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Ex vivo blood vessel bioreactor for analysis of the biodegradation of magnesium stent models with and without vessel wall integration

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A B S T R A C T

Current *in vitro* models fail in predicting the degradation rate and mode of magnesium (Mg) stents *in vivo*. To overcome this, the microenvironment of the stent is simulated here in an *ex vivo* bioreactor with porcine aorta and circulating medium, and compared with standard static *in vitro* immersion and with *in vivo* rat aorta models. In *ex vivo* and *in vivo* conditions, pure Mg wires were exposed to the aortic lumen and inserted into the aortic wall to mimic early- and long-term implantation, respectively. Results showed that: 1) Degradation rates of Mg were similar for all the fluid diffusion conditions (*in vitro* static, aortic wall *ex vivo* and *in vivo*); however, Mg degradation under flow condition (i.e. in the lumen) *in vivo* was slower than *ex vivo*; 2) The corrosion mode in the samples can be mainly described as localized (*in vitro*), mixed localized and uniform (*ex vivo*), and uniform (*in vivo*); 3) Abundant degradation products ($MgO/Mg(OH)_2$ and Ca/P) with gas bubbles accumulated around the localized degradation regions *ex vivo*, but a uniform and thin degradation product layer was found *in vivo*. It is concluded that the *ex vivo* vascular bioreactor provides an improved test setting for magnesium degradation between static immersion and animal experiments and highlights its promising role in bridging degradation behavior and biological response for vascular stent research.

Statement of Significance

Magnesium and its alloys are candidates for a new generation of biodegradable stent materials. However, the *in vitro* degradation of magnesium stents does not match the *clinical* degradation rates, corrupting the validity of conventional degradation tests. Here we report an *ex vivo* vascular bioreactor, which allows simulation of the microenvironment with and without blood vessel integration to study the biodegradation of magnesium implants in comparison with standard *in vitro* test conditions and with *in vivo* implantations. The bioreactor did simulate the corrosion of an intramural implant very well, but showed too high degradation for non-covered implants. It is concluded that this system is in between static incubation and animal experiments concerning the predictivity of the degradation.

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1. Introduction

Magnesium (Mg) and its alloys have received considerable attention as candidates for a new generation of biodegradable stent materials [1,2]. For example, absorbable Mg-based stents have been tested in various *in vitro* environments to evaluate their biosafety in vascular applications [3,4]. Meanwhile, they have been studied for several years in animal and clinical trials with encouraging results [5,6]. However, the degradation behavior tested in

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in vitro is not comparable with that in *in vivo* and there are obvious differences between *in vitro* and *in vivo* degradation rates and modes [7]. Although in 2014 ISO/TS 17137 has suggested extracorporeal and pre-clinical *in vivo* concepts to evaluate cardiovascular absorbable implants, no recommended method currently exists for *in vitro* analysis of the degradation behavior of absorbable metals to predict the *in vivo* situation due to the different test conditions and the complex reactions at the implant-tissue interface [8]. Up to now, there is an ongoing discussion about the reliability and predictability of the applied *in vitro* methods [9]. The challenges to build the systematic test system include i) the lack of *in vitro* models that sufficiently mimic the *in vivo* conditions [10]; ii) insufficient understanding of the factors that influence degradation behavior of Mg-based materials *in vitro*, which are keys to predict *in vivo* degradation [11]; and iii) a paucity of thorough knowledge of degradation mechanisms *in vivo*. The accurate determination of the degradation of bio-absorbable metals is a technologically important issue in science and biomedical applications [12]. A key step to develop current standards is to identify and test the biological, biochemical and biophysical microenvironment and develop parameters in test-systems for the specific applications.

Every medical device undergoes an interaction with the bio-environment, in which its surface is exposed to organic [4] and inorganic [13] constituents, followed by a complex molecular, blood [3], cell [14] and tissue response [15]. Also, the fluid dynamics [16], gas supply and diffusion [4], pH [17] and temperature have impact on the degradation [18]. This "physiological degradation" was defined by Willumeit et al. to describe degradation under cell culture conditions and the relevance of the influencing factors was determined by neural network analysis [19]. The degradation of a stent causes ion release and pH change, which in turn affect the host cell and biological microenvironment. Furthermore, the parameters from the complex remodeling of the vascular microenvironment critically influence the degradation behavior of absorbable stents. That is, the biodegradation behavior is modified by different microenvironments at the early- and long-term implantation process (i.e. the stages before and after endothelialization). In the physiologically hydrodynamic vascular system, the stent is exposed to the blood before the endothelialization on the stent. The fluid shear force acting on the vessel wall is transduced into biochemical and biophysical signals, leading to various degradation behaviors. In our previous studies, a couple of *in vitro* bioreactors, such as the varied flow bioreactor with computational fluid dynamic calculation [16] and an *in-situ* and *real-time* electrochemical monitoring bioreactor [20], have been developed to simulate the *in vivo* conditions and to reveal the biodegradation behavior and mechanism of Mg-based alloys and stents. It was demonstrated that the fluid flow has a significant impact on the degradation of absorbable metallic stents, including degradation kinetics, degradation modes, degradation rates, degradation products, and local pH changes [21,16]. After the stent is covered by neointima [22], it is no longer exposed to flow and degradation is dominated by diffusion of water, hydrophilic solutes and ions between vascular tissue and the stent. Besides hydrodynamics, the tissue response is also a vital factor to affect the degradation behavior of Mg-based stents [23]. For simulation of the degradation, it is of fundamental concern to control the *in vitro* environment in a way that approximates the *in vivo* physiological conditions during the early- and long-term implantation process [19].

Due to the complexity of the arterial wall structure and the difficulty to grow an artery *in vitro*, it is challenging to build an *in vitro* simulation model of this case. Therefore, investigation of a real artery cultured in a perfusion bioreactor might be a promising approach to provide a more simulated vascular environment. In this study, an *ex vivo* aorta model using a vascular bioreactor was developed to study Mg biodegradation. Results are systematically compared with *in vitro* standard immersion and *in vivo* assessments. This bioreactor was expected to determine Mg degradation behavior in more accurate parameters than the *in vitro* assay, for a better understanding of the *in vivo* degradation behavior of Mg-based stents.

2. Materials and methods

2.1. Pure magnesium wires preparation

As-drawn magnesium wire of 99.9% purity purchased from Goodfellow USA (Coraopolis, PA) was used as Mg implants. Impurities included <1 ppm Ag, 10 ppm Ca, 5 ppm Cu, <1 ppm Na, 100 ppm Si, and 100 ppm Fe. Samples were polished with 1200 grit abrasive paper, using isopropanol as a lubricant. The diameter of the wire was 250 ± 5 μm after polishing. The wire was cut into segments with the length of 1 cm. The wires were sterilized by ultraviolet radiation overnight prior to surgery.

2.2. *Ex vivo* model – a vascular bioreactor

The protocols for collecting aortas were taken from the international guidelines on animal experiments, and approved by Institutional Animal Care and Use Committee (IACUC). One-year old mixed Danish Landrace and Yorkshire pigs weighing approximately 200 kg were obtained from Piedmont Custom Meats (former Matkins meat processors, Gibsonville, NC, USA). The porcine abdominal aortas were immediately collected from the slaughtered pigs and perfused by phosphate buffer solution to remove the blood. The aortas were immediately stored in Dulbecco's modified Eagle's medium (DMEM, Lonza®, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS). The aortas were cut into segments with the length of 5 cm. All the aortas were maintained on ice before use.

The LumeGen bioreactor (TGT DynaGen® Series, USA) was chosen to provide a controllable environment for simulating physiological aortic conditions. The bioreactor consisted of blood-vessel chambers, variable-flow pump, hertz oscillator, flow control channel, culture medium, reservoir and incubator (Fig. 1a). A schematic diagram of the bioreactor system is shown in Fig. 1b. The entire system was sterilized by autoclavation.

Two Mg wires with the length of 1 cm were implanted into one aorta at two different positions (one exposed to the lumen and the other embedded in the wall) introduced by a syringe needle. The two implanted positions should simulate the stent surrounding environment at the early- and long-term implantation, respectively. A single aorta with two Mg wires was installed into each chamber (Fig. 1c), and the fluid through each aorta was from one reservoir (500 ml). DMEM with 10% FBS and 1% PS was chosen as a pseudo-physiological medium. The pH value of the solution was adjusted to 7.40 ± 0.05 prior to the test. Each chamber was filled with medium to a volume of 50 ml. The flow rate of the circulating medium was set to 100 ml/min. The luminal pressure oscillated in a range from 80 to 120 mmHg with a pulse frequency of 1 Hz, thus diastolic and systolic status of the aorta was simulated under the flow condition (Seen in Supplementary Video 1 and Fig. S1). As a control, another aorta with two Mg segments also was immersed into the chamber with static medium. Then, the setup was kept in the incubator at 37 °C and 5% CO_2 for 5 days. The culture medium was refreshed at the 3rd day, reproducing standard culture conditions. At 3rd and 5th day, all the samples were delicately removed from the bioreactor and transferred into a 5 ml centrifuge tube with DMEM for further analysis of the degradation behavior. Then, the aortas were fixed in 4% paraformaldehyde solution (Affymetrix USB 199431LT) overnight for

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