



Full length article

## *In vivo* monitoring the biodegradation of magnesium alloys with an electrochemical H<sub>2</sub> sensor



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### ABSTRACT

Monitoring the biodegradation process of magnesium and its alloys *in vivo* is challenging. Currently, this process is monitored by micro-CT and X-ray imaging *in vivo*, which require large and costly instrumentation. Here we report a simple and effective methodology to monitor the biodegradation process *in vivo* by sensing H<sub>2</sub> transdermally above a magnesium sample implanted subcutaneously in a mouse. An electrochemical H<sub>2</sub> microsensor was used to measure the biodegradation product H<sub>2</sub> at the surface of the skin for two magnesium alloys (ZK40 and AZ31) and one high purity magnesium single crystal (Mg8H). The sensor was able to easily detect low levels of H<sub>2</sub> (30–400 μM) permeating through the skin with a response time of about 30 s. H<sub>2</sub> levels were correlated with the biodegradation rate as determined from weight loss measurements of the implants. This new method is noninvasive, fast and requires no major equipment.

#### Statement of Significance

Biomedical devices such as plates and screws used for broken bone repair are being developed out of biodegradable magnesium alloys that gradually dissolve when no longer needed. This avoids subsequent removal by surgery, which may be necessary if complications arise. A rapid, non-invasive means for monitoring the biodegradation process *in vivo* is needed for animal testing and point of care (POC) evaluation of patients. Here we report a novel, simple, fast, and noninvasive method to monitor the biodegradation of magnesium *in vivo* by measuring the biodegradation product H<sub>2</sub> with an electrochemical H<sub>2</sub> sensor. Since H<sub>2</sub> rapidly permeates through biological tissue, measurements are made by simply pressing the sensor tip against the skin above the implant; the response is within 30 s.

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### 1. Introduction

Biodegradable implants such as plates and screws for bone repair offer many advantages over the permanent stainless steel and titanium implants used today [1–10]. Importantly, implants based on biodegradable materials eventually dissolve when no longer needed, eliminating the need for surgically removing the devices later if complications arise. Magnesium based alloys are

an exceptionally good candidate for several reasons. Magnesium is a light weight metal (1.74–2.0 g/cm<sup>3</sup>) that closely matches the density of bone (1.8–2.1 g/cm<sup>3</sup>) [11]. Magnesium based alloys are also typically 4.5 times and 3.3 times less dense than stainless steel and titanium based alloys, respectively [11–12]. Magnesium alloys have a modulus of elasticity of ~45 GPa, which is very close to that of human bone (45–57 GPa) [1,7,13–14]. These properties lessen the stress shielding and the associated loss of bone density. Moreover, magnesium is biocompatible, and a relatively large amount of magnesium is tolerated by the body without ill effects [15]. Additionally, due to functional roles and presence in bone tissues,

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magnesium has stimulatory effects on the growth of new bone tissue [11,16–19]. The rate of biodegradation can be controlled by using alloys or coatings to match the needs of the application [3,20].

Magnesium and its alloys degrade in aqueous environments, generating magnesium ( $Mg^{2+}$ ) and hydroxide ( $OH^-$ ) ions, as well as hydrogen ( $H_2$ ) gas. When the pH becomes sufficiently basic,  $OH^-$  reacts with  $Mg^{2+}$  to precipitate as magnesium hydroxide ( $Mg(OH)_2$ ) on the surface. One molecule of  $H_2$  is liberated for every mole of metallic Mg that biodegrades. Thus, measuring the evolved  $H_2$  allows a direct measurement reflecting the corrosion of magnesium [21]. Because 1 mL of  $H_2$  evolved approximately corresponds to 1 mg of magnesium dissolved [22], measuring  $H_2$  evolution rate is equivalent to measuring the biodegradation or corrosion rate of a magnesium alloy [23–24]. So far, the measurement of  $H_2$  evolution is mostly performed *in vitro* by measuring the generated  $H_2$  volume [21–23]. This method is cumbersome, and moreover, the  $H_2$  gas collection instruments often suffer leakage. Monitoring the biodegradation process *in vivo* can be done by micro-CT, which requires a major equipment investment, and by X-ray absorption, which is also expensive, involves exposure to radiation [8–9,25–30], and requires further time consuming complex analysis involving understanding the difference in X-ray absorption coefficients of the different corrosion products formed for quantization. Therefore, a simple and effective *in vivo*  $H_2$  sensing method is needed for animal testing of alloys and devices.

Monitoring biodegradation *in vivo* by sensing the corrosion product  $H_2$  has numerous advantages over sensing the other corrosion products  $OH^-$  and  $Mg^{2+}$ . First, only  $H_2$  has the possibility of being monitored noninvasively because of its high permeability through skin [31–33]. This is a significant advantage compared to the surgical insertion of a sensor as would be the case for pH or  $Mg^{2+}$ . Noninvasive measurement also means that the sensor would not be subject to possible interferences by components of biological fluids or biofouling. Second, no significant background level of  $H_2$  exists in mammals that needs to be corrected for. For example, the concentration of  $H_2$  in mouse blood is only ca. 1  $\mu M$  [34]. Background level is an issue with  $Mg^{2+}$  where a relatively high concentration exists *in vivo* ( $Mg^{2+}$  concentration in adult serum is 0.75–0.95 mM [35]). A sensor for  $Mg^{2+}$  would need to have sufficient precision to detect small increases due to biodegradation above this substantial background. Also, the commonly used electrochemical sensor for  $Mg^{2+}$ , an ion-selective electrode, suffers serious interference from  $Ca^{2+}$ , which exists *in vivo* at a higher concentration (ca. 2.0–2.6 mM in adult serum [35]). Third,  $H_2$  is relatively nonreactive in biological media, making it a robust biodegradation marker [36–40]. By comparison, released  $OH^-$  will be consumed by buffer, which severely compromises the measurement of pH for monitoring biodegradation. Furthermore,  $Mg^{2+}$  reacts with various anions such as  $OH^-$  and carbonate to form precipitates and with naturally occurring organic ligands (such as lactate and citrate) and proteins to form complexes that might obscure it from a sensor such as an ion-selective electrode [41–42]. Fourth, a commercially available electrochemical  $H_2$  sensor with excellent limit of detection and selectivity already exists [31,43].

The formation of gas cavities associated with implanted Mg alloys has been widely reported in literature [44–46]. However, the concentration of  $H_2$  in these cavities was not clearly known, as only a few studies using techniques not specific for  $H_2$  were done when magnesium was first tested as an implant over 60 years ago [47–48]. Nothing was reported since that time and most researchers assumed that these cavities contain primarily  $H_2$  [2,23,49–50]. We tested this assumption by implanting rapidly degrading Mg alloy (Mg-4 wt%Y-0.5 wt%Gd-2 wt%Nd-0.5 wt%Dy) discs subcutaneously in mice and determining the concentration of  $H_2$  gas using an electrochemical  $H_2$  sensor that was inserted into

the cavities through incisions made in the skin. The results were confirmed by gas chromatography-mass spectrometry (GC-MS) [31]. Two significant discoveries were made: (1) First, the concentration of  $H_2$  in the gas cavities was actually very low, even shortly after formation of the cavities. The  $H_2$  concentrations in the gas cavities measured with the sensor one, two and five days after surgery ranged from  $95 \pm 34 \mu M$  ( $0.22 \pm 0.08 \text{ vol.}\%$ ) to  $428 \pm 35 \mu M$  ( $0.97 \pm 0.08 \text{ vol.}\%$ ). These low levels of  $H_2$  were confirmed using GC-MS on samples collected from the gas cavities by a syringe. Thus, the levels of  $H_2$  in the gas cavities were found to be consistently less than only 1%. The balance of the gas was carbon dioxide and oxygen, both measured by GC-MS, and presumably nitrogen, which could not be quantified as it was the carrier gas in the GC. These results point to very fast transport of  $H_2$  directly through the biological tissue surrounding the implant. It should also be noted that  $H_2$  is not very soluble in water, the concentration at saturation being only 805  $\mu M$  at 20 °C at sea level [51–52]. The low  $H_2$  levels in the gas cavities can be explained by this fast transport combined with the low solubility of  $H_2$  in aqueous biological fluids and cells. (2) Second, measurements made noninvasively by just pressing the sensor tip against the skin covering the implant were similar to those made invasively by inserting the sensor tip inside the cavity. This observation confirms the extraordinarily fast transport of  $H_2$  through biological tissues such as skin. Most importantly from a practical point of view, this means that  $H_2$  levels *in vivo* can potentially be tracked noninvasively by a  $H_2$  sensor measuring transdermally by simply pressing it against the skin. Realizing that the skin is so permeable to  $H_2$  and that the electrochemical  $H_2$  sensor is extremely sensitive has indeed opened the pathway to develop non-invasive  $H_2$  sensing as an effective way to track *in vivo* biodegradation rates of magnesium implants [31].

Here we report the transdermal measurement of  $H_2$  from three biodegrading alloys implanted subcutaneously in mice with an electrochemical  $H_2$  sensor. We also correlate the  $H_2$  levels with the biodegradation rates obtained by weight loss of the explanted alloys. To the best of our knowledge, this is the first time that measuring  $H_2$  transdermally with a  $H_2$  sensor has been a routine part of a subcutaneous evaluation of Mg alloys. The measurements are rapidly made noninvasively by just pressing the sensor tip against the skin covering the implant. No major equipment is required such as with micro-CT and X-ray and moreover, there is no exposure to X-ray radiation. This simple method opens the way to developing non-invasive  $H_2$  sensing as an effective way to track biodegradation rates of Mg and its alloys *in vivo* and noninvasively.

## 2. Experimental

### 2.1. Implant materials

The Mg alloy ZK40 with Mg-4 wt% Zn-0.5 wt% Zr-1.4 wt% Cu-0.2 wt% Fe-0.3 wt% Mn-1.8 wt% Ni-0.7 wt% Si was prepared by a previously described method [6]. AZ31 alloy that contained 2.5–3.5 wt% Al, 0.6–1.4 wt% Zn and 0.2–1.0 wt% Mn with the remainder being Mg was purchased from Goodfellow (Oakdale, PA). The processing of the alloy was based on a previous method [4]. Typical preparation of the high purity magnesium single crystal Mg8H is described below. A crystal grower made by CVD Equipment Corporation was employed as a main tool. The initial poly-crystalline magnesium with purity of 99.95% (Alfa Aesar) was used as raw material to grow single crystals. A graphite crucible with a tapered shape was used to contain the molten material which was enclosed in an external holder made of a special grade stainless steel. The growth process took place in a vertical quartz tube under argon flow. The tube was surrounded by a vertical crystal furnace. The furnace had two temperature zones to create and control an appro-

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