Published in final edited form as:

Adv Healthc Mater. 2017 March ; 6(5): . doi:10.1002/adhm.201601163.

Ultrasound Detection of Regional Oxidative Stress in Deep Tissues Using Novel Enzyme Loaded Nanoparticles

Emilia S Olson^{1,*}, Inanc Ortac^{2,*}, Christopher Malone¹, Sadik Esener³, and Robert Mattrey^{1,**}

¹Department of Radiology, University of California, San Diego, 410 Dickinson St., San Diego, CA 92103

²Devacell, Inc., 6650 Lusk Blvd. Suite B105, San Diego, CA 92121

³Department of Nanoengineering, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093

Abstract

Oxidative stress is a powerful tool that is critical to immune mediated responses in healthy individuals, yet additionally plays a crucial role in development of cancer, inflammatory pathologies and tissue ischemia. Despite this, there remain relatively few molecular tools to study oxidative stress, particularly in living mammals. To develop an intravenously injectable probe capable of labeling sites of oxidative stress in vivo, we designed and fabricated 200 nm CATalase Synthetic Hollow Enzyme Loaded nanoSpheres (catSHELs) using a versatile enzyme nanoencapsulation method. CatSHELs catalyze H₂O₂ to water and oxygen producing microbubbles that can be detected and imaged using a clinical ultrasound system. CatSHELs were optimized in vitro to maximize ultrasound signal and their functionality was demonstrated in rat ischemic renal injury (IRI) model. Ischemic oxidative injury was induced in a single kidney of normal rats by clamping the renal artery for 1 hour followed by two hours of reperfusion. Imaging of both kidneys was performed following the intravenous bolus injection of 10¹² catSHELs of the optimized formulation. There was significant increase in ultrasound signal of the injured kidney relative to controls. Our method offers a novel intravenous approach to detect oxidative stress in deep tissues in living animals.

Graphical Abstract

^{**}Corresponding author: rmattrey@ucsd.edu.

^{*}These authors contributed equally to this work

E.S.O., C.M. and R.M. have nothing to disclose. I.O. and S.E. are founding members of Devacell Inc, a start-up based in part on SHELs technology. I.O. is an employee of DevaCell Inc.

Supporting Information Available: Video clips of AKI kidneys after injection with catSHELs and control SHELs. This material is available free of charge via the Internet at <http://pubs.acs.org>.

200 nm CATalase Synthetic Hollow Enzyme Loaded nanoSpheres (catSHELs) are intravenously injectable probes capable of labeling sites of oxidative stress in vivo. CatSHELs catalyze H_2O_2 to water and oxygen producing microbubbles that can be detected and imaged using a clinical ultrasound system. CatSHELs were optimized in vitro to maximize ultrasound signal and their functionality was demonstrated in rat ischemic renal injury (IRI) model in vivo.

Keywords

nanotechnology; nanoparticles; acute kidney injury; ischemia reperfusion injury; acute kidney injury

Introduction

Oxygen (O_2) free radical formation is an inevitable byproduct of O_2 metabolism. Oxidative stress occurs when the cellular defense mechanisms are unable to cope with the rate of O_2 free radical production resulting in elevated reactive oxygen species (ROS) levels, predominantly hydrogen peroxide (H_2O_2). Oxidative stress and its resultant bioeffects such as inflammation and DNA damage, are blamed for initiating many diseases including cancer¹, neurodegeneration, progression from hepatic steatosis to steatohepatitis^{2,3}, and is the hallmark of ischemic reperfusion injury (IRI)^{4,5}. Tissue ischemia and associated reperfusion injury adds billions of dollars in healthcare costs every year worldwide, manifesting in stroke, myocardial infarction, acute kidney injury (AKI) and vascular events⁶. AKI alone affects over 50% of patients admitted to intensive care units increasing mortality, and is the leading cause of chronic loss of renal function⁷. Elevation in ROS and superoxide radicals in IRI has been well established. H_2O_2 , the most common ROS, can increase by as much as five-fold in tissues injured by an ischemic event⁸. Although many attempts have been made at detecting H_2O_2 in vivo^{9–14}, translation of these methods remains elusive. Here, we introduce a novel translatable silica nanoshell as an ultrasound contrast agent to detect elevated levels of H_2O_2 in tissues. We present the in vitro optimization results of nanoshell properties to maximize the opportunity for the detection of pathophysiological levels of H_2O_2 in vivo. We then present the results of an in vivo study using a rat model of IRI of the kidney that results in AKI, which is characterized by elevated H_2O_2 levels^{15,16}.

Ultrasound is a clinical imaging modality that is widely available, portable, and exquisitely sensitive to microbubbles in vivo¹⁷. We designed a novel PEGylated 200 nm Synthetic Hollow Enzyme Loaded nanoSphere (SHELs) that is porous to water and small molecules, such as H_2O_2 , with a core filled with catalase (catSHELs). These nanoshells were recently

developed to trap enzymes used for enzymatic therapies to address the two major limitations of systemically administered free enzymes, short-lived plasma activity and induction of allergic reactions¹⁸. The nanoporous silica shell not only traps the enzyme in the core and allows it to interact with its small molecular weight substrate, but also prevents its interaction with immune cells, antibodies and plasma proteases that promote immune reactions, rapid clearance and neutralization. Briefly, hollow mesoporous shells are made of nanoporous silica grown around degradable uniform size templates as previously described¹⁸ (Fig. 1A).

The resulting hollow spheres are then suspended in a solution of the enzyme of interest that slowly fills their core through the large mesopores (Fig. 1B). The mesopores are subsequently sealed by nanoporous silica, trapping the enzyme within while allowing free diffusion of substrate (Fig. 1C). Using this novel packaging technique, we encapsulated catalase, a well-studied bioprotective enzyme that converts H_2O_2 into harmless water and O_2 (Fig. 2A). We hypothesized that this design, which allows the interaction of high local catalase concentration within the nanoparticle with H_2O_2 in tissues would release O_2 to saturate the surrounding fluid that is then nucleated to form microbubbles detectable by ultrasound (Fig. 2B).

Results

CatSHELS can be optimized for in vivo use by adjusting particle size, catalase concentration, surface chemistry, and particle concentration

In preparation for in vivo work, a series of in vitro experiments were performed in buffer to determine the optimal formulation. We evaluated the relationship of nanoparticle size, interior catalase concentration and catSHELS concentration on ultrasound signal generation. We observed a linear relationship between the amount of encapsulated enzyme calculated based on the measured activity using Amplex Red assay and the initial loading concentration of the enzyme in a particle concentration of 4×10^{12} pts/ml (Suppl. Fig 1A). Moreover, the catSHELS activity follows almost identical kinetics with free catalase, which supports our non-destructive encapsulation approach (Suppl. Fig 1B).

Ultrasound signal when using microbubble only imaging techniques is linear with change in microbubble concentration. Increasing the number of catSHELS while holding the catalase concentration and particle size constant resulted in higher ultrasound signal (Fig. 3A). Similarly, increasing the catSHELS particle size, while catalase and particle concentration remained constant, also increased signal (Fig. 3B). Although increasing the catalase concentration from 10mg/mL to 80 mg/mL increased signal, a further increase to 320 mg/mL did not have additional effect, likely due to consumption of H_2O_2 by catalase near the nanoshell's interior surface (Fig. 3C). Finally, in preparation for the in vivo experiment, we showed that attaching 10 kD PEG to the particle surface to prevent aggregation and increase intravascular dwell time, did not adversely affect signal generation (Fig. 3D).

Acute kidney injury causes hydrogen peroxide production in a rat model

To validate that catSHELs can allow ultrasound imaging to detect pathophysiologic levels of H_2O_2 in vivo was assessed using a rat AKI model known to increased H_2O_2 levels^{19,20}. Through a midline incision, the left renal artery of anesthetized rats was isolated and clamped for one hour followed by two hours of reperfusion (Fig. 4A).

Total occlusion and successful reperfusion were confirmed visually by assessing kidney color. The bladder was catheterized and urine samples collected from prior to renal artery occlusion to just post mortem to measure urine H_2O_2 levels. In a pilot study to confirm AKI, histologic analysis of the injured kidneys was performed at 24 hours after reperfusion that showed characteristic findings including pyknosis, eosinophilia, ghosting, flattening of the tubular cells and sloughing of the epithelium (Fig. 4B), which reverted to normal histology by three days post injury. Despite urine contribution from the normal contralateral kidney, H_2O_2 levels in the bladder measured by Amplex red assay, increased from $0 \pm 8 \mu\text{M}$ (range 0–13 μM) at baseline to $35 \pm 14 \mu\text{M}$, (range 15–50 μM) at 2 hours post reperfusion ($n=10$, $p<0.0001$) (Fig. 4C).

Intravenously injected catSHELs caused formation of microbubbles in the ischemic kidney

Two identical formulations of 200 nm catSHELs were used, one was filled with 80 mg/ml catalase, and the other was filled with fluid without catalase. Ten animals were subjected to AKI as described above, and catSHELs or control particles were injected intravenously at 2 hours following reperfusion, and both kidneys imaged by ultrasound alternately for 10–15 minutes. At real-time imaging, ultrasound signal increase was seen in only the injured kidneys and only after the injection of catSHELs resulting in echogenic enhancement (Fig. 5A). The degree of enhancement is best appreciated on real-time cine clips (Supplemental Video. 1).

Signal from the ischemic kidney differed from the contralateral kidney by up to 3-fold

Quantitative analysis of kidney brightness was calculated by subtracting the average kidney signal on multiple frames acquired at baseline from the average of multiple frames acquired at peak enhancement following nanoparticle administration. Signal of the injured kidney significantly increased compared to its normal contralateral kidney following the injection of catSHELs (10.1 ± 2.2 vs 0.6 ± 2.5 , $n=5$, $p=0.0005$) (Fig 5A), but not following the injection of control non-catalase-containing nanoshells (3.5 ± 2.5 vs 1.6 ± 2.5 , $n=5$, $p=0.3$) (Fig. 5B). Signal of the injured kidneys also increased following catSHELs (10.1 ± 2.2) relative injured kidneys following control nanoshells (3.5 ± 2.5 , $p=0.004$). We found that microbubble formation induced kidney enhancement starting within the first five minutes after catSHELs administration and continued for at least 10 minutes. Since the PEGylated 200nm catSHELs are expected to remain within the vascular space during the 10-minute observation period^{18,21}, cessation of microbubble formation is likely because the H_2O_2 that accumulated in the injured kidney was catalyzed to below detectable levels.

Discussion

CatSHELs are a novel intravenous molecular imaging agents for detecting H_2O_2 in vivo. In this study, we optimized particle size, concentration, and catalase content in vitro in order to detect microbubbles at near physiologic H_2O_2 levels to maximize the opportunity of detecting H_2O_2 in vivo. Note that, while increasing particle size and concentration increased ultrasound signal, increasing catalase content had a lesser effect. This is likely because increasing particle size or concentration increases their nucleation potential of the O_2 saturated suspension when exposed to ultrasound, and, 10mg/mL of catalase was sufficient to catalyze the available H_2O_2 , and the available particles were also sufficient to nucleate O_2 microbubbles in vitro. That the availability of nucleation sites is important for O_2 microbubble formation was demonstrated in our prior study using a non-translatable 500nm solid particle, where 80 times more free catalase was required to produce ultrasound detectable microbubbles at the same H_2O_2 concentration¹¹.

The contrast mechanism of catSHELs to produce a detectable ultrasound signal is markedly different from traditional contrast agents. Traditional agents carry the reporter and enhance tissues based on their concentration in the region of interest. With our approach, ultrasound is not detecting the nanoshells, rather, it is detecting the microbubbles generated when the catalase-containing nanoshells become exposed to a sufficient amount of H_2O_2 in vivo. In fact, during the first hour after injection in this study, the nanoshells are likely still in blood given that their vascular half-life is several hours¹⁸, and the normal kidney that should contain an equal amount of catSHELs as the injured kidney produced no signal.

We believe that catalase causes the release of O_2 , which saturates the fluid around the nanoshells. When exposed to ultrasound, O_2 microbubbles nucleate out of solution and are detected by ultrasound. Therefore, signal is related to the catalase activity provided by the nanoshells to generate O_2 faster than it is lost to saturate the fluid and generate microbubbles. Note that since we observed signal in vivo in the injured kidneys, the nanoshell properties optimized in vitro were sufficient to generate microbubbles from the H_2O_2 pool that accumulated during the reperfusion period. That signal was due to elevation of H_2O_2 levels in the injured kidney, was confirmed indirectly by detecting a large increase in urine H_2O_2 concentration. Since IRI was only induced in one kidney in our model, and IRI causes marked elevation in H_2O_2 ^{15,16}, the injured kidney was the only source of H_2O_2 and the only tissue that generated signal. Although urine output from the injured kidney is low shortly after reperfusion, it is likely that a small amount of H_2O_2 entered the circulation to be filtered by the normal kidney and accumulate in the bladder during the reperfusion period. Note that because there was no signal detected in the normal kidney, the level of H_2O_2 in circulation must have been below the detection limits of our current technique.

We chose AKI as proof of concept for in vivo detection of H_2O_2 instead of the more commonly used LPS peritonitis model^{10,12,14} because the kidney size in rats is sufficiently large to image with standard clinical equipment, oxidative stress is the hallmark of IRI^{19,20}, and IRI is the cause of AKI that is a real clinical entity. AKI is a very important disease that affects over 57% of patients admitted to intensive care units increasing mortality by 7 fold, and causing permanent loss of renal function in over 45% of those that survive⁷. In this

study, we clearly showed that the administration of catSHELs increased ultrasound signal of the injured but not normal kidneys and the signal increase is indicative of elevated H_2O_2 levels within the kidney with oxidative stress. The detection of H_2O_2 at 2 hours post IRI is important, since the most reliable diagnostic test of AKI – low urine output and elevated plasma levels of blood urea nitrogen and creatinine²², does not become abnormal until at least 24 hours after injury, when the injury is less reversible²³. Therefore, our method has the potential to serve as an early in vivo biomarker of AKI.

Signal of the injured kidney was no longer detected 15 to 20 minutes after catSHELs administration. This suggests that should H_2O_2 production continues after the initial H_2O_2 pool is catalyzed, the amount produced is below detection level. We expect that any amount produced is immediately catalyzed by available catSHELs, but O_2 produced cannot overcome O_2 losses to saturate the fluid. Although the measured overall renal enhancement caused by catSHELs was modest in this preliminary study, the characteristic flickering of microbubbles on real time ultrasound imaging allowed their detection in only injured kidneys (Supplemental Video 1). We expect further improvement in H_2O_2 detection following formulation and ultrasound imaging technique optimizations in vivo that are beyond the scope of this feasibility study.

Most molecular imaging advances have favored MRI and more so PET that have limited patient access and higher cost particularly in an inpatient setting. Given that ultrasound is frequently used in intensive care units, an ultrasound detectable molecular agent such as catSHELs, would not only be less costly, but also more practical in these critically ill patients that are attached to life saving monitors and support. Given the elevation of H_2O_2 in tissues with oxidative stress and the important role H_2O_2 plays in the pathogenesis of disease, future experiments will explore the association of elevated H_2O_2 with other diseases and the potential of using H_2O_2 elevation as a biomarker for early disease detection.

Methods

Preparation of Synthetic Hollow Mesoporous Nanoshells (SHMS)

The manufacturing details are available elsewhere⁸. Briefly, a 50 μl template particle solution was mixed with the corresponding amount of masking particle solution to prepare the desired ratio of particle concentrations. In order to generate the silica precursor and initiate the silica growth, 1 μl of tetramethoxysilane (TMOS) was added. The mixture was shaken overnight, and the suspended particles were collected by centrifugation (5 min at 14000 rpm), washed with deionized water and dried in vacuum overnight. To remove the organic compounds, a coverslide carrying the nanoparticle powder was placed over a hot plate and calcined overnight at 450°C. The calcined powder was transferred to a tube, suspended in 50 μl water and dispersed by gentle sonication.

Preparation of Synthetic Hollow Enzyme Loaded Nanoshells (SHELs)

SHMS was suspended in either PBS to produce the control SHELs or 50 μl of 80 mg/ml catalase solution in 1X PBS to produce catSHELs and incubated overnight. The solution was diluted with 1000 μl phosphate buffered saline and 50 μl 0.1% poly-L-lysine with a

molecular weight of 150–300 kDa. TMOS was added to 1 mM HCl in 74:500 volume ratio and mixed for a few minutes to make a silicic acid solution. 25 μ L of the silicic acid solution was added to the above SHMS solution immediately after dilution and shaken for 1 hour in order to close the pores and generate SHELS or catSHELS. The suspended hollow silica nanospheres were collected with centrifugation (5 min 14000 rpm) and washed several times with water. With this technique, some of the enzymatic load might end up exposed on the surface of the nanoparticles. To prevent this, any exposed catalase on the particle is removed by proteinase-K, a serine protease commonly used for general digestion of protein in biological samples. Samples were exposed to proteinase-K enzyme overnight at a concentration of 0.1 mg/ml in 1X phosphate buffered saline (PBS) solution at 37°C followed by removal of proteinase-K by successive washing again by 1X PBS by centrifugation (5 min 14000 rpm).

Characterization of SHELS Enzyme Encapsulation

Enzymatic activity of catSHELS after encapsulation quantified with Amplex Red enzyme assay (Molecular Probes, Eugene, OR). The assayed concentration of enzyme within a single SHELS determined to be ~78 mg/ml resulting in 97–100% match with the exterior loading concentration. This result also shows that there is no measurable loss of activity of enzyme during loading and sealing process or diffusion of substrate through the nanoporous shell in this interior concentration of enzyme.

Ultrasound phantoms and in vitro testing

Our method for testing and analyzing data from ultrasound based phantoms has been described elsewhere¹¹. Briefly, SHELS and catSHELS were concentrated to a stock solution of $1.82 \times 10^8/\mu\text{L}$ (500nm), $2.8 \times 10^9/\mu\text{L}$ (200nm), $2.8 \times 10^{10}/\mu\text{L}$ (100nm) and diluted in 1 $\times$ PBS prior to testing. Nanoparticles were placed into a transfer pipette modified to contain a port that could be pinned to the back of a water bath. 3mL phosphate buffered saline (PBS, Gibco) +0.04M sodium hydrate cholate (NaCH, Sigma) was added to the nanoparticles through the port. Samples were allowed to sit for approximately five minutes. The concentration of hydrogen peroxide was increased by factors of ten (eg. 8 μ M, 80 μ M, 800 μ M...) delivered in low volume to the top of the tube (3 μ L or 30 μ L) under real-time ultrasound observation operating in contrast imaging mode (GE LogiqE9, 6–15MHz linear transducer, MI<.20, 14 frames per second), catSHELS were tested side by side with SHELS of the same geometry but without catalase. All experiments were performed in triplicate.

Analysis

Regions were drawn near the bottom of the modified transfer pipette with care taken to avoid artifact associated with transducer motion. Each region was averaged over three ultrasound frames to obtain a “pre” value and over 20 consecutive frames to obtain a “post” value. The “pre” value was then subtracted from the “post” value to obtain the change in video signal due to addition of hydrogen peroxide. For animal experiments, regions of interest over the entire kidney were averaged over multiple frames pre and post injection of catSHELS. Statistical significance was assessed by an unpaired 2-tailed Student’s t-test.

Renal artery ischemia reperfusion injury (IRI) model

All animal experiments were overseen and approved by the Institutional Animal Care and Use Committee. Six week old, female Sprague-dawley rats (average weight=126.75g) were shaved, anesthetized with a ketamine/acepromazine cocktail injected IP and their urethra catheterized using the sheath of a 20G angiocatheter. Preoperative urine samples were taken. A midline incision was made from the xyphoid process to the level of the iliac crest after local injection of carbocaine (100uL) and SQ injection of carprofen. The intestines were exteriorized to expose the kidneys and kept warm and moist using sterile saline soaked gauze. The left renal artery was exposed, bluntly dissected from the renal vein and occluded using a vascular clamp (Fine Science Tools, cat. # 00400-03). Successful occlusion was confirmed by renal blanching. One hour later, urine samples were collected and the renal artery clamp removed. Complete reperfusion of the left kidney was confirmed by normalization of kidney color. The abdomen was then closed and the animals recovered.

Imaging experiments

1–2 hours after reperfusion, animal imaging was performed using a clinical ultrasound scanner (Siemens Sequoia 512, Mountain View, CA) equipped with the 15L8 transducer and a proprietary Contrast Pulse Sequence (CPS) algorithm for microbubble imaging transmitting at 7MHz. Concurrent images were taken in the CPS mode (tissue signal subtracted) and the standard B-mode (tissue signal not subtracted) for orientation.

Renal perfusion of the injured kidney was confirmed by detecting intra-renal blood flow on color Doppler imaging. 10^{12} catSHELS or control SHELS suspended in 1 ml were injected through the tail vein. 8–10 minutes post injection, real-time imaging was performed of the injured and normal kidneys. H_2O_2 concentration was measured in frozen urine samples using the Amplex Red assay performed according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Kidneys were fixed in formalin and submitted to the histopathology core for hematoxylin-eosin staining.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Drs. Olson and Malone were funded by a T32 grant (T32EB005970). Dr. Malone is the recipient of a Radiological Society of North America (RSNA) Resident Research Grant, RR1361. Nanoparticles were produced and supplied using DevaCell funds. The authors would like to acknowledge excellent technical assistance from Jackie Corbeil and Chris Barback. The authors are grateful to Ibrahim G. Yayla for his insightful comments.

References

1. Jezierska-Drutel A, Rosenzweig SA, Neumann CA. Role of oxidative stress and the microenvironment in breast cancer development and progression. *Adv Cancer Res.* 2013; 119:107–125. [PubMed: 23870510]
2. Domínguez-Pérez M, et al. Hepatocyte Growth Factor Reduces Free Cholesterol-Mediated Lipotoxicity in Primary Hepatocytes by Countering Oxidative Stress. *Oxid Med Cell Longev.* 2016; 2016:7960386. [PubMed: 27143995]

3. Satapati S, et al. Mitochondrial metabolism mediates oxidative stress and inflammation in fatty liver. *J Clin Invest.* 2015; 125:4447–4462. [PubMed: 26571396]
4. Li C, Jackson RM. Reactive species mechanisms of cellular hypoxia-reoxygenation injury. *Am J Physiol Cell Physiol.* 2002; 282:C227–241. [PubMed: 11788333]
5. Yu W, et al. H₂O₂ activates G protein, α 12 to disrupt the junctional complex and enhance ischemia reperfusion injury. *Proc Natl Acad Sci.* 2012; 109:6680–6685. [PubMed: 22493269]
6. Druss BG, Marcus SC, Olsson M, Pincus HA. The Most Expensive Medical Conditions In America. *Health Aff (Millwood).* 2002; 21:105–111. [PubMed: 12117121]
7. Hoste EAJ, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med.* 2015; 41:1411–1423. [PubMed: 26162677]
8. González-Flecha B, Cutrin JC, Boveris A. Time course and mechanism of oxidative stress and tissue damage in rat liver subjected to in vivo ischemia-reperfusion. *J Clin Invest.* 1993; 91:456–464. [PubMed: 8432855]
9. Guo J, et al. Nanoporous Metal–Phenolic Particles as Ultrasound Imaging Probes for Hydrogen Peroxide. *Adv Healthc Mater.* 2015; 4:2170–2175.
10. Lee D, et al. In vivo imaging of hydrogen peroxide with chemiluminescent nanoparticles. *Nat Mater.* 2007; 6:765–769. [PubMed: 17704780]
11. Olson ES, et al. Toward in vivo detection of hydrogen peroxide with ultrasound molecular imaging. *Biomaterials.* 2013; 34:8918–8924. [PubMed: 23958028]
12. Perng JK, et al. Ultrasound imaging of oxidative stress in vivo with chemically-generated gas microbubbles. *Ann Biomed Eng.* 2012; 40:2059–2068. [PubMed: 22562306]
13. Weinstain R, Savariar EN, Felsen CN, Tsien RY. In Vivo Targeting of Hydrogen Peroxide by Activatable Cell-Penetrating Peptides. *J Am Chem Soc.* 2014; 136:874–877. [PubMed: 24377760]
14. Yang F, et al. A Hydrogen Peroxide-Responsive O₂ Nanogenerator for Ultrasound and Magnetic-Resonance Dual Modality Imaging. *Adv Mater.* 2012; 24:5205–5211. [PubMed: 22811026]
15. Paller MS, Hoidal JR, Ferris TF. Oxygen free radicals in ischemic acute renal failure in the rat. *J Clin Invest.* 1984; 74:1156–1164. [PubMed: 6434591]
16. Chatauret N, Badet L, Barrou B, Hauet T. Ischemia-reperfusion: From cell biology to acute kidney injury. *Progres En Urol J Assoc Francaise Urol Soc Francaise Urol.* 2014; 24(Suppl 1):S4–12.
17. Cui W, et al. Neural progenitor cells labeling with microbubble contrast agent for ultrasound imaging in vivo. *Biomaterials.* 2013; 34:4926–4935. [PubMed: 23578557]
18. Ortac I, et al. Dual-Porosity Hollow Nanoparticles for the Immunoprotection and Delivery of Nonhuman Enzymes. *Nano Lett.* 2014; 14:3023–3032. [PubMed: 24471767]
19. Munshi R, Hsu C, Himmelfarb J. Advances in understanding ischemic acute kidney injury. *BMC Med.* 2011; 9:11. [PubMed: 21288330]
20. Zweier JL, Talukder MAH. The role of oxidants and free radicals in reperfusion injury. *Cardiovasc Res.* 2006; 70:181–190. [PubMed: 16580655]
21. Coley BD, Mattrey RF, Mitten RM, Peterson T. AUR Memorial Award 1990. The physiologic basis of the radiodense renal medulla after the administration of blood pool contrast agent PFOB. *Invest Radiol.* 1990; 25:1287–1293. [PubMed: 2279907]
22. Murray PT, et al. Potential use of biomarkers in acute kidney injury: report and summary of recommendations from the 10th Acute Dialysis Quality Initiative consensus conference. *Kidney Int.* 2014; 85:513–521. [PubMed: 24107851]
23. Murray PT, et al. A framework and key research questions in AKI diagnosis and staging in different environments. *Clin J Am Soc Nephrol CJASN.* 2008; 3:864–868. [PubMed: 18287251]

Fig. 1. Synthesis of catSHELs. Nanosized hollow mesoporous silica shells are fabricated following a previously reported nanomasking procedure¹⁸ (A). Hollow empty mesoporous silica shells are filled with catalase through an equilibrium process (B). The pores are then sealed with nanoporous silica. The concentration of catalase inside the particles is dependent on the concentration of the catalase solution (C).

Fig. 2. Microbubble formation depends on the properties of the catSHELLS used as well as the immediate chemical microenvironment. CatSHELLS are nanoporous silica nanoSHELLS that trap macromolecules in their core while allowing free exchange of small molecules such as H₂O₂ across the shell (A). In the presence of sufficient levels of H₂O₂, catalase converts H₂O₂ into oxygen and water causing the formation of ultrasound detectable oxygen microbubbles (B).

Fig. 3.

Microbubble formation increased with increasing number of particles (pts), increasing particle size, and catalase concentration. Exposing increasing numbers of catSHELs to graded H_2O_2 concentration while holding particle size and internal catalase concentration constant (A) increased ultrasound signal as measured using a contrast specific imaging mode on a clinical ultrasound machine. Note that at the highest particle concentration significant increased signal was detected at 10uM H_2O_2 (inset of (A)). Similarly, increasing particle size at the same particle and catalase concentration (B) or internal catalase concentration while holding particle size and concentration constant (C) also increased signal. PEGylation of particles to allow for systemic administration did not affect signal generation (D).

Fig. 4.

The left renal artery was surgically exposed in anesthetized rats and selectively clamped for one hour. Successful clamping and reperfusion were confirmed visually by observing the blanching of the affected kidney and the re-normalization of color (A). Hematoxylin-eosin staining of an injured kidney 24 hours after reperfusion showed expected ischemic changes including pyknosis, eosinophilia, ghosting, flattening of the tubular cells and sloughing of the epithelium while the normal contralateral kidney architecture is histologically normal (B) (bar = 200 μm). Following ischemic injury and reperfusion, urine H₂O₂ increased by over four-fold (C).

Fig. 5.

Microbubbles were seen on ultrasound throughout the renal parenchyma but slightly more so at the corticomedullary junction following injection with catSHELS, but not with control particles. Ten animals with unilateral ischemic kidney injury were given either catSHELS (n=5) or control SHELS (n=5) intravenously and imaged periodically for ten minutes. The renal parenchyma of injured kidneys enhanced to a greater degree than the normal contralateral kidneys in animals injected with catSHELS (A), but did not enhance following the injection of control non-catalase containing SHELS. These differences were statistically significant (B).