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Antimicrobial Properties of Biodegradable Magnesium for Next Generation Ureteral Stent Applications

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***Abstract*— Bacterial infection often causes clinical complications and failure of indwelling medical devices. This is a major problem of current ureteral stents, which are used clinically to treat the blockage of ureteral canals. This study investigates the effectiveness and applicability of magnesium as a novel biodegradable ureteral stent material that has inherent antimicrobial properties. Incubating *Escherichia coli* with the magnesium samples showed a decrease in the bacterial cell density as compared with the currently used commercial polyurethane stent. Magnesium degradation in the immersion solutions (artificial urine, luria bertani broth, and deionized water) resulted in an alkaline pH shift. Antimicrobial and biodegradation properties of magnesium make it an attractive alternative as next-generation ureteral stent material.**

I. Introduction

Bacteria can irreversibly adhere to solid surfaces and accumulate on biomedical devices, forming biofilms. The outer surface of the biofilm is composed of dead bacteria, which protect the growing interior colonies from antimicrobial treatments. Within the interior of the biofilms, the bacteria adhere to each other and to surfaces of medical devices through extracellular polysaccharide linkages [1, 2].

One specific type of medical device, ureteral stents, is highly affected by the prevalence of bacterial infections. Ureteral stents help to facilitate urine flow from the kidney to the bladder in blocked or wounded ureter ducts [3]. Although ureteral stents are effective in treating blockages postoperatively, they have three major problems: (1) infection due to the accumulation of bacteria; (2) encrustation due to organic mineral deposition by bacteria; and (3) painful removal which can lead to further damage to the ureteral canal [4-6]. Approximately 10–50% of patients, who undergo short-term catheterization develop urinary tract infections (UTI) due to the adherence and accumulation of bacteria on the stent [7, 8]. In addition, all patients with an indwelling ureteral stent in place for more than 30 days contract UTI [7, 8].

Ureteral stents maintain an open passage between the kidney and bladder, allowing bacteria to enter the urinary tract through the urethral tube. More specifically, ureterovesical junction valves, which normally close off the canal, are held open by ureteral stents [9]. The most common bacteria, known to infect the urinary tract, is *Escherichia coli* (*E. coli*) [7]. Bacterial attachment and proliferation in the ureter result in the formation of biofilm on stent surface, which cause irritation of ureteral canal. Bacterial colonies cause mineral deposition such as calcium phosphates and struvite (magnesium ammonium phosphate) on the stent, which can lead to the build-up of solid crystals and eventually result in encrustation [4, 10, 11]. Stent encrustation damages to ureteral canal and causes pain during stent removal because sharp edges of crystals can tear the ureteral and urethral tissues.

Surface coating is a common approach currently used in preventing bacterial infection on polyurethane stents. Different coating materials and methods have been investigated, including anti-adhesion or drug delivery coatings [12, 13]. For example, heparin or other charged species, are used on polyurethane stent to inhibit bacterial adhesion [14]. However, heparin is an anti-coagulant and can cause complications with healing of open wounds [12]. Recently, it has been shown that a coating of chitosan/poly(vinyl alcohol) can reduce friction and protein adsorption on polyurethane stents and prevent bacterial adhesion to stents [15]. As the currently used ureteral stents are all made out of permanent materials, they require removal, which causes pain and discomfort for many patients. Alternatively, biodegradable stents eliminate the need of a secondary procedure for their removal and hence, decrease patient pain, risk of complications, and health care cost [16]. Magnesium is biodegradable in physiological environments and the degradation products are not toxic [17]. Additionally, previous work has shown that magnesium metal turnings have similar antibacterial effect as the antibiotic drug, fluoroquinolone [18].

Therefore, the objective of this study is to investigate antimicrobial and biodegradable properties of magnesium for ureteral stent applications. This study focused on: (1) bacterial growth inhibition in the presence of magnesium as compared with a commercially available polyurethane stent; and (2) degradation of magnesium in artificial urine solution and controls measured by change in mass and pH.

II. Methods

A. Preparation of Magnesium Samples and Controls

Magnesium strips (98% purity, Miniscience, NJ) were used for this study. A Universa™ soft polyurethane ureteral stent (Cook Medical, IN) was used as control. Fisherbrand plain glass slides (Fisher Scientific, IL) and polystyrene

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plate (BD Falcon) as references. The samples were cut into a consistent length to give a total surface area of 208 mm^2.

Since the polyurethane stent is tubular, the stent was flattened in between two glass slides in order to increase the exposed surface area. The polyurethane stent was rolled out flat in between two taped glass slides. A change in extreme temperature was used to flatten the tubular polyurethane stent into a rectangle. Specifically, the stent was submerged in 100 °C water for 20 minutes, cooled to room temperature for 5 minutes, submerged again in 100 °C water for 10 minutes, placed in the freezer for 5 minutes, and allowed to dry overnight. A thread was tied to hold the stent in a U-shaped position to optimize surface area exposure.

To clean all the samples, the magnesium, glass, and polyurethane stent were sonicated in 200 proof pure ethanol for 5 mins. After drying overnight, samples were exposed to ultraviolet (UV) irradiation for at least 1 hr on each side.

B. Artificial Urine Solution Preparation

To prepare 500 mL of Artificial Urine (AU) solution, deionized (DI) water was heated to 37 °C and the following chemicals were added: 0.375 g urea, 0.75 g creatine, 0.12 g calcium chloride, 0.43 g ammonium phosphate dibasic, 0.5 g magnesium chloride hexahydrate, 1 g potassium chloride, 1 g sodium sulfate (Sigma-Aldrich). The pH was adjusted to rest between 5-8 with Tris buffer or uric acid [19].

A 0.22 micron polyethersulfone filter (Fisher Scientific, Barrington IL) was used to filter the AU solution. The AU solution was UV sterilized in a glass bottle for 24 hrs. The AU solution was stored at 4 °C.

C. Bacterial Co-culture with Materials

E. coli (ATCC 25922) was grown in 6 mL of luria bertani broth (LBB) (Fisher Scientific, IL) in a 37 °C shaker incubator for 16 hrs. The bacteria were washed in Tris-buffer solution, centrifuged for 5 mins at 8,000 RPM, and resuspended in AU solution (wash step completed 3 times).

To determine the antimicrobial properties of magnesium in a clinically relevant condition, the magnesium sample was placed in the AU solution with *E. coli*. Three samples each of magnesium, polyurethane stent, glass slide were placed into separate wells of a 12-well non-tissue culture treated polystyrene plate. The last three wells had no samples and were used as a reference. Approximately 2 mL of 7.8×10^6 bacterial cells/mL AU solution were added into each well. The 12 well plate was incubated in a shaker-incubator for 16 hrs at 250 RPM and 37 °C. After 16 hrs immersion with the test substrates, bacteria cell density was determined using a hemocytometer.

D. Degradation in Artificial Urine Solution and Controls

The degradation rate of magnesium was compared in three different solutions: AU, LBB, and DI water. Prior to experiment, all samples were weighed using an atomic scale and then sterilized for at least 1 hr under UV exposure. Samples were immersed in AU solution, LBB solution, and DI water for 72 hrs (24 hrs shaker-incubator, 24 hrs stationary-incubator, 24 hrs shaker-incubator). After 72 hrs, the samples were dried overnight at room temperature and weighed. Solution pH was measured using a pre-calibrated pH meter (Mettler Toledo).

III. Results & Discussion

A. Antibacterial Activity of Magnesium

After the 16 hr incubation period, the bacterial cell density was quantified (Fig. 1). Specifically, in the presence of magnesium, the bacterial cell density dropped almost by a factor of two. In contrast, the other two test substrates (polyurethane stent and glass slide) and polystyrene reference did not inhibit bacteria growth and the cell density actually doubled. Additionally, bacterial cell density decreased for bacteria exposed to magnesium indicating an antibiotic property of magnesium. These results showed that magnesium inhibited bacterial cell growth while the polyurethane stent, glass slide, and polystrene plate control promoted bacteria growth.

Initially about 7.8×10^6 bacterial cells/mL AU solution were added to the test substrates (magnesium, polyurethane stent, and glass slide) and polystyrene plate control. This seeding density was a factor of ten times greater than that for a confirmed urinary tract infection ~10^6 colony-forming units/mL [20]. This seeding density was utilized to account for the lack of nutrition of the AU solution. Specifically within the ureteral canal, bacteria thrive while feeding on umbrella cells in addition to the proteins and amino acids released from the nephrons of the kidneys. For the polystrene plate control, we observed that the bacteria immersed in artificial urine solution were able to proliferate and increase in cell density after a 16 hr incubation period (Fig. 1). From this observation, the *E. coli* were able to still thrive in an environment with high salt concentration. This confirmed that it was the magnesium, and not the immersion media that affected bacterial cell growth.

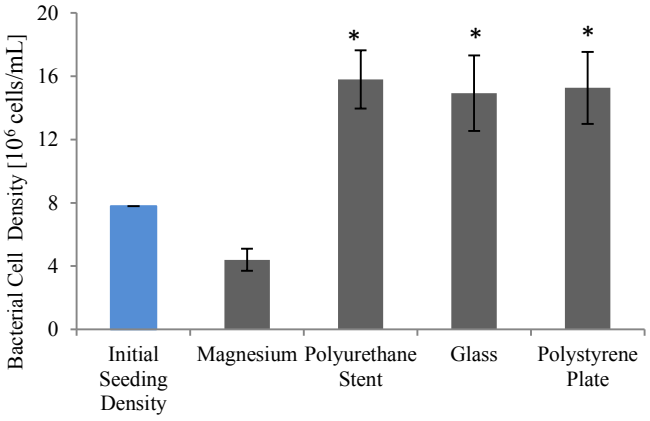

Figure 1. Bacterial cell density after 16 hours of incubation with the test substrates of interest and controls in artificial urine (AU) solution. The initial bacterial seeding density was 7.8×10^6 cells ml^{-1}. Bacterial cell density decreased when incubated with magnesium, but increased when incubated with the polyurethane stent, glass, and polystyrene plate. Additionally, bacterial cell density was significantly less on magnesium as compared to the controls, i.e., polyurethane stent, glass, and polystyrene plate. Data are ± SEM (N = 3). *p<0.05 compared to magnesium.

B. Magnesium Degradation in Artificial Urine

We investigated the degradation of magnesium in three different solutions (i.e. AU solution, LBB, and DI water) and ascribed magnesium oxidation to the presence of water molecules in the solutions. Magnesium reacts with water to form magnesium hydroxide and hydrogen gas, resulting in a more basic solution [21]. Magnesium hydroxide can further interact with physiological anions (i.e. Cl^-) to form soluble magnesium salts. The precipitation of such salts depends upon solubility and how well anions interact with the hydration sphere around the magnesium ion. Although magnesium degrades by this oxidation process, the unique composition of each solution also affected the rate and process of magnesium degradation. The presence of electrolytes in AU solution yields a higher ionic strength when compared with DI water and affects how magnesium ions dissociate [22, 23]. Proteins present in LBB chelate magnesium and lead to magnesium dissolution [23, 24].

Within the 72 hrs degradation period, there was only a small increase or decrease in weight before and after immersion in the different solutions (Fig 2). Although the magnesium degrades by reacting with water, salt precipitates also form on the magnesium surface. Thus, the short 72 hrs degradation period did not result in any significant mass change due to the simultaneous mass increase by precipitates and loss by dissolution.

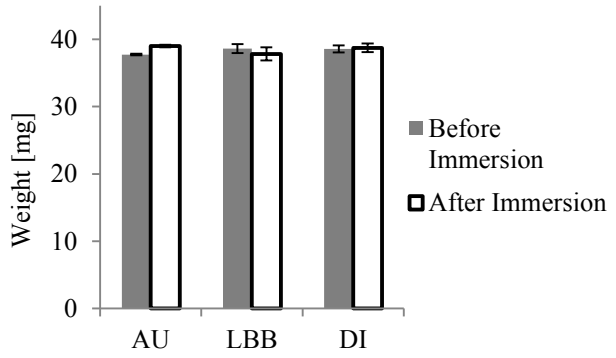

Figure 2. The mass of magnesium samples before and after 72 hrs of immersion in artificial urine (AU) solution, luria bertani broth (LBB), and deionized (DI) water. The short immersion did not result in any significant change in sample mass, even though the occurrence of degradation was observed after immersion. It is speculated that the precipitation of insoluble degradation products and solution salts onto the samples may have canceled out any mass loss of soluble degradation products during the initial degradation period. Data are ± SEM (N = 3).

Magnesium oxidation results in a more basic solution due to the increase in hydroxide ions. It has been previously reported that magnesium corrosion increases the pH of the solution, which may contribute to the antibacterial property of magnesium [18, 25]. From the degradation studies, a similar trend was observed in all three solutions where the pH was increased after 72 hrs of incubation (Fig 3). For the AU solution, the pH increased from 5.7 to 9.1. The pH of LBB solution increased from 6.9 to 10.0. The pH of DI water increased from 7.9 to 10.1. There was a significant increase of 2-3 pH units when comparing each solution before and after immersion.

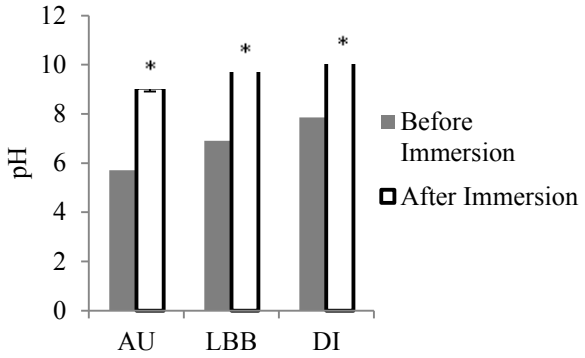

Figure 3. pH measurements of artificial urine (AU) solution, luria bertani broth (LBB), and deionized (DI) water before and after immersion of magnesium samples for 72 hours. In all three solutions, the magnesium samples resulted in the pH increase, that is, a more alkaline solution. Data are ± SEM (N = 3). *p<0.05 compared to the pH measurement before sample immersion.

C. Magnesium Degradation and Inhibition of Bacterial Cell Growth

Following magnesium degradation in AU solution for 72 hrs, the solution becomes more basic and there is an increase of magnesium ions in the solution. These two factors are correlated to the decrease in bacterial cell counts after 16 hrs of exposure to magnesium in AU solution. A similar trend was previously published in which magnesium turnings had an inhibitory effect on the growth of *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (*S. aureus*) [18]. Robinson et al. studied the inhibitory effect of the magnesium turnings on bacteria growth in Mueller-Hinton broth. Additionally, they showed that simply increasing magnesium chloride salt concentration alone did not inhibit bacterial growth [18]. In our investigation, we studied the growth of *E. coli* in AU solution to analyze how magnesium can be utilized as a novel stent material for urological applications. In both studies, magnesium showed an inhibitory effect on *E. coli* bacteria growth and resulted in an alkaline immersion solution due to magnesium degradation.

Additionally, Ren et al. examined how a silicon coating on magnesium affected *E. coli* and *S. aureus* growth [25]. From their experiments, the porous silicon coating reduced the degradation rate while still maintaining the antibacterial property of magnesium [25]. Coating thickness and porosity can be altered to control the rate of magnesium ion diffusion from the substrate into the bacterial solution. Although we examined the degradation of uncoated magnesium, our group has investigated how magnesium degradation can be tuned through surface treatment [26, 27]. Specifically, electrodeposition of hydroxyapatite coating on a magnesium alloy (Mg-4.0Zn-1.0Ca-0.6Zr (wt %)) had a slower degradation rate as compared to uncoated magnesium alloys [27].

Although it is believed that alkalinity of magnesium degradation contributed to bacteria death, it may not be the sole reason. There may be other mechanisms yet to be explored. For example, magnesium hydroxide precipitates may contribute to bacteria death as well because recent report showed antimicrobial property of magnesium

nanoparticles. Magnesium oxide nanoparticles inhibited the growth and resulted in bacterial cell death of both foodborne *E. coli* O157:H7 and *Salmonella* [28]. Jin and He observed that the magnesium oxide nanoparticles resulted in significant changes in cell morphology and membrane integrity causing cell death [28]. A magnesium fluoride nanoparticle also inhibited bacterial growth in a similar manner. Specifically, Lellouche et al. found that magnesium fluoride nanoparticles attached to the surface and penetrated *E. coli* cells [29]. The underlying antibacterial mechanisms of these magnesium nanoparticles need to be further studied in order to understand how such magnesium-based nanoparticles inhibit bacterial growth and biofilm formation.

The objective of this initial study was to determine how magnesium degrades in AU solution and to characterize the material interaction with *E. coli*. This information provides crucial data for the design and fabrication of surface coatings to regulate magnesium degradation *in vivo* while still maintaining its antibacterial properties.

IV. Conclusion

Magnesium samples showed significantly less bacterial growth when compared with the FDA approved polyurethane ureteral stent. Additionally, magnesium degradation resulted in an alkaline shift of the immersion media. Magnesium-based ureteral stents can be designed to be fully biodegradable in a controllable manner *in vivo*. Thus, the antimicrobial property coupled with the biodegradation of magnesium presents a new avenue for its use as next-generation ureteral stent materials.

Acknowledgments

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