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Reviews

Targeted microbubbles: a novel application for the treatment of kidney stones

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Kidney stone disease is endemic. Extracorporeal shockwave lithotripsy was the first major technological breakthrough where focused shockwaves were used to fragment stones in the kidney or ureter. The shockwaves induced the formation of cavitation bubbles, whose collapse released energy at the stone, and the energy fragmented the kidney stones into pieces small enough to be passed spontaneously. Can the concept of microbubbles be used without the bulky machine? The logical progression was to manufacture these powerful microbubbles *ex vivo* and inject these bubbles directly into the collecting system. An external source can be used to induce cavitation once the microbubbles are at their target; the key is targeting these microbubbles to specifically bind to kidney stones. Two important observations have been established: (i) bisphosphonates attach to hydroxyapatite crystals with high affinity; and (ii) there is substantial hydroxyapatite in most kidney stones. The microbubbles can be equipped with bisphosphonate tags to specifically target kidney stones. These bubbles will preferentially bind to the stone and not surrounding tissue, reducing collateral damage. Ultrasound or another suitable form of energy is then applied causing the microbubbles to induce cavitation and fragment the stones. This can be used as an adjunct to ureteroscopy or percutaneous lithotripsy to aid in fragmentation. Randall's plaques, which also contain hydroxyapatite crystals, can also be targeted to pre-emptively destroy these stone precursors. Additionally, targeted microbubbles can aid in kidney stone diagnostics by virtue of being used as an adjunct to traditional imaging methods, especially useful in high-risk patient populations. This novel application of targeted microbubble technology not only represents the next frontier in minimally invasive stone surgery, but a platform technology for other areas of medicine.

Keywords

targeted, microbubbles, kidney stone, minimally invasive

Introduction

Lifetime incidence of urinary stones is ≈10% for men and 7% for women, which corresponds to a prevalence of ≈2–3% in the general population. In all, 50% of patients with previous urinary stones will experience a recurrence within 5 years [1–3]. Major intra-abdominal surgery was mainstay in the treatment of kidney and ureteric stones before the 1980s, but was fraught with morbidity and mortality, even among experienced urologists [4,5]. However, over the last few decades there has been great expansion in minimally invasive techniques that has led to the dramatic decrease in open stone surgery [4,6]. ESWL was the first major breakthrough in stone surgery, which fragmented stones via acoustical pulses generated by a machine located outside a patient's body [7]. This technology enabled urologists to treat patients with urinary stones without the morbidity and invasiveness of open surgery. As technology and optics improved, other minimally invasive methods for symptomatic urinary stone treatment have been developed including percutaneous nephrolithotomy (PCNL) and ureteroscopy (URS), but their comparative discussion is beyond the scope of this review.

ESWL

ESWL was initially developed in 1980 by Dornier Medizintechnik GmbH (now Dornier MedTech Systems, Germany) and has been widely used since the introduction of the first commercial lithotriptor Dornier Human Model 3 (HM3) in 1983 [8]. It has been used in the non-invasive treatment of many types of stones including kidney, bladder, salivary and biliary, using thousands of focused shockwaves generated outside the body to shatter stones into small fragments. Urinary stones pass spontaneously, but biliary stones typically require secondary procedures for removal. Lithotripters differ from one another in the method

(electromagnetic, electrohydraulic, piezoceramic) used to generate shockwaves, but they all produce similar acoustic waves. Shockwaves are characterised by a rapid high energy peak, which differs from ultrasonic sinusoidal waves by its extremely large pressure amplitude. Additionally, ultrasound usually consists of a periodic oscillation, whereas a shockwave is a single pulse [8]. The focusing mechanism (fluoroscopy and/or ultrasonography) of the lithotriptor directs the shockwaves to a fixed second focal point (F2) target whereby the shockwaves becomes additive at the same location where the patient and their stone/s are positioned for treatment [9].

Mechanism of ESWL Stone Breakage

Cavitation is the primary mechanism by which shockwaves break stones into small pieces [8]. Shockwaves are focused onto a stone and the interaction between the shockwaves and the stone created a negative pressure tail that induces the formation of strongly collapsing cavitation bubbles [10–13]. The bubble nucleus is initially compressed by the shockwaves and then rapidly expands and then collapses (cavitation), which liberates energy resulting in high-speed micro jets with strong erosion abilities to fragment nearby stones [14–16]. Cavitation plays a critical role in the generation of small stone fragments during lithotripsy. The lithotriptor machine is necessary to provide extracorporeal energy that generates shockwaves that are additive upon convergence at the F2 that create cavitation microbubbles [8]. Is it possible to deliver these microbubbles to offending urinary stones without the need for a large, expensive and bulky machine?

The Diminishing Role of ESWL

The original Dornier HM3 and other older general lithotripters had the most optimal coupling and resulted in the most efficient stone fragmentation [17]. Newer generation lithotripters have smaller F2 zones in hopes of reducing pain and potential renal injury; unfortunately, stone fragmentation rates have been significantly compromised [18,19]. Additional factors that influence the efficacy of ESWL include stone composition, skin to stone distance (body mass index), presence of anomalous renal anatomy, stone location, and associated hydronephrosis [4,8]. Typical side-effects include post-procedural gross haematuria, subcapsular haematoma (0.9%), occasional acute kidney injury, and rarely damage to surrounding organs [10,17,18,20,21]. Unconfirmed associations with hypertension and diabetes mellitus have been suggested [22,23].

Other minimally invasive techniques, such as PCNL and URS, have supplemented ESWL in treating kidney and ureteric stones with improved optics, smaller instruments and laser lithotripters that allow for direct visualisation of stone fragmentation [4]. Unlike ESWL, these other endoscopic approaches frequently require postoperative drainage with JJ ureteric stents and/or percutaneous nephrostomy tubes [4]. Could the principles of microbubble cavitation be leveraged during endoscopic approaches, including URS and PCNL, without the need for a large lithotriptor machine to optimise stone fragmentation? If this could be applied, it would provide a logical extension to making the treatment of stones more minimally invasive.

Microbubble Technology

Microbubbles have played a growing and significant role in medical therapeutics and diagnostics as contrast agents for ultrasonographic imaging [24–32]. The first use of this technology was in radiographic imaging to identify cardiac structural anomalies. Carbon dioxide (CO_2) encapsulated microbubbles were first used as contrast agents in the venous circulation to delineate the right heart for evaluation of suspected ventricular septal defects. These microbubbles were comprised of perfluorocarbon gases, and injected into the systemic circulation. Subsequent echocardiography was performed to detect the presence of these microbubbles in the left ventricle, providing an ultrasonographic method for identifying the presence and magnitude of cardiac shunts [33]. Microbubbles have been used as an imaging agent for ultrasound in various other parts of the body with great success. Some have investigated its use in targeted destruction of tissue [34,35] or the restoration of some vital tissue such as myocardium [36]. Recently, targeting ligands have been attached to the surface of the microbubbles, which have been widely used in the cardiovascular system, as well as for tumour diagnosis and therapy [37–39]. Others have combined microbubbles and ultrasound for drug delivery to brain tumours [40–42] and to other immunologically privileged areas. Other emerging applications of this technology include the effective opening of the blood–brain barrier, and for the therapeutic treatment of antimicrobial films [37].

Microbubble Synthesis and Preparation

Various microbubble products are available commercially; including microbubbles marketed under the trade names DEFINITY® (Lantheus Medical Imaging, Inc., N. Billerica, MA, USA) and OPTISON® (General Electric Imaging, Fairfield, CT, USA). The preparation of these Food and Drug Administration (FDA) approved commercially available non-targeted microbubbles is carried out according to already established and approved procedures, with appropriate modifications as necessary [39]. Tagged microbubbles are self-assembled with a phospholipid surface and a perfluoronated carbon gas centre. These microbubbles have an average diameter between 0.1 and 10 μm. The contents of the microbubble can vary with application. For example, the bubble contains air, CO_2, a fluorinated or perfluorinated gas, another gas, or mixtures of various gasses. Moreover, the microbubbles may initially be at a temperature such that a

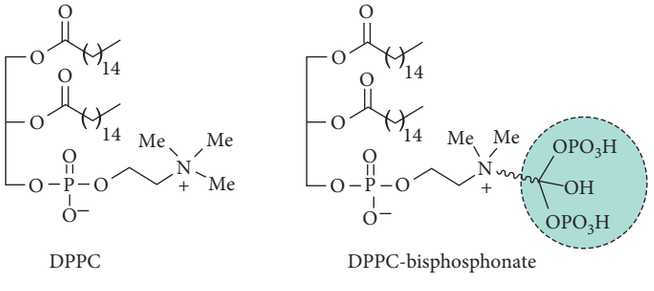
deflated microbubble may be injected into the patient, but will inflate as it heats to physiological temperatures (≈37 °C). These microbubbles can be filled partially or completely with a payload other than a gas, such as a pharmaceutically active agent, a cytotoxic agent, an imaging agent, or the like and delivered to a targeted organ or mass. To target urinary stones these stable, short-lived microbubbles (15–20 min) are synthesised with bisphosphonate surface tags to facilitate selective attachment to hydroxyapatite. After attaching the bisphosphonate chemical tags to the biocompatible microbubbles, the microbubbles are then delivered into a patient.

Our current approach is inspired by a microbubble solution developed by DEFINITY, which is comprised of a mixture of commercially available and FDA approved phospholipids. DEFINITY microbubbles encapsulate perfluoropropane, a gas which has been shown to be exhaled from the lungs with no toxic effects [43]. Specifically, our strategy involves chemical modification of the major phospholipid component present in the DEFINITY mixture, dipalmitoylphosphatidylcholine (DPPC) [44]. Initially, synthetic efforts are directed towards chemical modification of one of the methyl substituents on the amino group of DPPC, as the corresponding bisphosphonate derivatives can be readily accessed from commercially available starting materials using standard transformations (Fig. 1). Furthermore, chemical modification of the amino group in this fashion results in a minimal structural change to DPPC. It is reasonable to expect that these new bisphosphonate analogues will result in similar physical properties, such as solubility as well as improved stability *in vivo* when incorporated in microbubble solutions, and will retain the biocompatibility exhibited by DPPC.

Microbubbles and Diagnostics

Targeted microbubbles can be used in the diagnosis of kidney stones. Targeted microbubbles as contrast materials require a small dosage and show excellent detection sensitivity [27–29]. CT is the 'gold standard' in radiographic diagnosis of kidney stones providing the highest sensitivity, but some stones (i.e. drug stones) are invisible even on CT [6]. Targeted microbubbles can bind to specific drug targets, revealing them on radiography. Plain X-ray is poor at visualising radiolucent stones (i.e. uric acid, cystine), but these stones can be specifically targeted to allow detection using simple plain radiographs. Stones in the parenchyma of the kidney can be differentiated from ones in the collecting system, thereby proving a more accurate measurement of stone burden. Traditionally MRI is poor at visualising stones [4], but microbubbles can be equipped with MRI-detectible ligands that have an affinity for kidney stones, thereby aiding in MRI detection. This may have a value in high-risk patient populations, such as pregnant women or children. Additionally, specific ligands (i.e. sulfhydryl groups) can be used to tag the microbubbles to detect specific stone types, providing a unique, non-invasive method in the diagnosis of kidney stones.

Fig. 1 Structures of phospholipid-based microbubble forming compounds.



DPPC DPPC-bisphosphonate

Targeted Microbubbles and Urological Applications

Lipid-coated microbubbles can be labelled to target specific tissue [27,36,45–47]. Microbubbles can be generated *ex vivo* with a functional group that is able to specifically target a particular substance or tissue. These microbubbles would subsequently bind selectively at the target site (i.e. kidney stone). The microbubbles would be induced to cavitate through the use of variety of energy sources. The rapid collapse of these microbubbles would release energy only at the site of interest. This minimally invasive technology has the potential to replicate the microbubbles generated *in vivo* from ESWL that can cavitate and fracture stones. The key is labelling the microbubbles to bind only onto the specific surface of the stones to minimise or eliminate complications and increase efficiency. How does one specifically target the urinary stones using microbubbles to direct their cavitation energy only to the stone? We explore observations that answer these questions.

Development of Kidney Stone Targeting

Based on X-ray diffraction, infrared spectroscopy, and chemical analysis hydroxyapatite is regarded as the principal inorganic constituent of bone mineral, built of crystals containing mainly calcium and phosphate [48–50]. Bisphosphonates are compounds that are used to treat or slow the progress of osteoporosis and bone-related events, by inhibiting osteoclastic bone resorption by attaching to hydroxyapatite binding sites on bony surfaces. They have a high affinity for calcium phosphate (hydroxyapatite or apatite) surfaces in the inorganic matrix of human bone where they preferentially attach [51–53]. Bone scanning is routinely performed with ^{99m}Tc-labelled diphosphonates that are similar to the bisphosphonates used for therapeutic applications. The principle uptake mechanism involves

adsorption onto or into the crystalline structure of hydroxyapatite after i.v. administration [54]. Quantitative bone scintigraphy using a γ-camera allows for kinetic modelling to evaluate aspects of bone perfusion and metabolism, including conditions with diffuse alteration to bone remodelling (such as primary hyperparathyroidism, renal osteodystrophy, and osteoporosis), and for assessment of bone perfusion, regional metastasis, bone (graft) vitality and osteonecrosis [55–57]. Can this same affinity of bisphosphonates to hydroxyapatite be exploited in urinary stone disease?

Most urinary stones are calcium based, and a significant portion is composed of hydroxyapatite. Many think that most biomineralisation starts with hydroxyapatite crystals. Additionally, these stones contain a number of cavities irregularly distributed throughout the entire interior that entomb small spheres of hydroxyapatite in the lattices of crystal sheets [58–61]. Theoretically with microbubbles tagged with bisphosphonates, the urinary stones can be specifically targeted; and can be used as an alternative minimally invasive treatment for stone fragmentation. A microbubble can have a specific targeting moiety (such as a bisphosphonate ligand) created *ex vivo*, that will have an affinity for hydroxyapatite in urinary stones after being injected into the urinary system.

Randall's plaque is thought to be the initial nidus for many stones. Dr Alexander Randall [62] hypothesised that these papillary interstitial plaques were composed of calcium phosphate (hydroxyapatite), not calcium oxalate, and served as a nidus for subsequent stone formation. By injecting microbubbles that preferentially bind to the hydroxyapatite of these papillary plaques, one could theoretically cavitate and destroy them in hopes of reducing the nidi for future stone formation.

Other Urological Applications

An investigational technology called histotripsy is a novel technique that uses pulsed ultrasound that causes rapid cycles of compression and expansion, which in turn form microbubbles that have been used to fragment and homogenise unwanted tissue. It has been developed by a University of Michigan research team as a potential treatment for benign prostatic hyperplasia with good results in animal models. Human studies are pending [63–65]. Histotripsy shows the versatility and power of microbubbles technology, but specific tissue targeting is performed by an external machine, but the individual microbubbles are not target specific.

Delivery of Microbubbles

This microbubble technology can be quickly prepared in the outpatient or the inpatient setting. These microbubbles can be injected into the urinary system and last about 15–20 min before spontaneous dissolution. These targeted bisphosphonate laden microbubbles can concentrate and attach to the surfaces and inner crevices of urinary stones. Any excess bubbles not attached to the desired target can be washed away using a combination of a diuretic and/or fluid irrigation. This is important because excess bubbles can shield any applied energy source, interfering with the effect of the locally bound microbubbles. The passage of excess bubbles will allow for selectivity of the targeted stone and avoid collateral injury.

Targeting the Microbubbles

Prior medical applications of cavitation have used extracorporeal energy sources to create and collapse microbubbles in the tissue [32,63,66–68]. This new technology differs from such procedures by using application-specific, gas-containing microbubbles that are manufactured *ex vivo*. The manufactured microbubbles contain targeting tags (e.g. bisphosphonates) that allow them to concentrate on or near the targeted tissue (e.g. urinary stones). They are then specifically delivered to the surface or vicinity of the desired target.

Energy Sources for Cavitation

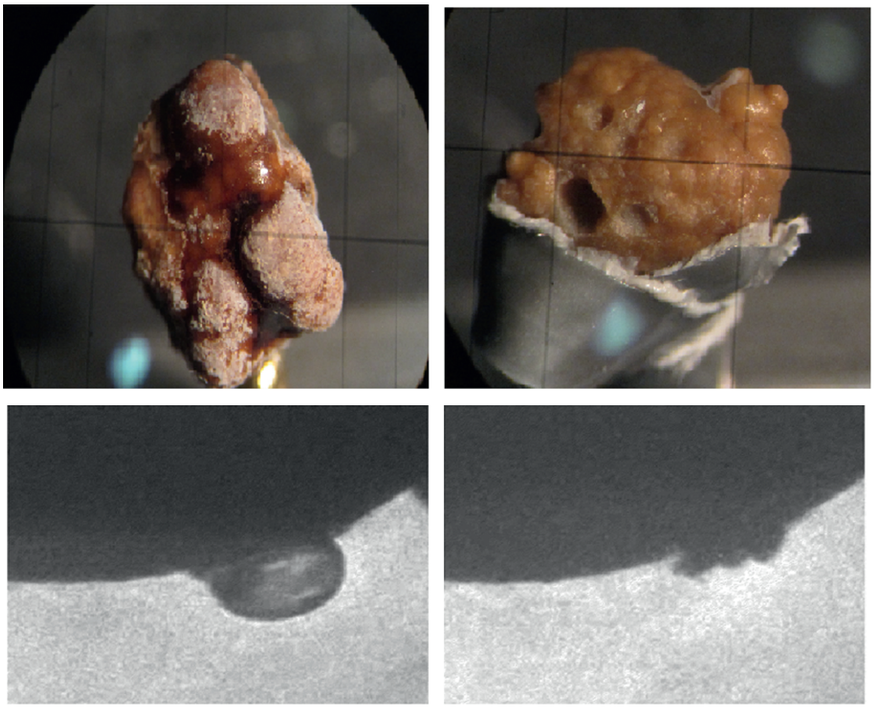
Energy required to cause cavitation can be delivered in the form of electromagnetic radiation (e.g. radio or microwaves), or ultrasound waves. Due to its low electrical conductivity, electromagnetic frequencies between 400 and 10 000 kHz may be suitable because it propagates through tissue without strong interactions, while focusing on the intended target [69]. For example, standard ultrasound units are applied within or adjacent to the body with sufficient power to initiate cavitation of the pre-positioned bubbles.

Microbubbles for the Treatment of Kidney Stone Disease

Preferential Targeting of Kidney Stones

The bisphosphonate tags on the microbubbles, as previously described, have an affinity for the hydroxyapatite present in most urinary stones such that microbubbles bind to the target and not to surrounding fluid or tissue. Energy from a nearby source (ultrasound, radio frequency energy, or the like) is then applied to induce cavitation. The engineered microbubbles act as a cavitation nucleus upon interaction with the delivered energy and can fragment the targeted stone (Fig. 2 and accompanying Videos S1 and S2). Theoretically, when treating a patient with kidney or ureteric stones, the urologist can deliver these tagged microbubbles to a site within the patient (ureter or kidney) using routine endoscopes.

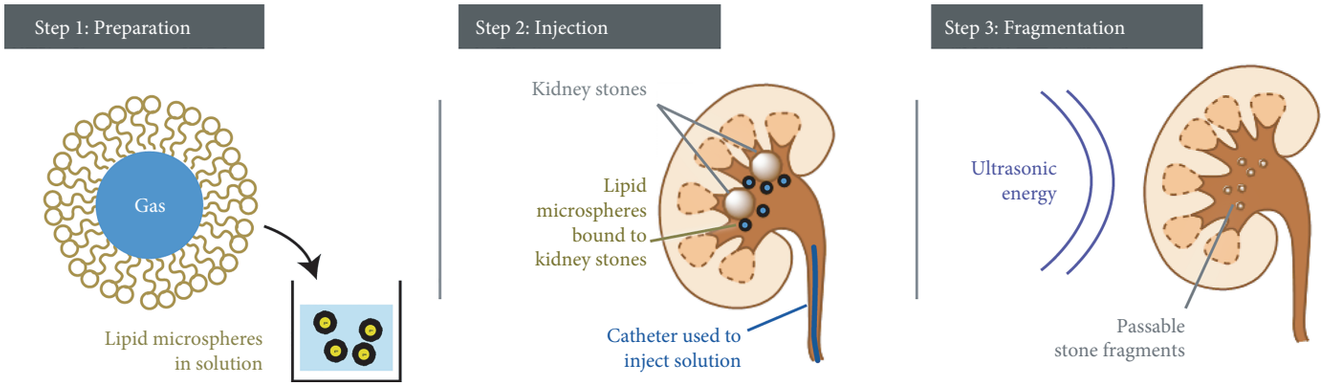
Fig. 2 *Ex vivo* cavitation (top images) of stone. The same cavitation captured with rapid-shutter speed 1/1 000 000 second camera (below images). The microbubble coated calcium-based urinary stones show excellent fragmentation.



Delivery of Microbubbles to Kidney Stones

Delivery of the microbubbles into or near the targeted urinary stone can be achieved by various means. With ureteric stones, these microbubbles can be injected directly into the ureteric orifice using a flexible scope or even onto the stone using a small catheter placed up to the stone. If the stone is in the kidney, one can inject the microbubbles in a retrograde or an anterograde percutaneous fashion depending upon patient anatomy and location of stone fragments (Fig. 3). Randall's plaques, which are precursors to calcium-based urinary stones, can also be targeted pre-emptively at

Fig. 3 Synthesise microbubbles and inject into collecting system. External energy source used to cavitate attached microbubbles and break stones into small pieces.



the time of PCNL or URS. Many urologists have a sense of foreboding after endoscopic lithotripsy knowing these plaques will probably become recurrent stones – it is just a matter of time [62,70]. Microbubbles theoretically can be used to target these plaques at the time of URS or PCNL to pre-emptively destroy them; therefore, potentially reducing stone recurrence. Additionally, this technology can be used as an adjunct to URS or PCNL, where stones initially can be fragmented by traditional means, and microbubbles can subsequently be deployed to complete the conversion of these stone remnants into dust. This would be a manner in which to attempt to recreate the 'popcorn effect', where small fragments are obliterated into dust or gravel that would pass spontaneously.

Energy Source for Cavitation of Kidney Stones

Energy needed to initiate cavitation can be delivered *ex vivo* as in traditional lithotripters. Alternatively a micro-energy source can be applied from the tip of a catheter or endoscope, which can be directed under fluoroscopic guidance or direct vision. This would enable the urologist to observe the resultant fragmentation in real-time. These catheters are readily available and widely used in other minimally invasive medical fields [71,72].

Platform Technology

Application of this targeted microbubble technology can be broadened outside of urological indications. Depending on specific needs, various formulations and preparations may be constructed to unique targets using surfactants or other additives for dispersal [73]. Delivery of specifically tagged microbubbles can be delivered through natural orifices such as the mouth, nose, eyes, vagina, urethra, and ears. It can also be delivered by s.c. injection and/or spray [74].

Conclusions

The novel application of targeted microbubble technology represents the next frontier in minimally invasive stone surgery, and our team envisions this as a platform technology in medicine. Traditional ESWL uses an extracorporeal energy source that creates microbubbles at the targeted stone, and subsequent cavitation leads to stone fragmentation. Targeted, tagged microbubbles eliminate the need for a large, bulky machine, and these unique microbubbles can be delivered directly to the offending stones. An energy source applied from either an extracorporeal or intracorporeal source can initiate the cavitation process, leading to stone fragmentation. This is the obvious extension of minimally invasive stone treatment. We envision the principles of this technology to be applied to other commonly appreciated pathological conditions in medicine.

Conflicts of Interest

D.L. reports personal fees from Applaud Medical, Inc. outside the submitted work. M.L.S. reports having a patent Provisional Patent pending. R.H.G. reports having a patent Provisional Patent pending. All other authors have nothing to disclose.

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Abbreviations: DPPC, dipalmitoylphosphatidylcholine; FDA, USA Food and Drug Administration; HM3, Human Model 3; PCNL, percutaneous nephrolithotomy; URS, ureteroscopy.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Videos S1 and S2. Real-time cavitation of tagged microbubbles as captured by an ultra-fast shutter camera.