vitro and in vivo studies in lithotripsy research. U-30 stones are simple to prepare, have physical properties in the range of natural stones, show a predictable dose response to the parameters of SW delivery (SW number, voltage, rate), exhibit damage due to spallation and to cavitation, are significantly less variable in their breakage than natural stones, and are suitable for short-term implantation in the pig kidney. Fragmentation of U-30 stones can be quantitated by several sensitive, accurate methods. This is one example of an artificial stone that can be used in vitro to model SW action in vivo.

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