Design of Impedance-Driven Flow Device for Studying Magnesium Degradation in Simulated Body Fluid

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ABSTRACT

A flow apparatus using an impedance pump was devised to investigate the effect of liquid flow on magnesium (Mg) degradation. Mg has potential to be an effective biomaterial for use inside the human body due to its biocompatibility and biodegradability. Mg undergoes electrochemical reactions with the surrounding fluid causing a loss of Mg from the bulk and increasing the pH of the surrounding fluid. To quantify this degradation process, a flow apparatus consisting of a small flow chamber and an impedance pump was constructed. The setup is low-cost and flexible enough such that Mg degradation can be observed in both flow and static conditions, as well as to take measurements. The pump design has not been used for this kind of investigation before. The average flow rate in the flow apparatus was about 2.8 mL/s. In flow conditions, Mg samples lost their mass at more than three times the rate of the mass loss of the Mg samples under static conditions. Starting with a pH of 7.4, the Mg degradation increased the pH to greater than 8.0 after several days of degradation in the flow chamber, although the samples subjected to flow conditions had a higher rate of pH increase compared to the static condition. This information helps in designing Mg-based implants that may be subject to fluid flow in the human body.

Keywords: Magnesium, Degradation, Corrosion, Impedance Pump, Flow Device, Mass, pH

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Professor Liu's research is to understand cell-biomaterial and tissue-biomaterial interactions in 2D and 3D and to develop better tissue substitutes and medical implant materials using biodegradable polymers, ceramic nanoparticles, polymer/ceramic nanocomposites and bioresorbable metals.

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Elbert Mai is a fourth-year undergraduate student who will graduate summa cum laude in 2013 with a Bachelor of Science in Bioengineering. He is a recipient of a Graduate Research Fellowship Program award from the National Science Foundation and the Marlan and Rosemary Bourns College of Engineering Award from UCR. He has also received Dean's Academic Distinction Awards each year during his undergraduate career at UCR. He is a member of the Tau Beta Pi Engineering Honor Society, California Alpha Beta chapter where he also serves as treasurer for the 2012-2013 academic year. In addition, he is an active member of the Biomedical Engineering Society at UCR. Through these organizations he contributes to outreach and volunteer activities on and off campus. He plans to pursue a Ph.D. in bioengineering at UCSD. Elbert personally thanks his faculty mentor Dr. Huinan Liu for her unwavering support, encouragement, and tutelage during his time at UCR.

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Elbert David Mai

Introduction

The biocompatibility and biodegradability of magnesium (Mg) makes it an excellent candidate for use in biomedical applications and demands further investigation of its properties.[1,2] For example, biodegradable ureteral stents may be prescribed to avoid forgotten indwelling ureteral stents made of non-biodegradable materials.[3] Mg has similar mechanical properties as bone, making them attractive for use in orthopedics.[4] Temporary prostatic stents have been shown to be beneficial to men with micturition problems without significant complications.[5] Because of the biodegradability of Mg, there is no need for a second surgical operation to remove an implant made of Mg after the initial surgical emplacement of the stent.

Mg degrades in two ways: corrosion and embrittlement. Mg is easily oxidized in water such that Mg dissolves in solution as Mg ions.[6] Also, the redox reaction produces hydrogen gas and hydroxide, which increases the local pH and causes embrittlement of the metal due to the hydrogen gas generated.[7] Both these processes cause a mass loss in the metal, which is of interest since it affects the integrity of the bulk substance. Also, the increase in the local pH is of particular interest since pH is tightly regulated in biological systems.[8] Physiologically relevant ions such as chloride ions can induce pitting, while bicarbonate and sulfate ions have been observed to stimulate the corrosion of Mg.[9] Interestingly, phosphate ions inhibits Mg corrosion,[9] reflecting the complex nature of Mg degradation in physiological conditions and emphasizes the need for empirical studies of its degradation.

Since these changes in mass and pH affect implant performance and local cell health, we aim to study Mg degradation subjected to flow conditions using an impedance pump design, which has not been used before for investigating material properties in this manner. The results of such experiments can be used to guide future *in vivo* experiments as part of preclinical tests.[10]

Previous papers have investigated Mg degradation *in vitro* under various conditions. Witte, *et al.* have used ASTM standard immersion tests for Mg degradation, although they do not include physiological flow and does not agree with observed *in vivo* behavior.[11] Other papers have presented flow devices involving Chandler loops,[12] peristaltic pumps,[13] and centrifugal pumps[14] for investigating material properties. However, Chandler loops require a loop that is partially filled with air for generating thrombi.[15] Peristaltic pumps tend to be large and costly, and moving centrifugal pump parts are in contact with the working fluid, making sterilization difficult. Chen, *et al.* devised an elegant method to subject materials to flow conditions, but the method is suitable for only cylindrical-like samples and cannot accept arbitrary sample geometries.[16] Our flow device is designed to address these weaknesses.

Materials and Methods

Figure 1 shows the schematic for the flow apparatus, and Figure 2 shows a realization of the flow apparatus. The components are connected together by 1/4 inch inner-diameter PVC tubing and matching polypropylene barbs throughout the system due to their wide availability, high chemical resistance and low cost. The barbs allow interchangeability of the components. The tubing and components create a closed-loop fluid conveyance for the working fluid, called the flow loop for the rest of this paper.

The working fluid used for the degradation experiments is simulated body fluid (SBF). SBF was developed by Kokubo, *et al.*[17, 18] and its preparation has been covered in previous papers. SBF was chosen since it mimics the ion composition of blood plasma.[17] The pH of the SBF was adjusted to a pH of 7.4 using TRIS buffer to match physiological pH. 1 liter of SBF was prepared and filtered through a 0.22 micron polyethersulfone filter (Fisher Scientific, Barrington IL) and stored at 4 °C. 1 liter was deemed to be a sufficient quantity to perform the experiments in triplicate for flow and static conditions plus a few rehearsal trials.

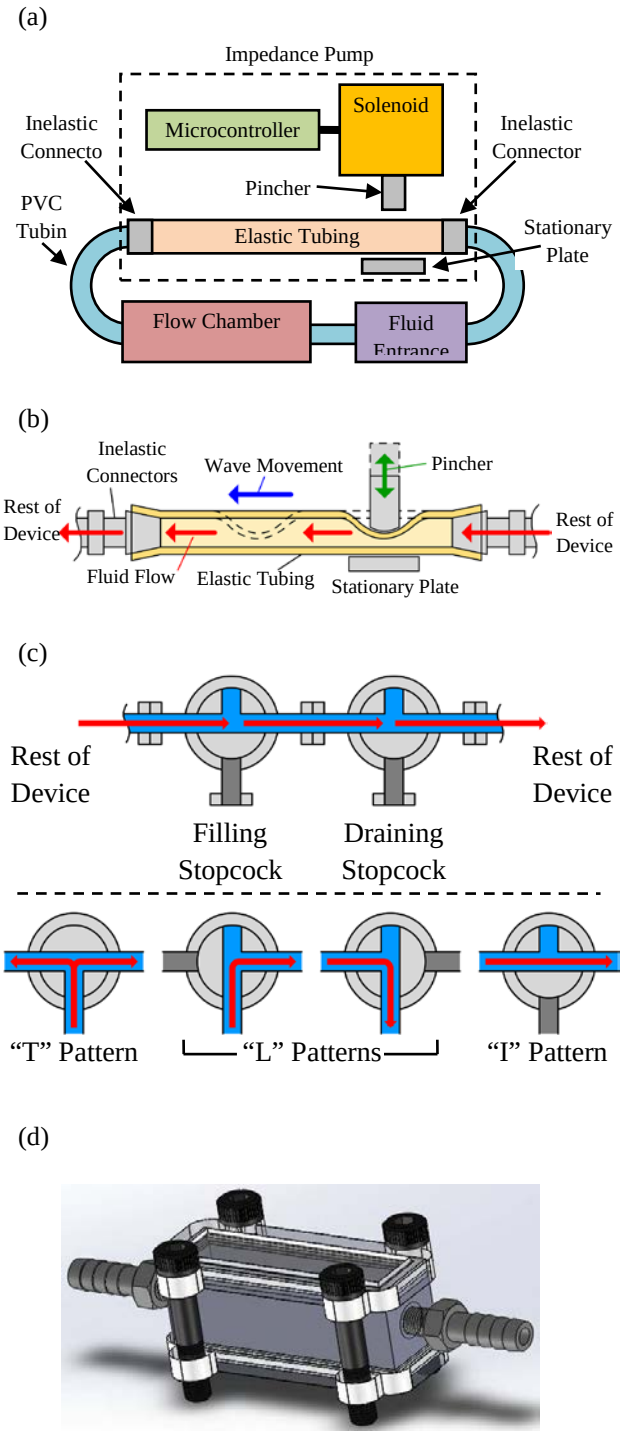


Figure 1. The schematic of the flow device. (a) Overview (b) An illustration of the principle behind the impedance pump. The illustration is not to scale. (c) The dual stopcock configuration that forms the fluid entrance. (d) 3D CAD drawing of the flow chamber used in this flow device.

The flow chamber will hold the Mg sample in place while fluid is being pumped through it. The flow chamber can be made to mimic the target application (e.g. blood vessels). In this study we investigate Mg degradation without regard to any specific body part, thus a simple "box"-type chamber is used (see Figures 2d, 2e, and 2f). Multiple identical chambers were made to allow for triplicate experiments to be performed simultaneously. The chamber is made of anodized aluminum. The outside dimensions of the chamber were 1 × 3 × 1 inch. 3D printed ABS brackets hold glass slides on the top and bottom in place while silicone gaskets were used to prevent leakage between the glass and aluminum components. The entire assembly was secured using four stainless steel hex screws and hex nuts.

The fluid entrance consists of two four-way stopcocks connected in series with the flow loop as shown in Figure 1c. One stopcock is used to deliver the working fluid into the flow loop, while the other stopcock is used to vent the flow loop to allow the fluid to displace any fluid in the loop. This scheme was chosen since it allows for easy fluid transport in and out of the loop without disconnecting any part of the loop and minimizes air bubbles

For the pump, we created a design based on the impedance principle in a novel application. The impedance pump has even less moving parts than a peristaltic pump and thus can be made less expensive and more compact. Figure 1b provides an illustration of the impedance principle. The impedance pump and its properties have been investigated in prior studies.[19, 20, 21] Briefly, an impedance pump consists of a length of elastic tubing with both ends connected to inelastic tubing. A pincher device is placed near one end of the tube and repeatedly pinches the tube at a certain frequency and duty cycle. This particular configuration produces a net flow of the fluid without requiring any valves or many moving parts.[19] The elastic tubing used in these experiments were soft (Shore A35) latex tubing with an inner diameter of 1/4 inch and a wall thickness of 1/32 inch.

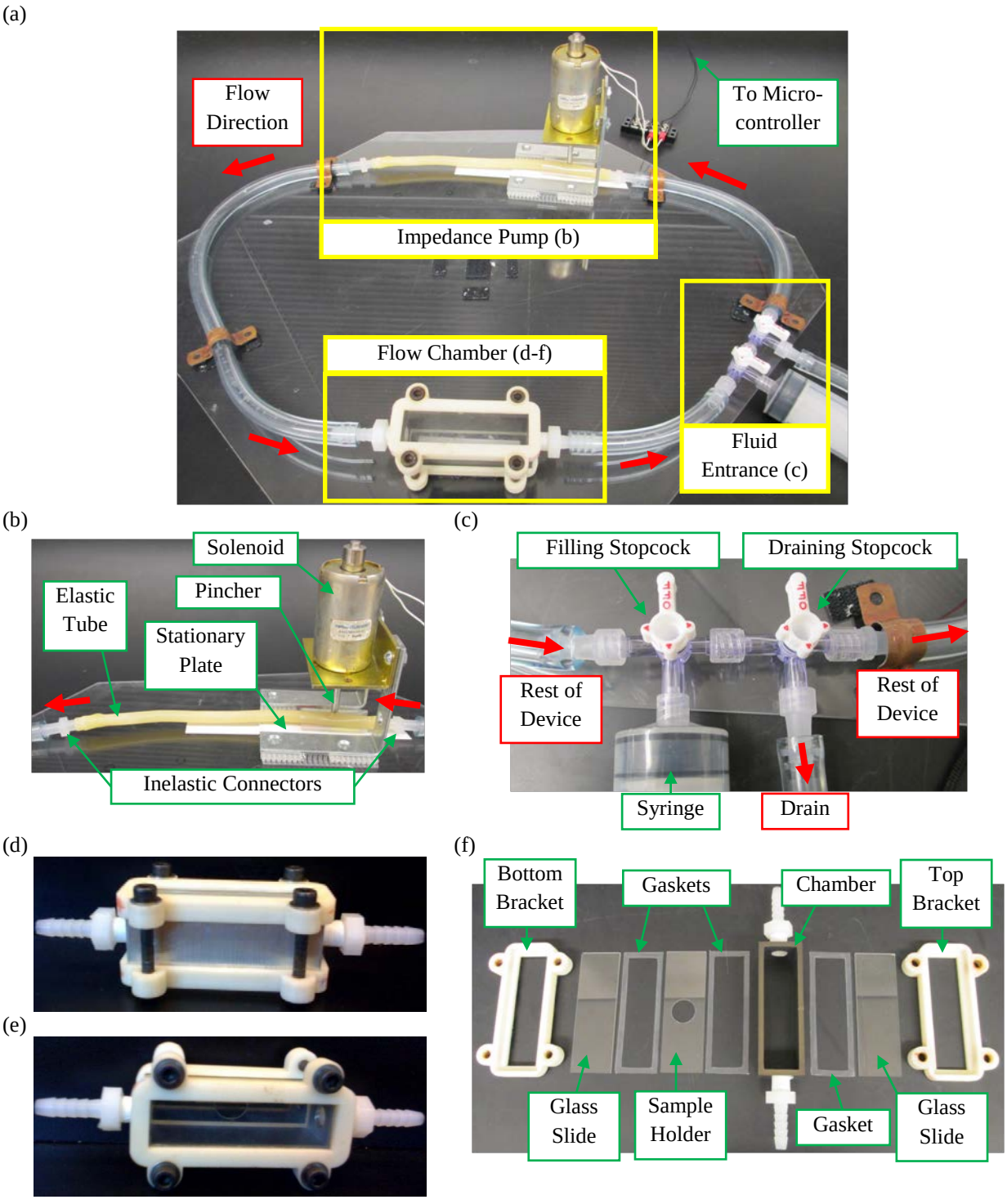


Figure 2: The implementation of the flow device: (a) Overview, (b) Impedance pump, (c) Fluid entranc,e (d) Flow chamber, side view, (e) Flow chamber, top view, and (f) Flow chamber components.

The pump uses an electromagnetic solenoid (Guardian Electric) to drive the pincher. This solenoid is rated for 24 VDC continuous duty use and controlled via a transistor circuit, which is in turn controlled by an Arduino microcontroller. The microcontroller is set to repeatedly activate the solenoid for 45 milliseconds (pinching the tube) then deactivate the solenoid for 250 milliseconds (releasing) the tube, resulting in a frequency of about 3.39 Hz with a duty cycle of about 15.25%. This pumping behavior is fixed for all experiments.

To determine the flow rate given a pump frequency and duty cycle, we used a procedure based on the same flow rate determination principle used for calibrating flow meters in industrial contexts.[22] Briefly, the flow chamber is replaced with a tube section of a known volume and a plastic bead with about the same diameter as the inner diameter of the tube section was inserted. The flow loop was filled with SBF and the pump was turned on. The time it takes for the bead to clear the tube was measured several times to verify the reproducibility of the clearance time. Based on the average measured time and the tube volume, the estimated average flow rate for the current experimental setup is calculated to be about 2.8 mL/s.

The degradation protocol is as follows. Six 98% pure Mg samples were carefully cut from a coil of Mg to produce 5 × 17 mm rectangles. Pictures of the samples were taken and their masses were determined (the "hour 0" mass) Three of the samples were subjected to flow conditions in separate flow loops (the dynamic flow loops), while the other three were subjected to static conditions in flow loops without the pump running (the static flow loops).

Each flow loop is disinfected using 70% ethanol. The ethanol solution is loaded into each flow loop via the fluid entrance and allowed to circulate through the entire loop for several minutes. The ethanol solution was discarded afterwards. The Mg samples were first UV sterilized for 45 minutes then loaded into their corresponding flow chambers. Once the flow chambers are secured, approximately 50 mL of SBF was injected into the flow loops via the fluid entrance. The pumps were turned on the dynamic flow loops, while the pumps were turned off for static flow loops. This starts a single 24-hour cycle. The samples in the static flow loops were allowed to sit. After 24 hours, the pumps were turned off, the working fluid was unloaded from the loop for pH measurements, and the chamber was disassembled to remove the Mg samples. This ends the 24-hour cycle. While the samples may continue to degrade and form oxide layers outside the working fluid, the rate at which this happens will be negligible when placed in the atmosphere compared to being immersed in the working fluid while inside the flow chamber.

The samples were placed in a vacuum chamber to dry overnight. After this drying period, the masses of the samples and images of both front and back of the samples were taken. These images are shown in Table 1. The samples were then UV sterilized for 45 minutes again and the process repeats using fresh SBF for as long as desired. The replacement of SBF in this manner mimics the elimination of degradation byproducts by the circulation in the human body.[23] The experiments were stopped after 4 cycles of the above procedure as we have deemed it to be sufficient time to show significant degradation.

Results and Discussion

Figure 3a shows the mass of the Mg samples with respect to the total time spent in the flow chamber. The time does not include time spent during UV sterilization and drying overnight. The mass is expressed as a percentage of their original starting masses. The rate of Mg degradation on the basis of mass is clearly higher under flow conditions than in under static conditions. At the conclusion of the experiment, the mean mass of the Mg samples was 84.6% of the starting masses in flow conditions, while the mean mass of the Mg samples was 95.5% of the starting masses in the static conditions. Assuming that the rate of change of Mg degradation is constant, the mean rate of percentage change of masses of the samples subjected to flow conditions were −0.00169 %/hour (R^2 = 0.984), while the mean rate of change of masses of the samples subjected to static conditions were −0.000478 %/hour (R^2 = 0.982).

We determine from the two-sample *t*-test (with $n = 3$ and degrees of freedom = 4) that the *p*-value is much less than 0.05. Thus we conclude that the data supports the fact that the flow rate had a significant effect on the degradation rate of the Mg. In fact, the mean mass loss rate of the samples subjected to flow conditions were about 354% of the mean mass loss rate of the samples subjected to static conditions. This is due to the fluid flow sweeping the reaction products away from the degradation sites allowing the reactions to proceed at a relatively fast rate.

Figure 3b shows the pH of the working fluid at the conclusion of each 24-hour cycle described previously. The pH of the working fluid became more basic relative to the initial preparation of SBF in all trials. Specifically, the pH of the working fluid from the dynamic flow loops increased from 7.4 to 7.89, 8.21, 8.49, and 8.11 at cycles 1, 2, 3, and 4 respectively. The pH of the working fluid from the static flow loops increased from 7.4 to 7.77, 7.87, 7.99, and 8.11 at cycles 1, 2, 3, and 4 respectively. The general trend is that the pH increases with the length of time the Mg samples have been subjected to flow or static conditions. This is due to the formation of hydroxide ions as a result of the redox reactions discussed earlier.

An exception to the trend was the pH of the working fluid from the dynamic flow loops at cycle 4, which is actually less than the pH at cycle 3. Xin, *et al*. have observed that bicarbonate ions found in SBF and in the plasma of living things (as part of the natural plasma buffering system) speed up the corrosion of Mg by consuming the hydroxide produced by the degradation reaction[24] discussed earlier, shifting the equilibrium in favor of the degradation products. Xin, *et al*. also notes that bicarbonates promote the precipitation of insoluble carbonates on the surface of the Mg; effectively passivating it and reducing the reaction rate.[24] The passivating surface precipitation can be seen in Table 1, especially after an incubation time of 96 hours. The combination of these two mechanisms helps explain the rising then falling trend in the pH under flow conditions. As discussed earlier, the flowing fluid sweeping away the degradation products enhance the reaction rate, allowing the passivation to occur earlier than under static conditions. Clearly, the degradation process is a complex phenomenon which must be taken into account when designing Mg-based implants.

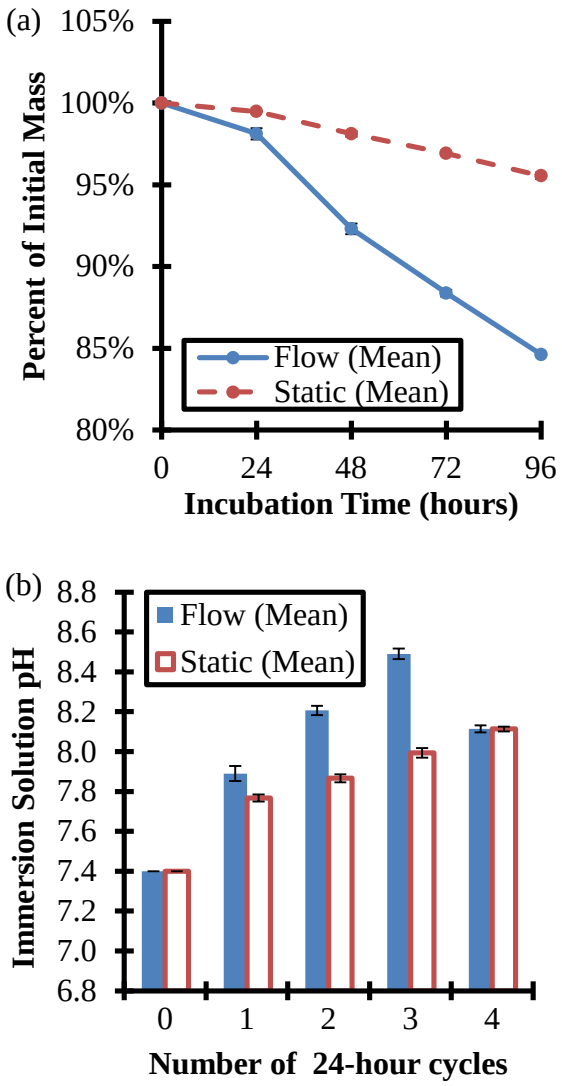


Figure 3: (a) Mass change of Mg samples in flow and static conditions (b) pH change of SBF working fluid in flow and static conditions at the end of individual 24-hour cycles (see text). Each data point in both graphs represents the mean of the three samples under the specified conditions. Note the incubation time does not include time spent UV sterilizing or drying overnight. The error bars represent ± standard error of the mean (n = 3 for each data series).

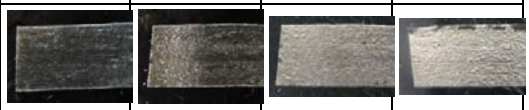
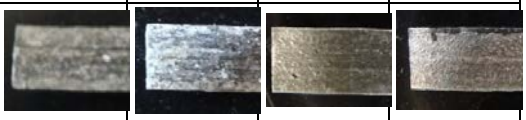
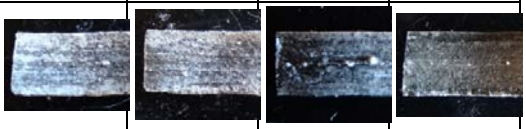
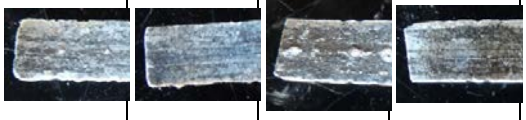
Representative Mg Appearance under Flow and Static Conditions				
Incubation Time (hours)	Flow		Static	
	Front	Back	Front	Back
0				
24				
48				
72				
96				

Table 1: Images of Mg samples at various incubation times (does not include time spent UV sterilizing or drying overnight). The times correspond with the x-axis of Figure 3a. The images at hour 0 were taken before the start of the experiments. The rest were taken after overnight drying.

Conclusion

We were able to construct a flow apparatus based on the impedance principle to investigate Mg degradation under controlled conditions; namely, the average flow rate and the working fluid. This flow apparatus was relatively simple to construct and required no particularly expensive parts, thanks to the straightforward construction of the impedance pump. Using the flow apparatus we were able to obtain the mass of the Mg samples (Figure 3a), pH of the working fluid (Figure 3b), and images of some of the Mg samples themselves (Table 1). This demonstrates the viability of the impedance pump-based design of the flow apparatus and represents a novel application of such a design.

We have shown that Mg placed in flow conditions degraded at a significantly higher rate than Mg placed in static conditions. We find that the Mg degraded more than three times faster under a flow rate of about 2.8 mL/s than in static conditions. This degradation characteristic is especially relevant in high flows such as blood flow in arteries. We have also shown that the pH of the working fluid became more basic at a higher rate under flow conditions than in static as the Mg degraded. It is clear that flow conditions have a significant and complex effect on Mg degradation and should be considered in any biomedical application of Mg.

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