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In vitro antibacterial properties of magnesium metal against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* ☆

Duane A. Robinson [a,1], Ronald W. Griffith [b], Dan Shechtman [c,2], Richard B. Evans [d,3], Michael G. Conzemius [a,*]

[a] Veterinary Clinical Sciences Department, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA
[b] Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA
[c] Materials Science and Engineering, College of Engineering, Iowa State University, Ames, IA 50011, USA
[d] Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA

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ABSTRACT

Bacterial infections are a costly sequela in any wound. The corrosion properties of 0.15, 0.30, 0.45 and 0.60 g of Mg metal were determined in Mueller–Hinton broth by serially measuring the Mg^{2+} concentrations and pH over 72 h. In addition, the effect of Mg metal, increased Mg^{2+} concentration and alkaline pH on the *in vitro* growth of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were evaluated in three separate experiments. The primary outcome measure for culture studies was colony-forming units/ml compared to appropriate positive and/or negative controls. Regardless of the mass of Mg added, there was a predictable increase in pH and Mg^{2+} concentration. The addition of Mg and an increase of pH resulted in antibacterial effects similar to the fluoroquinolone antibiotic; however, a simple increase in Mg^{2+} concentration alone had no effect. The results demonstrate an antibacterial effect of Mg on three common aerobic bacterial organisms, the mechanism of which appears to be an alkaline pH.
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1. Introduction

Regardless of the source or location, bacterial infections represent significant hurdles in the management of both surgical and traumatic wounds and contribute considerably to increased morbidity and mortality. The continued advancement of the field of medicine has emphasized these problems and underscores why the development of biomaterials that prevent and/or treat infection remains a major unsolved medical problem [1,2]. The significance of this problem is underscored when one considers that orthopaedic implant-related infections occur in nearly 112,000 human patients annually and create a $2 billion burden for the US healthcare industry [1]. Historically, preventative and therapeutic management of wound-related infections has utilized antimicrobials and adherence to aseptic technique; however, this approach is fraught with problems and has had incomplete success [3,4].

Bacterial–host interactions are complex and multifactorial. Although host defense mechanisms (i.e. a competent immune response) are important, the virulence characteristics of the bacterial organisms must also be considered. Together these determine whether an organism is merely a contaminant or is able to establish an infection [3,4]. When attempting to develop biomaterials aimed at preventing/treating infection, it is prudent to understand that the bacterial population of a wound is not static; instead, the complex and dynamic interactions between different species of microorganisms and the physiological changes occurring in the host result in alterations in the population as healing progresses [3]. Thus, some important characteristics of a bioabsorbable antifouling device are: (i) it should have a broad spectrum of antibacterial activity in the prevention/treatment of an infection; (ii) it should be nontoxic to the host; and (iii) it should not be susceptible to the development of microbial resistance.

Current biomaterial research in the area of device-associated infection has focused on many diverse areas including polymers [5–7] and metals [8–13]. Thus the concept of a metal possessing antibacterial properties is not new. The antibacterial activity of silver was first demonstrated in the 19th century and the use of various forms of silver as a topical agent is commonplace [8]. Silver has also been evaluated as an antimicrobial coating on both orthopaedic and vascular devices with some success [9,14,15]. One significant problem with the use of silver is that it is nonessential to

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* Corresponding author. Present address: Veterinary Clinical Sciences Department, 1352 Boyd Avenue, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN 55108, USA. Tel.: +1 612 625 3147; fax: +1 612 624 0751.
E-mail address: conze012@umn.edu (M.G. Conzemius).
[1] Present address: Veterinary Clinical Sciences Department, 1352 Boyd Avenue, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN 55108, USA.
[2] Present address: Materials Engineering, Technion – Israel Institute of Technology, Haifa, Israel.
[3] Present address: Veterinary Clinical Medicine Department, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802, USA.

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the host and can be toxic [9,16,17]. Zinc [10,11,18] and copper [12,13] have reported antibacterial activity; however, while considered essential to normal homeostasis, both have the potential for toxic sequelae.

Magnesium (Mg) is (i) an inexpensive and readily available metal; (ii) an abundant cation (Mg^{2+}) in mammals, most of which is in bone; and (iii) essential to many processes in eukaryotic cells [19–21]. Since its first use in orthopaedic surgery in the early part of the 20th century [20], Mg and Mg alloys have been evaluated for both orthopaedic and cardiovascular applications [20,22,23]. When one considers the material properties of orthopaedic implants commonly used, none have physical and mechanical properties that closely resemble those of cortical bone, whereas Mg does [20]. Clinical investigations utilizing Mg and Mg^{2+} have included alloys for cardiovascular stenting [22,24–26], orthopaedic applications [20,23,27], and as an oral therapy in treating disorders such as autism [28], attention deficit hyperactivity disorders [29] and multiple sclerosis [30].

The first objective of this study was to characterize the effects of the corrosion products of Mg on pH and Mg^{2+} concentrations in a microbial culture broth. The second objective was to evaluate the effects of the corrosion products on the *in vitro* growth of *Escherichia coli* (Gram-negative), *Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Finally, in an effort to identify a mechanism of action for the antibacterial properties of Mg, we evaluated the effects of Mg^{2+} alone and pH on bacterial growth. Our hypotheses were: (i) as Mg metal corrodes in culture broth the increase in Mg^{2+} concentration will parallel that of the pH; (ii) when added to the growth media, Mg corrosion products will inhibit the growth of *E. coli*, *P. aeruginosa* and *S. aureus*; (iii) the addition of Mg^{2+} alone will not inhibit bacterial growth; and (iv) increasing the alkalinity (i.e. higher pH) will inhibit bacterial growth.

2. Materials and methods

2.1. Magnesium corrosion

To determine the corrosion properties of Mg metal in bacterial growth media 0.15, 0.30, 0.45 and 0.60 g of Mg metal turnings (Fisher Scientific, NJ, USA) with an approximate surface area of 816, 1728, 2544 and 3300 mm^2, respectively, were placed into 8.0 ml of sterile Mueller–Hinton (MH) broth (Beckton Dickinson Diagnostic Systems, MD, USA) in duplicate. The concentration of Mg^{2+} and the pH of the broth were serially measured over a 72 h period. The Mg^{2+} concentrations were measured using an automated serum chemistry analyzer (Hitachi 912 Automatic Analyzer, Boehringer Mannheim Roche, IN, USA) and converted to mmol l^{-1}. The measurement of Mg in this automated analyzer is accomplished using a reaction with xylidyl blue. Xylidyl blue forms a purple complex with Mg and the concentration of Mg is then measured by the decrease in absorbance. This test principle is based on the methodology published by Mann and Yoe [31]. The pH was measured using a portable pH meter (Horiba Instruments Inc., CA, USA).

2.2. Test materials

Magnesium turnings (0.30 g) (Fisher Scientific) were used as the source of Mg metal and served as an implant test group. 316L stainless steel intramedullary pins (316LSS) (Imex Veterinary Inc, TX, USA), 25 mm long and 6.4 mm in diameter, were used as an implant-negative control material. All test materials were sterilized prior to use in the experiments.

Enrofloxacin (Baytril Injectable, Bayer Animal Health, KS, USA) was used as a treatment positive control for a bactericidal agent. A final concentration of enrofloxacin of 10 $\mu g\ ml^{-1}$ was used in the culture vials.

To increase the Mg^{2+} ion concentration $MgCl_2$ salt (1.7 mg ml^{-1}) (Fisher Scientific) was added to the MH broth in an attempt to achieve a final concentration of 6.58 mmol l^{-1} (16.0 mg dl^{-1}) prior to sterilization. To evaluate the effect of adding an ionic salt to the culture broth, the same amount of NaCl (1.7 mg ml^{-1}) (Fisher Scientific) was added prior to sterilization. After sterilization, a sample of each was collected, the pH was measured and the Mg^{2+} concentration determined.

To adjust the pH of the MH broth, 1 N NaOH (Fisher Scientific) was added to achieve a pH of 8.0, 9.0 or 10.0. The pH was verified prior to and after sterilization.

Finally, all bacteria were cultured with no additive to serve as a treatment negative control.

2.3. Bacterial cultures

The bacterial inoculum consisted of one of the following organisms: *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) (both of which are Gram-negative organisms) and *S. aureus* (ATCC 25923) (which is a Gram-positive organism) (American Type Culture Collection, VA, USA). These bacterial strains were chosen to represent a spectrum of organisms encountered and because they are used as control strains in quality-control susceptibility testing, for media testing and for susceptibility disc testing. Thus they are well-established cultures and strains in the *in vitro* setting. Pure cultures of *E. coli*, *P. aeruginosa* and *S. aureus* were aerobically cultured for 24 h at 37 °C on bovine blood agar plates. Samples of each of the pure cultures were collected and suspended in 10.0 ml of sterile MH broth. The cultures were incubated in a waterbath at 37 °C with agitation for approximately 10 min and then in a dry incubator at 37 °C with agitation at 220 rpm for 1 h. 4.67×10^5, 5.75×10^5 and 1.95×10^5 colony-forming units (CFU) of *E. coli*, *P. aeruginosa* and *S. aureus*, respectively, were used to inoculate the culture vials.

2.4. Incubation of bacteria with test materials

Culture vials were prepared for each experiment as outlined below. After the addition of bacteria, the optical density was monitored using a control culture vial for each organism starting at approximately 90 min post-inoculation. The optical density (OD) at 590 nm was used to monitor bacterial growth and to determine when samples would be collected for analysis. The goal was to start sampling when the OD was approximately 0.2 and continue until it was $\geqslant 0.6$. The number of samples collected was dependent on bacterial growth but sampling was performed a minimum of three separate times.

2.4.1. Magnesium turnings

Culture vials were prepared such that each contained 8.0 ml of sterile MH broth. Mg turnings, 316LSS or antibiotic (10.0 $\mu g\ ml^{-1}$ of enrofloxacin) were added to the culture vials. Culture vials with a 316LSS coupon only were used to monitor the sterilization process. The culture vials were incubated aerobically in a waterbath at 37 °C with agitation at 220 rpm for 3.0 h after the addition of test materials. At this time the vials were inoculated with bacterial suspension and returned to the incubator.

2.4.2. Addition of Mg^{2+}

Culture vials were prepared such that each contained 8.0 ml of sterile MH broth, MH + $MgCl_2$ broth or MH + NaCl broth. The culture vials were incubated aerobically at 37 °C for 3.0 h prior to inoculation with bacterial suspension.

2.4.3. Adjustment of pH

Culture vials were prepared such that each contained 8.0 ml of sterile MH broth with a pH of 7.4 (control), 8.0, 9.0 or 10.0. When mixed for normal use, the MH broth has a pH of approximately 7.4. For this experiment the broth was mixed as per the manufacturer's instructions and 1.0 N NaOH was added to increase the broth pH. The culture vials were incubated aerobically at 37 °C for 3.0 h prior to inoculation with bacterial suspension.

2.5. Microtiter dilution and viable bacterial counts

Microtiter dilutions were performed using a modification of a previously described technique [32,33]. The number of CFUs in each tube was determined in quadruplicate by aseptically collecting a sample from each tube at the desired time point. Tenfold dilutions were made (10^{-1}–10^{-7}) using phosphate-buffered saline in 96-well round-bottomed microtiter plates. 20 μl was collected from each well and streaked across a tryptic soy agar (Beckton Dickinson Diagnostic Systems, MD, USA) plate in a uniform manner. The plates were incubated aerobically at 37 °C for 24 h, at which time the number of colonies was counted.

2.6. Statistical analysis

Dilutions with up to 30 colonies present were used to calculate the median CFU ml^{-1}. Summary statistics were calculated and are presented as the median CFU ml^{-1}. The error bars in the figures represent the 25th and 75th percentile. At each time point the distributions of CFU ml^{-1} were compared for each pair of groups using the least-significant difference method to protect against Type I error inflation [34], followed by pairwise Wilcoxon rank sums test for nonparametric data. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Magnesium corrosion

The concentration of Mg^{2+} rapidly increased within the first 3 h to an average of 6.64 mmol l^{-1} (16.14 mg dl^{-1}) regardless of the mass of Mg turnings added. At 24, 48 and 72 h, the average Mg^{2+} concentration was 6.60 mmol l^{-1} (16.05 mg dl^{-1}), 6.60 mmol l^{-1} (16.05 mg dl^{-1}) and 6.96 mmol l^{-1} (16.91 mg dl^{-1}), respectively (Fig. 1). The pH of the broth rapidly increased over the first 3 h to an average of 9.49 regardless of the mass of Mg turnings added and there was no apparent dose response. At 24, 48 and 72 h the average pH was 9.94, 10.10 and 10.15, respectively (Fig. 1).

3.2. Incubation of bacteria with magnesium turnings

All three organisms grew as expected under the experimental growth conditions as indicated by the CFU ml^{-1} recovered from the control culture vials. At all time points and for all three organisms no CFUs were recovered from any of the culture vials where only 316LSS was added. In addition, no CFUs were recovered from any culture where enrofloxacin was added. The median CFU ml^{-1} recovered from the control culture vials and those with enrofloxa-

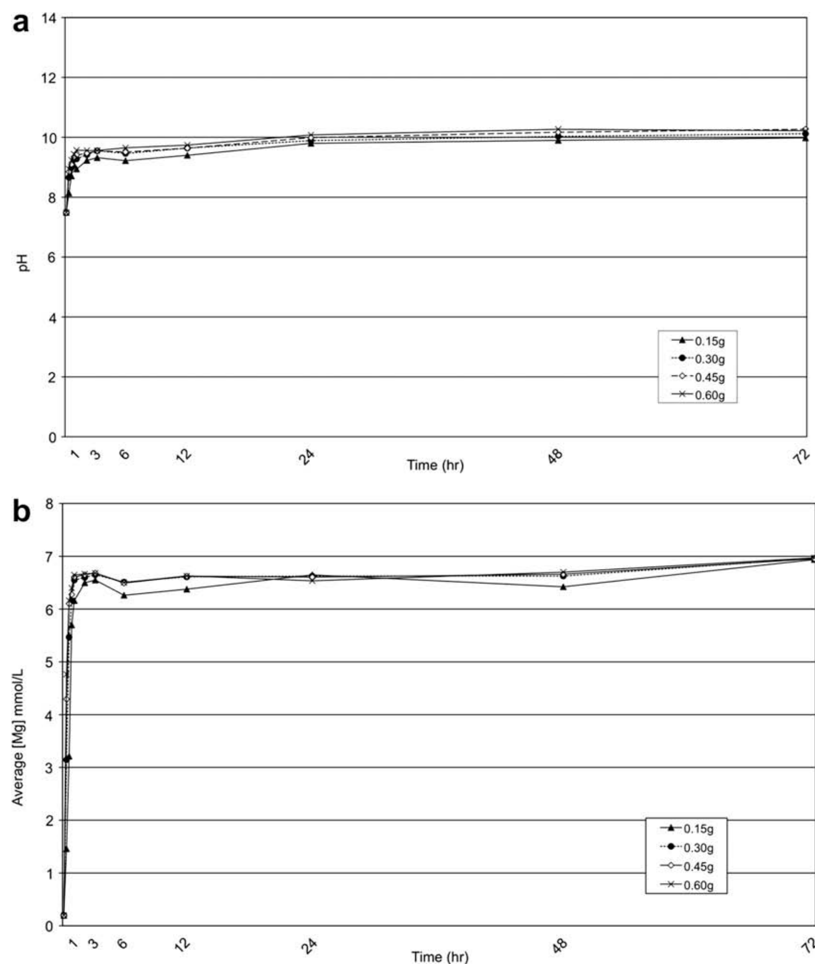

Fig. 1. Results of (a) pH measurement and (b) Mg^{2+} concentration (mmol l^{-1}) following the addition of Mg metal turnings to bacterial culture broth.

cin, 316LSS and Mg turnings at all sampling points are reported in Fig. 2. There was a statistically significant difference in the CFU ml^{-1} recovered across all treatment groups at all time points. When the CFU ml^{-1} in the Mg group was compared independently to the control and 316LSS groups, a statistically significant difference was found at all time points for all three organisms (with the exception of *P. aeruginosa* at the first time point). Similarly, there was an overall trend (P = 0.05–1.0) towards no significant difference between the Mg and Enrofloxacin groups or between the 316LSS and control groups (Fig. 2).

Overall, the addition of Mg turnings to the culture broth resulted in a lower number of CFU ml^{-1} recovered at all time points for *E. coli*, *P. aeruginosa* and *S. aureus* as compared to the stainless steel and control (no additive) groups.

3.3. Incubation of bacteria with $MgCl_2$ and NaCl

The pH of the $MgCl_2$ and NaCl solutions after sterilization was 7.35 and 7.43, respectively. The Mg^{2+} concentration of the $MgCl_2$ and NaCl solutions was 5.95 mmol l^{-1} (14.45 mg dl^{-1}) and

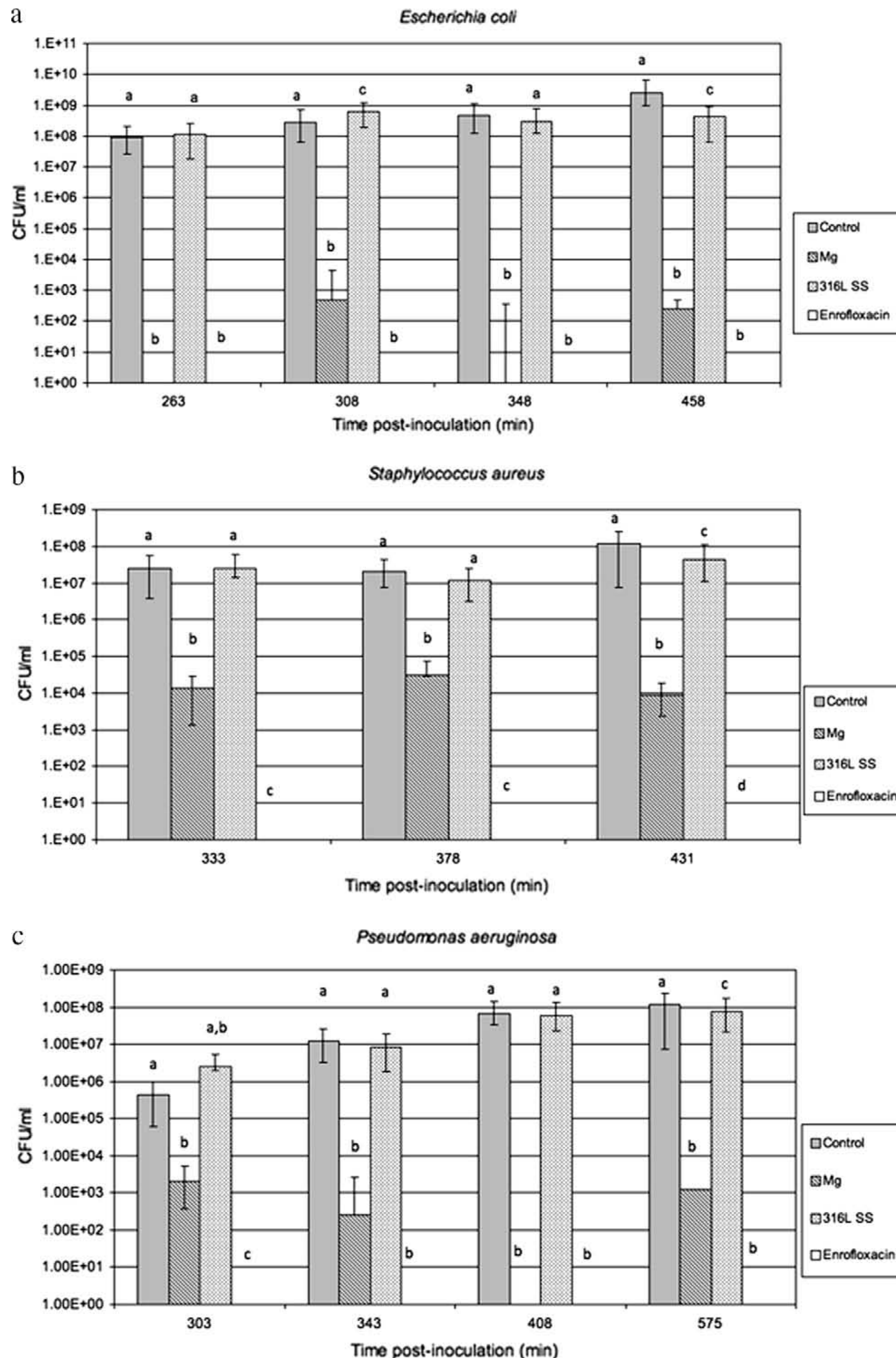

Fig. 2. Culture plate counts for (a) *Escherichia coli*, (b) *Pseudomonas aeruginosa* and (c) *Staphylococcus aureus* with control, Mg, 316LSS and enrofloxacin treatment groups. Control, no additive; Mg, Mg metal turnings; 316LSS, 316L stainless steel; Enrofloxacin, antibiotic. Data are presented as median CFU ml^{-1} with the error bars representing the 25th and 75th percentiles. Columns labeled with the same letter were not significantly different ($P > 0.05$) at the given time point.

0.16 mmol l^{-1} (0.38 mg dl^{-1}), respectively. As indicated by the CFU ml^{-1} recovered from the control vials, all three organisms grew as expected. The median CFU ml^{-1} recovered from the control, $MgCl_2$ and NaCl vials at all sampling points are reported in Fig. 3. With the exception of the third time point for *E. coli* and the second time point for *S. aureus* there was no statistically significant difference across all three treatment groups at the various time points. The overall trend was that the increase in Mg^{2+} concentration, via the addition of $MgCl_2$, did not result in an appreciable effect on the CFU ml^{-1} for *E. coli*, *P. aeruginosa* or *S. aureus*. Similarly, there was no statistically significant difference between the $MgCl_2$ and NaCl groups for all three bacteria at all time points

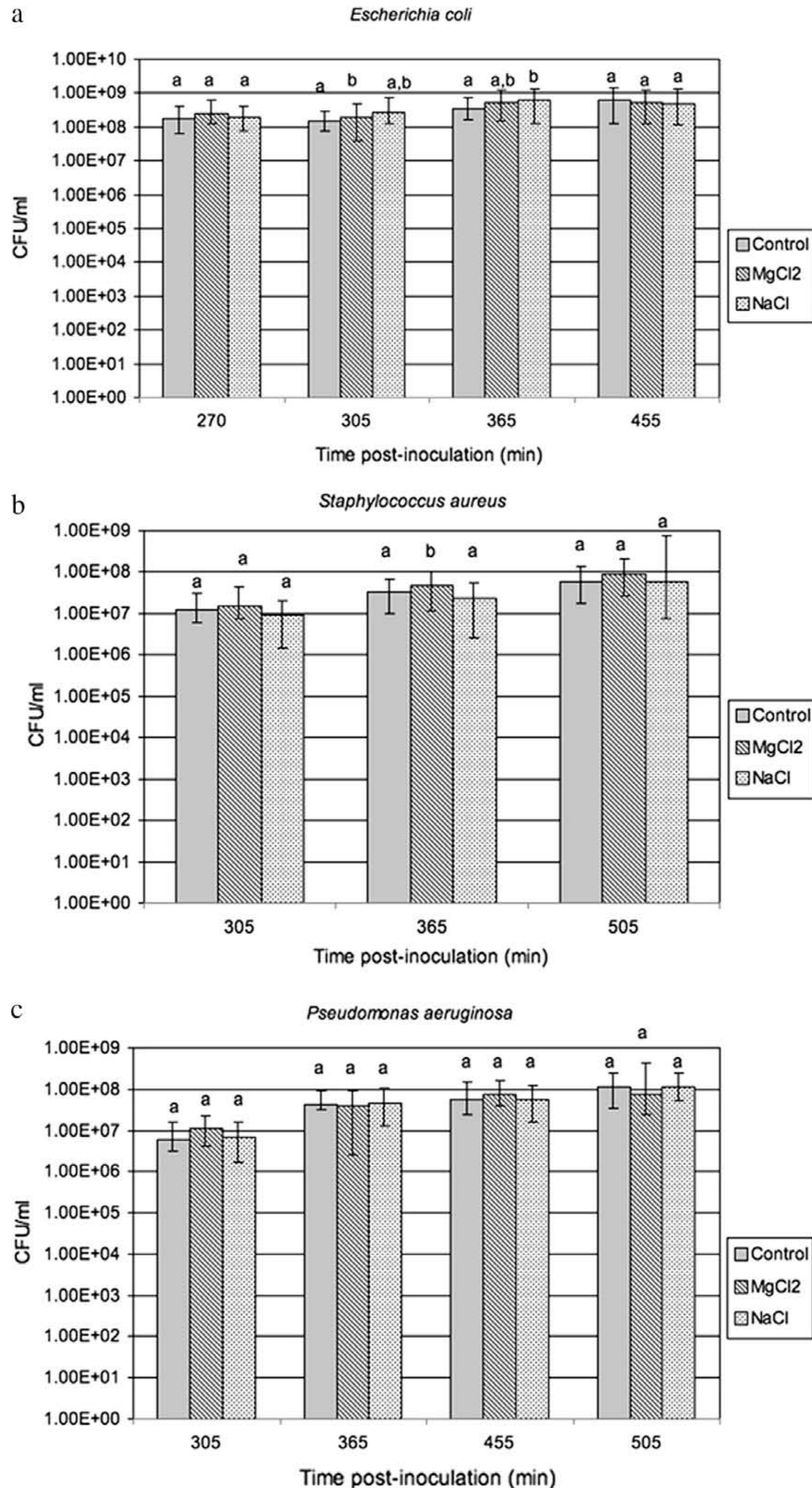

Fig. 3. Culture plate counts for (a) *Escherichia coli*, (b) *Pseudomonas aeruginosa* and (c) *Staphylococcus aureus* with control, $MgCl_2$, and NaCl treatment groups. Data are presented as median CFU ml^{-1} with the error bars representing the 25th and 75th percentiles. Columns labeled with the same letter were not significantly different ($P > 0.05$) at the given time point.

(with the exception of the second time point for *S. aureus*). In general, the addition of an ionic salt, in the form of NaCl, also did not have a measurable effect on the CFU ml^{-1} (Fig. 3).

3.4. Incubation of bacteria at various pH

The pH of the four MH groups after sterilization was 7.4, 8.0, 9.0 and 10.0, respectively. All three bacteria grew as expected as indicated by the CFU ml^{-1} recovered from the pH 7.4 group (considered the control). The median CFU ml^{-1} recovered from all four treatment groups at all time points is reported in Fig. 4. There was a statistically significant difference in the CFU ml^{-1} recovered across all treatment groups at all time points. When the CFU ml^{-1} from the pH 7.4 group was compared to the pH 9.0 and 10.0 groups there was a statistically significant difference at all time points for all three bacteria (Fig. 4). Thus an increase in pH to $\geqslant 9$ resulted in a measurable effect on the CFU ml^{-1} recovered in this *in vitro* model.

4. Discussion

The results of this study have demonstrated two important characteristics of Mg metal. First, when added to bacterial culture media Mg metal corrodes in a predictable fashion, resulting in both an increase in pH and Mg^{2+} concentrations, a finding that supports our first hypothesis. In a pure aqueous environment, Mg undergoes an electrochemical reaction with water, producing hydrogen gas

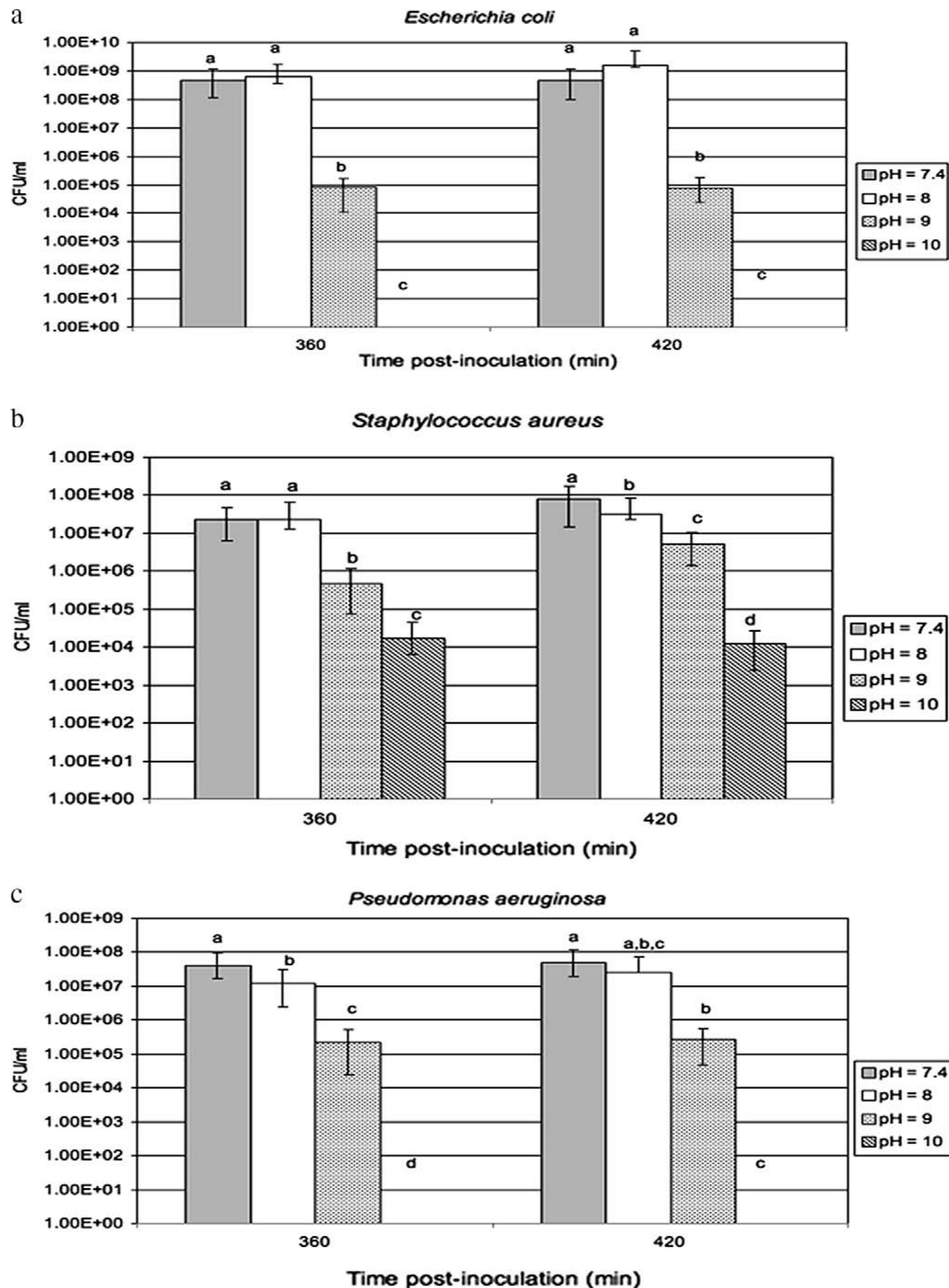

Fig. 4. Culture plate counts for (a) *Escherichia coli*, (b) *Pseudomonas aeruginosa* and (c) *Staphylococcus aureus* with pH 7.4 (control), 8, 9 and 10 treatment groups. Data are presented as median CFU ml^{-1} with the error bars representing the 25th and 75th percentiles. Columns labeled with the same letter were not significantly different ($P > 0.05$) at the given time point.

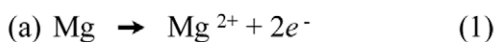
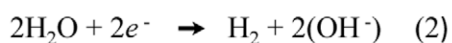
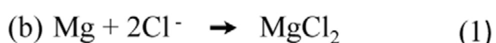

Fig. 5. Corrosion reactions for Mg metal (a) in a pure aqueous environment [20,35] and (b) in the presence of an activating ion (i.e. Cl^-) [20].

and magnesium hydroxide (Fig. 5a). In the presence of an activating anion (i.e. chloride) both magnesium and magnesium hydroxide can react further, producing a magnesium salt (Fig. 5b) [20,35]. Almost immediately after placing the Mg in the broth, gas formation could be observed on the surface of the metal, and by the end of the incubation period a precipitate could be observed in the bottom of the vials. While we did not analyze either of these reaction products, the equations in Fig. 5 would estimate their occurrence. In general, Mg corrodes very quickly in the normal physiologic environment (i.e. high chloride concentration and pH of 7.4–7.6) [20], resulting in a rapid increase in pH and Mg^{2+} concentration. This susceptibility of Mg to rapid corrosion is not surprising and can be predicted in part by its low position in the galvanic series for seawater [36,37]. The specific corrosion rate of a Mg implant, however, will also depend on the surface area, temperature, anatomical location and composition of the implant [20,23,27]. Within the first 24 h both the pH and Mg^{2+} concentration reached a plateau. Passivity of Mg is believed to occur via formation of a $Mg(OH)_2$ film which is thought to be stable at a pH $\geqslant$ 11.[23,35,36] With this in mind, the plateau observed in pH and Mg^{2+} concentration is also predictable, especially since this was a closed environment. As the pH of the broth increased further corrosion was prevented by stabilization of the protective $Mg(OH)_2$ film. Although many of the contributing factors to Mg corrosion have been described, any Mg biomaterial should be evaluated by *in vivo* testing as predicted corrosion behavior cannot be based on *in vitro* testing alone. [27].

The second characteristic observed was that when added to a closed *in vitro* culture system, Mg metal has an effect on the CFUs of both Gram-negative and Gram-positive bacteria recovered that is similar to those of a bactericidal fluoroquinolone antibiotic (enrofloxacin). Our hypothesis was that when added to the growth media, Mg corrosion products would inhibit the growth of *E. coli*, *P. aeruginosa* and *S. aureus*. The results reported here do not provide sufficient data to confirm this hypothesis; however, we would suggest that there is adequate data to support an overall trend towards an inhibitory effect of Mg corrosion products on the growth of these three bacteria.

While the antimicrobial properties of other metals have been reported previously [8–18], to the best of our knowledge this is the first publication to describe and evaluate the antimicrobial properties of Mg.

In an attempt to characterize the mechanism responsible for the effect of Mg on bacterial growth, we undertook two additional experiments. In the first we increased the concentration of Mg^{2+} and did not detect a measurable effect, which supports our hypothesis that the addition of Mg^{2+} alone would not inhibit bacterial growth. It is well known that Mg is important in the normal homeostatic mechanisms of eukaryotic cells [20,21] and was first identified as an essential requirement for growth of *E. coli* in 1968 [38]. Since that time the importance of Mg in prokaryotic metabolism and the molecular mechanisms of Mg regulation have been further characterized [39,40]. Although it has been reported that the Mg^{2+} concentration affects the virulence of *Salmonella typhimurium* [39] and the susceptibility of some Gram-negative and Gram-positive aerobic bacteria to fluoroquinolone antibiotics [41], it does not appear to have an appreciable effect in our planktonic model.

In the second experiment we increased the alkalinity of the culture broth prior to inoculation with the bacteria. We hypothesized that increasing the alkalinity (i.e. higher pH) would inhibit bacterial growth, which is supported by the finding that there was a decrease in CFU ml^{-1} recovered when the pH was $\geqslant$9 for all three organisms. pH and charge gradients are important in prokaryotic physiology in terms or generating a proton motive force that is then used to do useful work [42]. Nonetheless, most organisms have a pH range in which preferential growth occurs and several sophisticated systems operate that can affect both the intra- and extracellular environment [42]. The ability of a biomaterial to produce an alkaline pH as an antibacterial mechanism has been suggested in previous studies [43–45]. In one study the antibacterial effects of a bioactive glass paste was evaluated. The bioactive glass resulted in a pH of 10.8 and loss of bacterial viability by 60 min post-inoculation [43]. In another series of experiments Allan et al. exposed a range of oral bacteria to particulate Bioglass®. The presence of the Bioglass® in the culture media resulted in a pH of 10.0 or greater after 3 h and a kill rate of 94% or greater for the majority of bacterial evaluated [44]. Finally, Hu et al. demonstrated that increasing concentrations of 45S5 Bioglass® resulted in an increase in aqueous pH (pH of 9.8 and 10.3 at 50 and 100 mg ml^{-1} of 45S5 Bioglass®, respectively) and accompanying increase in bactericidal percentage against three pathogenic bacterial species (*S. aureus*, *S. epidermidis* and *E. coli*) [45]. Given the results of these and the study reported here it would seem plausible that an alkaline pH as generated by a biomaterial could be responsible for the antibacterial effect observed.

Based on the overall results of our study we would suggest that the alkaline pH, and not the increased Mg^{2+} concentration, is responsible for the effect on CFU ml^{-1} found in this closed *in vitro* model.

In considering Mg as an implant, one must also address the possibility of Mg toxicity and the issue of biocompatibility. Although toxicity can occur, hypermagnesiumia is rare. The daily requirements for an adult are between 300 and 400 mg day^{-1} [46], and unlike other antimicrobial metals, Mg is efficiently managed by the normal mammalian kidney and gastrointestinal tract [20,21]. Another determinant with respect to Mg toxicity is the rate of corrosion. As discussed above, the mechanism and determinants of Mg corrosion are multifactorial and require *in vivo* evaluation when being considered for use in an implant [27]. With respect to biocompatibility, some would suggest that the alkaline pH produced by an Mg-based implant would be detrimental to host cells and tissues. While not evaluated in the study presented here, there are a number of both *in vitro* and *in vivo* studies in the literature that contradict this assumption. For example, L. Li et al. found that there was no evidence of morphological changes or inhibition of cell growth by alkali-heat-treated Mg on mouse marrow cells [47] and Z. Li et al. found that L929 cells demonstrated better growth in extraction media from an Mg–Ca alloy than in the control media [48]. During *in vivo* evaluation of Mg alloys, Witte et al. found that when compared to a polymeric implant, a Mg alloy resulted in enhanced bone formation around an implant that had been placed in a guinea-pig femur [23]. Similarly, Zhang et al. evaluated an Mg–Zn–Mn alloy that was implanted in the femur of a rat and found that these implants were associated with new bone production [49]. While the data reported here is intriguing it is prudent to remember that this was an *in vitro* model and the performance of Mg in an *in vivo* model is required before any conclusions can be made regarding the behavior in a biological system. The next step would undoubtedly be *in vivo* analysis whereby the buffering capacity of biological systems is addressed.

Bacterial wound infections represent a substantial burden to the healthcare system in terms of financial costs and effects on patient morbidity and mortality [3]. In 2004 the projected number of infections associated with cardiovascular, orthopaedic, neurosurgical, urological and plastic implants was 149,130 cases with average infection rates ranging from 2% to 40% [1]. These numbers are impressive but become even more so when one considers the recent rise in antimicrobial-resistant infections associated with methacillin/oxacillin-resistant organisms that respond poorly to traditional therapies. Our results demonstrate that in this *in vitro* model, Mg, an inexpensive metal, can reduce the growth of three common aerobic bacteria. Given that the mechanism of action of Mg metal is related to changing local pH, we would argue that Mg could represent an antibacterial agent that is not susceptible to the traditional mechanisms of microbial resistance (i.e. enzymatic inactivation of the antibacterial agent, modification of the antibacterial target, failure to activate the antibiotic).

5. Conclusions

In this *in vitro* study Mg metal was shown to have a predictable corrosion reaction and had an activity that was comparable to a fluoroquinolone antibiotic against *E. coli*, *P. aeruginosa* and *S. aureus*. The growth-limiting property of Mg metal described here is not typical of conventional antibacterial agents and we would suggest that these results add to the body of knowledge in support of the use of Mg as a biomaterial.

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References

[1] Darouiche RO. Treatment of infections associated with surgical implants. N Engl J Med 2004;350:1422–9.
[2] Clutterbuck AL, Cochrane CA, Dolman J, Percival SL. Evaluating antibiotics for use in medicine using a poloxamer biofilm model. Ann Clin Microbiol Antimicrob 2007;6:2.
[3] Ryan TJ. Infection following soft tissue injury: its role in wound healing. Curr Opin Infect Dis 2007;20:124–8.
[4] White RJ, Cutting K, Kingsley A. Topical antimicrobials in the control of wound bioburden. Ostomy Wound Manage 2006;52:26–58.
[5] Niemela SM, Ikaheimo I, Koskela M, Veiranto M, Suokas E, Tormala P, Waris T, et al. Ciprofloxacin-releasing bioabsorbable polymer is superior to titanium in preventing *Staphylococcus epidermidis* attachment and biofilm formation in vitro. J Biomed Mater Res B 2006;76:8–14.
[6] Bryers JD, Jarvis RA, Lebo J, Prudencio A, Kyriakides TR, Uhrich K. Biodegradation of poly(anhydride-esters) into non-steroidal anti-inflammatory drugs and their effect on *Pseudomonas aeruginosa* biofilms in vitro and on the foreign-body response in vivo. Biomaterials 2006;27:5039–48.
[7] Smith AW. Biofilms and antibiotic therapy: is there a role for combating bacterial resistance by the use of novel drug delivery systems? Adv Drug Deliv Rev 2005;57:1539–50.
[8] Chopra I. The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern? J Antimicrob Chemother 2007;59:587–90.
[9] Silver S, Phung le T, Silver G. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. J Ind Microbiol Biotechnol 2006;33:627–34.
[10] Sheng J, Nguyen PT, Marquis RE. Multi-target antimicrobial actions of zinc against oral anaerobes. Arch Oral Biol 2005;50:747–57.
[11] Petrini P, Arciola CR, Pezzali I, Bozzini S, Montanaro L, Tanzi MC, Speziale P, et al. Antibacterial activity of zinc modified titanium oxide surface. Int J Artif Organs 2006;29:434–42.
[12] Neel EA, Ahmed I, Pratten J, Nazhat SN, Knowles JC. Characterisation of antibacterial copper releasing degradable phosphate glass fibres. Biomaterials 2005;26:2247–54.
[13] Baena MI, Marquez MC, Matres V, Botella J, Ventosa A. Bactericidal activity of copper and niobium-alloyed austenitic stainless steel. Curr Microbiol 2006;53:491–5.
[14] Gosheger G, Hardes J, Ahrens H, Streitburger A, Buerger H, Erren M, Gunsel A, et al. Silver-coated megaendoprostheses in a rabbit model – an analysis of the infection rate and toxicological side effects. Biomaterials 2004;25:5547–56.
[15] Ueberrueck T, Zippel R, Tautenhahn J, Gastinger I, Lippert H, Wahlers T. Vascular graft infections: in vitro and in vivo investigations of a new vascular graft with long-term protection. J Biomed Mater Res B 2005;74:601–7.
[16] Schierholz JM, Lucas LJ, Rump A, Pulverer G. Efficacy of silver-coated medical devices. J Hosp Infect 1998;40:257–62.
[17] Poon VK, Burd A. In vitro cytotoxity of silver: implication for clinical wound care. Burns 2004;30:140–7.
[18] Cho YH, Lee SJ, Lee JY, Kim SW, Lee CB, Lee WY, et al. Antibacterial effect of intraprostatic zinc injection in a rat model of chronic bacterial prostatitis. Int J Antimicrob Agents 2002;19:576–82.
[19] Romani AM, Maguire ME. Hormonal regulation of Mg^{2+} transport and homeostasis in eukaryotic cells. Biometals 2002;15:271–83.
[20] Staiger MP, Pietak AM, Huadmai J, Dias G. Magnesium and its alloys as orthopedic biomaterials: a review. Biomaterials 2006;27:1728–34.
[21] Saris NL, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium: an update on physiological, clinical and analytical aspects. Clin Chim Acta 2000;294:1–26.
[22] Waksman R, Pakala R, Kuchulakanti PK, Baffour R, Hellinga D, Seabron R, et al. Safety and efficacy of bioabsorbable magnesium alloy stents in porcine coronary arteries. Catheter Cardiovasc Interv 2006;68:607–17. discussion 618–9.
[23] Witte F, Kaese V, Haferkamp H, Switzer E, Meyer-Lindenberg A, Wirth CJ, et al. In vivo corrosion of four magnesium alloys and the associated bone response. Biomaterials 2005;26:3557–63.
[24] Di Mario C, Griffiths H, Goktekin O, Peeters N, Verbist J, Bosiers M, Deloose K, et al. Drug-eluting bioabsorbable magnesium stent. J Interv Cardiol 2004;17:391–5.
[25] Bose D, Eggebrecht H, Erbel R. Absorbable metal stent in human coronary arteries: imaging with intravascular ultrasound. Heart 2006;92:892.
[26] Heublein B, Rohde R, Kaese V, Niemeyer M, Hartung W, Haverich A. Biocorrosion of magnesium alloys: a new principle in cardiovascular implant technology? Heart 2003;89:651–6.
[27] Witte F, Fischer J, Nellesen J, Crostack HA, Kaese V, Pisch A, Beckmann F, et al. In vitro and in vivo corrosion measurements of magnesium alloys. Biomaterials 2006;27:1013–8.
[28] Mousain-Bosc M, Roche M, Polge A, Pradal-Prat D, Rapin J, Bali JP. Improvement of neurobehavioral disorders in children supplemented with magnesium–vitamin B6. II. Pervasive developmental disorder-autism. Magnes Res 2006;19:53–62.
[29] Mousain-Bosc M, Roche M, Polge A, Pradal-Prat D, Rapin J, Bali JP. Improvement of neurobehavioral disorders in children supplemented with magnesium–vitamin B6. I. Attention deficit hyperactivity disorders. Magnes Res 2006;19:46–52.
[30] Rossier P, van Erven S, Wade DT. The effect of magnesium oral therapy on spasticity in a patient with multiple sclerosis. Eur J Neurol 2000;7:741–4.
[31] Mann CK, Yoe JH. Spectrophotometric determination of magnesium with sodium 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)-naphthalene-1′-(2-hydroxybenzene-5-sulfonate). Anal Chem 1956;28:202–5.
[32] Cutler SA, Rasmussen MA, Hensley MJ, Wilhelms KW, Griffith RW, Scanes CG. Effects of Lactobacilli and lactose on *Salmonella typhimurium* colonisation and microbial fermentation in the crop of the young turkey. Br Poult Sci 2005;46:708–16.
[33] Johannsen SA, Griffith RW, Wesley IV, Scanes CG. *Salmonella enterica serovar typhimurium* colonization of the crop in the domestic turkey: influence of probiotic and prebiotic treatment (*Lactobacillus acidophilus* and lactose). Avian Dis 2004;48:279–86.
[34] Ramsey F, Schafer DF. The statistical sleuth: a course in methods of data analysis. Florence (KY): Cengage Learning; 2002. p. 816.
[35] Makar GL, Kruger J. Corrosion of magnesium. Int Mat Rev 1993;38:138–53.
[36] Ferrando WA. Review of corrosion and corrosion control of magnesium alloys and composites. J Mater Eng 1989;11:299–313.
[37] Jones D. Galvanic and concentration cell corrosion. In: Anonymous principles and prevention of corrosion, 2nd ed. Upper Sadle River (NJ): Prentice Hall; 1996. p. 168–98.
[38] Lusk JE, Williams RJP, Kennedy EP. Magnesium and the growth of *Escherichia coli*. J Biol Chem 1968;243:2618–24.
[39] Garcia Vescovi E, Soncini FC, Groisman EA. Mg^{2+} as an extracellular signal: environmental regulation of Salmonella virulence. Cell 1996;84:165–74.
[40] Nelson DL, Kennedy EP. Transport of magnesium by a repressible and a nonrepressible system in *Escherichia coli*. Proc Natl Acad Sci USA 1972;69:1091–3.
[41] Auckenthaler R, Michea-Hamzehpour M, Pechere JC. In vitro activity of newer quinolones against aerobic bacteria. J Antimicrob Chemother 1986;17(Suppl B):29–39.
[42] White D. Membrane bioenergetics: the proton potential. In: The physiology and biochemistry of prokaryotes, 3rd ed. New York: Oxford University Press; 2007. p. 83–119.
[43] Stoor P, Soderling E, Salonen JI. Antibacterial effects of a bioactive glass paste on oral microorganisms. Acta Odontol Scand 1998;56:161–5.

[44] Allan I, Newman H, Wilson M. Antibacterial activity of particulate bioglass against supra- and subgingival bacteria. Biomaterials 2001;22: 1683–7.
[45] Hu S, Chang J, Liu M, Ning C. Study on antibacterial effect of 45S5 Bioglass. J Mater Sci Mater Med 2009;20:281–6.
[46] Yates AA, Schlicker SA, Suitor CW. Dietary reference intakes: the new basis for recommendations for calcium and related nutrients, B vitamins, and choline. J Am Diet Assoc 1998;98:699–706.
[47] Li L, Gao J, Wand Y. Evaluation of cyto-toxicity and corrosion behavior of alkali-heat-treated magnesium in simulated body fluid. Surf Coat Technol 2004;185:92–8.
[48] Li Z, Gu X, Lou S, Zheng Y. The development of binary Mg–Ca alloys for use as biodegradable materials within bone. Biomaterials 2008;29:1329–44.
[49] Zhang E, Xu L, Yu G, Pan F, Yang K. In vivo evaluation of biodegradable magnesium alloy bone implant in the first 6 months implantation. J Biomed Mater Res A 2008. doi:10.1002/jbm.a.32132 [Epub ahead of print].