Magnesium stents – fundamentals, biological implications and applications beyond coronary arteries

Abstract: Permanent metallic stents have improved the treatment of diseases like coronary heart disease. Although superior to balloon angioplasty, the persistent presence of a metallic stent limits their application and yields several problems like late thrombosis, restenosis and chronic inflammation reactions. Biodegradable magnesium stents have been introduced to solve these problems. Additionally, in pediatric cardiology or for advanced applications like minimally invasively implanted tissue engineered heart valves, the use of degradable stents is mandatory for best success of the treatment. After implantation of a stent, the healing process starts in the vessel. In many cases, the stent is only needed during the initial phase of this process (up to around 6 months). When the stent is degraded, complete healing, return of vasomotion and growth of the vessel are possible. Several magnesium stents have been tested with promising results. However, there is still a huge demand in further research on new alloys and stent designs. Beside coronary stents, other applications should be addressed as well. A better understanding of the interaction between body and stent as well as correlating in vitro and in vivo degradation tests in a predictive manner are needed.

Keywords: biodegradable; in vitro-in vivo correlation; pediatric cardiology; wall shear stress.

Introduction

Before the introduction of stents into clinical practice, balloon angioplasty was the only percutaneous treatment available for atherosclerotic cardiovascular disease. Balloon angioplasty carries risk of acute restenosis caused by recoil, thrombosis, neointimal (NI) hyperplasia or constrictive remodeling. Bare metal stents (BMS) were introduced to overcome these problems. The stents prevent acute recoil and constrictive remodeling, but are susceptible to acute or late thrombosis, and they inhibit positive remodeling and vasomotion. Drug-eluting stents (DES) were invented to reduce NI proliferation and subsequent clinical restenosis [1, 2]. The incorporated drug (e.g., paclitaxel or sirolimus) suppresses smooth muscle cell (SMC) proliferation or migration, resulting in less NI proliferation and less restenosis. However, this also leads to a delayed healing [3]. Although DES show better performance than BMS, they have an increased incidence of late stent thrombosis because of poor re-endothelialization, inflammation reactions, and the degradation of the drug-containing polymer [4]. Also, the presence of the permanent metallic cage still precludes normal vessel physiology [5].

The next logical step was the development of biodegradable stents (BDS). BDS prevent early vascular recoil like BMS or DES, but when no longer needed, they gradually lose their scaffolding ability followed by complete degradation. When no stent material is left, late positive remodeling, biological vasomotion and growth should be possible. Also, future interventions and surgical procedures can be carried out unhindered [6, 7]. It has been shown that NI proliferation is limited to the first weeks after balloon angioplasty, thus there is no longer need for anti-proliferative drugs [8].

The exact time the mechanical integrity of a stent is needed is not known exactly. Negative remodeling seems to be limited to the first 6 months, thus mechanical scaffolding is required only during this period [9]. The remodeling process of the vessel until healing is expected to go
on for another 6 months. This gives a desired degradation rate as depicted in Figure 1. The stent should have a constant mechanical strength for 6 months, and then gradually lose its scaffolding ability while slowly degrading. After 12 months, the remodeling process is finished and only stent remnants are left [10]. However, the exact time points are not known and, as will be shown later, also depend on the intended application and the target vessel.

Most research on BDS focusses on coronary applications [1, 2]. The first degradable polymer stent implanted in humans was the Igaki-Tamai stent. It was made from poly-l-lactic acid, and 25 stents were placed in the coronary arteries of 15 patients [11]. Some other applications like scaffolding the left pulmonary artery of a preterm baby [12] have also been investigated, with the size of the stent still being in the coronary range. However, the application of BDS is not limited to coronary interventions. The use of degradable magnesium (Mg) stents for critical limb ischemia has been reported [6, 13], and tracheal [14, 15], esophageal [16] or urethral [17] stent applications may be possible.

Advantages of biodegradable stents in pediatrics

Stent implantation in pediatric patients is an established method for treatment of stenotic vessels or vascular malformations [18]. Most often they are indicated in patients were balloon dilatation alone fails. In congenital heart disease patients undergo more frequent stent implantations including stenting of the pulmonary artery and the aorta [18]. Different stents and sizes are available and they may be used according to age and size of the patient. However, the majority of these stents are not labeled for these indications. For example, bare metal stents intended for coronaries are used in small children and newborn, peripheral stents from adults are used for children of older age and up to adolescents. In adult patients bigger stents (CP-, Andra XL- or XXL-, Advanta V12- or IntraMax or Mega-Ev3-stents) may be used with an expansion range of 10−24 mm [18−20]. The stent material differs and includes cobalt chromium, stainless steel and platinum iridium (Cheatham Platinum, CP-Stent) [18, 19]. Stents covered with extruded polytetrafluoroethylene (e-PTFE) are also used in order to seal the vessel at the site of the stent implantation and to treat aneurysms. Covered CP-stents (NuMed Inc., Hopkinton, NY, USA) and Advanta V12 (Atrium Medical Corporation, Maquet Getinge group) stents may cover wide ranges of implantation possibilities and sizes [19, 20].

Stents implanted in pediatric patients may work as efficiently as in adults for a while. With ongoing growth of the patient, a BMS becomes a problem as it does not grow, resulting in a stenosis of the stented lesion. Re-dilation of balloon expandable stents may be an option for a while, but most of the stents show only restricted possibilities for further expansion. In regard to a growing patient and vessel, especially in younger aged patients, the stent options are limited in order to achieve a long lasting effect and surgical removal may become necessary. The presence of a stent straightens the vessel and affects the blood flow pattern up- and downstream of the treated lesion. The resulting wall shear stress (WSS) can have severe influence on vessel growth [21, 22].

Beside the treatment of stenosis, stents can be implanted as carrier of prostheses like tissue patches or heart valves. Some implants like decellularized xenogous [23], decellularized homologous [24] or living tissue engineered autologous [25] heart valves have the possibility to grow. In combination with a degradable stent these prostheses can be implanted minimally invasively and, after degradation of the stent, grow with the patient.

The main advantage of BDS in pediatrics may be their potential of enabling growth. However, especially in congenital heart disease, imaging techniques like computed tomography (CT) or magnetic resonance imaging (MRI) represent important diagnostic tools. Due to the low atomic weight of magnesium, Mg stents are not visible in fluoroscopy. Although complicating the implantation, this is a huge advantage for later cardiac imaging as the stent does not cause artifacts in X-ray or CT [8]. The paramagnetic properties contribute to good MRI quality without severe artifacts caused by an Mg-stent [6].

![Figure 1: Idealized progress of degradation (solid line) and loss of mechanical integrity (dashed line). Reprinted from [10] with permission from Elsevier.](image-url)
Why Magnesium?

Polymers and metals are the two basic material classes available for BDS production. First investigations focused on polymeric materials, mostly polylactic and polyglycolic acid copolymers or polycaprolactone [2, 10, 26]. However, polymers have several shortcomings when implanted in the vascular system. They can cause high inflammation response [27], and when compared to metals, the poor mechanical properties like lower Young’s modulus, lower ultimate tensile strength (UTS) or higher elastic recoil become evident [10, 28]. In order to achieve the same radial stiffness as metallic stents, polymeric struts have to be thicker, increasing the risk of side-branch occlusion [1]. Another shortcoming of polymers is the long time until complete degradation. In one study, 28 months after implantation of the ABSORB stent (polylactide; Abbott Vascular, Santa Clara, CA, USA), a patient developed critical restenosis. At this time point most struts were still present [5].

The other material class is biodegradable metals which can be divided into two alloying systems, either iron (Fe) or magnesium (Mg) based. On the one hand, the mechanical properties of Fe (Young’s modulus ~200 GPa, elongation ~40%) are close to SS316L (Young’s modulus ~193 GPa, elongation ~40%), which can be considered a benchmark material for stents. On the other hand, the in vivo degradation of pure iron stents is too slow. Alloying with manganese seems to be a promising path to shorten degradation time [10]. However, the degradation products can emerge as voluminous flakes, bearing a risk of inducing new lesions [29].

Mg is an essential trace element involved in more than 300 cell reactions and generally considered non-carcinogenic [30]. Its physiologic plasma content lies between 0.70 and 1.05 mmol/L, and it has a toxicity threshold between 2.5 and 3.5 mmol/L. Considering that the weight of a coronary stent is between 3 and 6 mg, the toxicity level will not be reached [26]. Even a large stent with a length of 30 mm and a crimped diameter of 6 mm will not exceed 450 mg of Mg, which is close to the recommended daily oral intake.

Magnesium itself has medical application. A high-dose infusion of Mg can cause vasodilation and recruitment of collaterals during ischemia [31]. Mg has antiarrhythmic properties [32]. By contrast, Mg depletion is attributed to cardiac arrhythmia, development of atherosclerosis, coronary vasoconstriction and increased blood pressure [33]. Although lower than Fe or SS316L, the mechanical properties of Mg alloys are in the same order of magnitude as conventional stents [10, 34]. In addition, Mg and its alloys have the ability to completely degrade in a physiological environment [35].

In addition to the above-mentioned physiological advantages and the resulting excellent biocompatibility, its long history as a medical implant makes Mg a promising candidate for stents. In 1878 Edward C. Huse used Mg as a ligature in three patients. Within the following decades, several attempts have been made to establish Mg as an implant material. However, due to the lack of controllable and reproducible degradation time, Mg proved unsuitable for regular clinical application [36]. With ongoing research on Mg alloying and production technology the quality of Mg was improved. In recent years, several Mg-stents have been tested in animal and clinical trials yielding promising results [6, 8, 12, 28, 32, 37, 38].

Magnesium properties

The corrosive and mechanical properties of a metal are mostly determined by its crystal structure and microstructure. The crystal structure depends on the alloy composition, whereas the microstructure can also be influenced by the production process and post-treatment (e.g., heat treatment). Therefore, alloying and processing are the two parameters that strongly influence the properties of a stent precursor.

Mechanics

The mechanical properties of Mg and its alloys are typically weaker than those of common stent materials like SS316L or cobalt chromium (CoCr). Although the Young’s modulus of about 44 GPa is sufficient, the plastic deformation capability of 2% (WE43, T6) [10] seems to limit the application of Mg alloys as a stent material. This poor formability at room temperature is due to the hexagonal close-packed (hcp) crystals of Mg and most of its alloys [39]. In hcp-materials, the active slip system at room temperature lies in the basal plane of the crystals. The only deformation mechanism along the c-axis (i.e., the normal of the basal plane) is twinning. In contrast to slip, twinning has a limited deformation capability. After the whole crystallite undergoes twinning, this mechanism cannot be activated again [40]. Alloying Mg with lithium (Li) changes the crystal structure to body center cubic (bcc) [41]. This results in a higher formability because in bcc-materials more slip systems can be activated requiring less energy than with hcp-crystals.

In addition to the crystal structure, the microstructure, namely grain size and orientation, has a major
influence on the mechanical properties of metals. The stress necessary to deform metals depends on the grain size (Hall-Petch relation). Small grains directly result in a higher required stress, as the larger amount of grain boundaries represents more obstacles for slip. Especially in hcp-materials, however, with an increasing number of grains, the amount of grains with preferentially oriented slip systems rises. Thus the Hall-Petch relation cannot be applied. The material becomes stronger without losing its plasticity. Also, with smaller grains, grain boundary slip becomes more likely [40].

As mentioned before, the orientation of the basal plane plays a crucial role in the plasticity of hcp-materials. Thus, oriented microstructures can accommodate preferential deformation directions. Different production techniques (e.g., drawing or extrusion) can be used for preparing stent precursors like wires or tubes. By controlling the parameters, oriented microstructures can be achieved [42].

Another processing method is equal channel angular pressing (ECAP), where a work piece is pressed through a channel without dimensional change. At some point the channel changes its direction at a certain angle, causing huge plastic deformation within the processed material. In contrast to direct extrusion, where the normal of the basal planes become preferably oriented perpendicular to the extrusion direction, the normal of the basal planes become oriented perpendicular to the shear direction induced by ECAP. Several passes through the channel, with a certain rotation of the work piece before each pass, can result in fracture strains up to 50% (A291, twice as much as conventionally extruded while UTS remains almost unchanged) [43]. However, this plasticity is reported to be highly anisotropic with some directions even having ductility significantly lower than the conventionally processed material [44].

Precipitations are another factor influencing the mechanical properties of metals. They can act as obstacles for dislocation movement with the severity depending on their lattice parameters, size and shape. Wang et al. analyzed the influence of the neodymium (Nd) content on the precipitations and plastic deformation capability of an Mg alloy (2 Zn, 0.46 Y, xNd with x=0, 0.5 and 1.0 weight-%; average grain size 3–4 μm). With no Nd, the grains were inhomogeneous with granular precipitations. Adding Nd to the alloy resulted in homogeneous and equiaxed grains. Along with grain size distribution, the shape and composition of the precipitations changed. Precipitations in the 0.5%-Nd material showed a rod-like structure (Nd/Zn=1/2, atom-%), yielding good plastic deformation capability. Increasing the Nd-content to 1.0%, rod-like precipitations were found within the matrix accordingly (Nd/Zn=1/2, atom-%). In addition, line-shaped particles were present along the grain boundaries (Nd/Zn=1/2.5, atom-%). This resulted in higher strength but significantly reduced [45]. Generally, rare earths (RE) increase both the strength and corrosion resistance of Mg-alloys [41].

Corrosion

Mg shows poor corrosion resistance, mainly due to two reasons. One is the initial galvanic corrosion caused by impurities or second phases. The other is the quasi-passive hydroxide film formed on the Mg-surface. This film is less stable than on other metals (e.g., aluminum), resulting in low pitting resistance of Mg and its alloys. The corrosion of Mg follows the overall chemical reaction Mg (s) + 2 H₂O (aq) ↔ Mg(OH)₂ (s) + H₂ (g), with the anodic reaction Mg (s) ↔ Mg²⁺ (aq) + 2e⁻ and the cathodic reaction 2 H₂O (aq) + 2e⁻ ↔ H₂ (g) + 2 OH⁻ (aq). As the grain boundaries have cathodic properties relative to the grains, no intergranular corrosion occurs [46]. Generally, 2-phase alloys have significantly larger corrosion rates than 1-phase alloys. One of the phases always acts as the cathode, the other as the anode yielding micro-galvanic elements [47].

Stress corrosion cracking (SCC) can also proceed transgranularly [46]. The sites of pitting corrosion can become initiation sites for corrosion fatigue and SCC [33]. Mg is susceptible to SCC and the mechanical stress yielding material failure during SCC usually is much lower than without corrosion. This mechanism should be taken into account during the development of a degradable stent, especially as the surrounding conditions of the target site for the stent are not completely known [26]. In stent applications, SCC can have a huge impact on the lifetime of the implant. The frequency of the mechanical burden is rather low compared to typical technical applications, but the amplitude of the deformation can become large (i.e., when the stent is implanted in or close to the heart).

Corrosion protection

The corrosion resistance of an Mg-alloy is determined by its microstructure [48]. Precipitates lead to localized or pitting corrosion [49], and coarse grains suffer the risk of large particle release accompanied by inhomogeneous corrosion [50]. Therefore grain refinement results in a more homogenous corrosion and, by minimizing precipitations, in a higher corrosion resistance [33, 51]. This can
be achieved by alloying elements acting as grain refiners (e.g., Zr, Ca, Sr, REs) [33] or suitable processing routes. For example, the severe plastic deformation induced by ECAP yields grain refinement [50]. Also, heat treatment can change the ratio of different phases present in the metal, thus changing corrosion properties [33]. The addition of Nd to an Mg-alloy (2 Zn, 0.46 Y, xNd with x = 0, 0.5 and 1.0 weight-%) improved the corrosion properties from severe pitting to a more homogeneous attack. The line-shaped precipitations occurring at 1.0% Nd might enhance corrosion initially but may form corrosion protection barriers later as Nd raises the electrochemical potential [45].

Impurities have a low solubility resulting in pronounced galvanic corrosion [52]. A low corrosion rate is usually found in high-purity (HP) alloys. However, due to the manufacturing process, particles originating from machines, lubricants and the like can be embedded in the surface of even HP materials. These surficial remnants have to be removed by organic or inorganic pickling [52, 53].

The corrosion resistance can be modified by different surface treatments including protective layers. Blawert et al. compared different surface layers applied by different physical vapor deposition (PVD) methods (magnetron sputtering, ion beam sputtering and vacuum deposition). As PVD utilizes very high cooling rates, the method is less limited by kinetics than classic alloying. Thus new alloys can be prepared as homogeneous solid solutions showing no miscibility gap. Also materials with big differences in melting temperature can be combined with this method and even cathodic corrosion protection is possible [54]. The absence of precipitations and the homogenous distribution of all alloying elements results in very homogenous corrosion [49].

Zirconium (Zr) is maybe the most versatile element when it comes to corrosion protection of Mg via alloying. It acts as a grain refiner [51] and precipitates Fe-impurities and, to a lesser amount, nickel (Ni) impurities. Also, SCC does not occur in Zr-containing alloys, except when the resulting stress approaches the yield stress [46].

**Biocompatibility**

Beside general functionality any implant material has to be biocompatible to be tolerated by the body and ensure safety for the patient. It is first tested in vitro and includes cytotoxicity and inflammation response, both depending on dose, release rate and solubility of the particular material [28]. Drynda et al. calculated the medium daily release of RE from Mg-coronary stent as 6–12 μg assuming a stent weight of 10 mg, 5–10% RE and a linear degradation for 3 months. All REs (except the radioactive Pm) were analyzed in vitro using primary human vascular SMCs. At such doses no dysfunctions of SMC proliferation were found. At high doses, the metabolic activity of the cells decreased but no apoptosis or necrosis was observed [55]. Other groups also tested the in vitro properties of important alloying elements like Li, Y, Zr, and some REs using different cell lines (MG63, HUCPV, RAW264.7). La and Ce showed the highest cytotoxicity, and Gd and Dy seemed to be tolerated better than Y. The inflammatory response on Gd was elevated for 1000 μmol Gd but unremarkable for 500 μmol. At the moment there is no standard testing protocol for biodegradable implant materials available. For cytotoxicity testing, EN ISO 10993:5 and 10993:12 apply for conventional materials. However, the preparation of extracts from degradable metals accordingly yields very high osmolarities and pH-values so that the test cells die of osmotic shock [56].

In 1965, Sawyer et al. replaced parts of the descending aorta of dogs with tubes made from different metals (Mg, Al, SS, Cu, Pt). In the Mg-tubes, no thrombosis was found during follow-up of 293 days. Steel- and Pt-tubes showed severe thrombosis after a short time (mean time to thrombosis: steel 28 days, Pt 1 day) with only one steel-tube being patent after 250 days. In previous works the same group has shown in vitro that the deposition of erythrocytes and leukocytes on metals depends on the metal’s standard electrode potential. If it exceeded about +0.33 V, deposition occurred. Thus, metals at the non-noble end of the electromotive series seem to be less thrombogenic. In electrochemical settings representing implantation sites, these metals have the tendency to release positively charged ions into the electrolyte (i.e., the metal corrodes), forming an electrical double-layer. The electrons left behind in the bulk material result in a negatively charged metal surface. The favorable outcomes with the implanted Mg-tubes can be attributed to these effects. As Mg has a low electrochemical potential, it starts releasing ions right after implantation and is thus less likely to promote the deposition of blood components or thrombus formation than positively charged surfaces [57]. However, thrombus formation is not only related to the considered material but also depends on the flow pattern of the blood. This can be altered significantly when a stent is implanted as will be shown later.

After implantation in the body, Mg starts to corrode and the degradation products become metabolized. Mg dissolves into its salts (chloride, oxide, sulphate or phosphate), macrophages will digest hydroxyapatite, which evolves as a by-product of degradation [1]. Proteins
can change the corrosion rate, for example by forming surface layers [33], as well as the change of the surrounding medium from blood to neointima during the healing process. During degradation, the remaining metallic Mg is surrounded by calcium and oxygen rich material, presumably \( \text{MgCO}_3 \) and \( \text{Mg(OH)}_2 \) [38]. After complete degradation, amorphous calcium phosphate has replaced the former stent struts accompanied by infiltrating cells [12, 38].

**Alloys proposed for degradable stents**

Considering the results mentioned before, Mg alloys containing REs are widely studied for stent applications. Particularly WE alloys containing yttrium and REs have been widely investigated [38, 41, 58, 59]. Although the processing is difficult, Li is suggested as alloying element for its positive effects on deformability [60]. However, the processing of Li-containing melts is difficult, and it generally lowers the corrosion resistance of the Mg alloy [61]. Other materials like ZM21 (zinc and manganese), AZ31 (aluminum and zinc) and AE21 (aluminum and REs) are also subject to research activities [28, 58, 59, 62–64]. More detailed information about Mg-based alloying systems for cardiovascular stents can be found in recent reviews [65, 66].

**Influence of stent design on the mechanical and degradation properties**

The mechanical and degradation behavior of BDS depend on the material properties as well as on the geometrical design, the manufacturing parameters and post-treatment of the stent. Unfortunately, manufacturers do not publish detailed information about their production process and parameters [64]. Most stents available are laser-cut from tubes. However, as it alters the microstructure, the production of a tube can change the mechanical and degradation properties of stents significantly. Fang et al. tested methods for the production of Mg-tubes (ZM21 alloy) as stent precursors. During hot drawing, the geometrical tolerances are high and a coarsening of the microstructure can occur. Starting with hot-extruded hollow billets, cold drawing with moving mandrels, multiple passes and intermediate heat treatment was performed. Annealing recrystallizes the twinned grains arising from cold deformation into fine grains. By varying the temperature, different average grain sizes of 15 \( \mu \text{m} \) (250°C), 30 \( \mu \text{m} \) (300°C) and 60 \( \mu \text{m} \) (400°C) could be achieved. Increasing the duration of the heat treatment did not influence the grain size but yielded a more homogeneous microstructure [64]. Ge et al. produced ultrafine-grained Mg-tubes (grain size about 0.5 \( \mu \text{m} \)) of the same alloy by combining ECAP with subsequent cold-drawing. Laser-cutting with a fiber laser was possible with no relevant changes in the microstructure [63].

The geometry of a BDS plays a crucial role regarding both mechanical and degradation properties. As the properties of Mg alloys are different from the materials used for BMS, a different design is required for achieving comparable mechanical characteristics. The effective stresses induced in a BDS make the stent subject to SCC. Beside the implant environment these stresses largely depend on the stent design.

The first available BDS based on an Mg alloy was the Magic stent (Biotronik SE & Co.KG, Bülach, Switzerland). It was made of a WE43 alloy containing 7% REs. The rectangular strut design was laser cut from a tube, followed by electro-polishing. Later, the stent design was refined employing a modified WE43 alloy and square-shaped struts for slower degradation and better scaffolding ability [13, 38, 67].

With ongoing research on BDS, several new designs and optimizations using finite-element-simulation (FEM) were developed. Wu et al. conducted shape optimization of the Magic stent utilizing FEM modelling. Four alloys were included in the analysis (AZ80, AZ31, ZM21, WE43). While strut thickness was kept constant, the optimized design had increased and non-uniform strut width (at least +68%). The resulting maximum principal stress and strain were lower compared with the initial geometry (163 MPa instead of 230 MPa, 0.132 instead of 0.153, WE43 alloy). Although having almost identical mechanical properties, the analysis of the optimized design showed different values for maximum principal stress and strain and elastic recoil for ZM21 and WE43 alloys. This led to the conclusion that the stent geometry needs to be designed in concordance with the material selection [59]. For the AZ31 alloy, the new geometry resulted in less recoil (70% instead of 8.1%), lower maximum principal stress (163 MPa instead of 183 MPa) and a more homogeneous stress distribution. The degradation behavior was modelled as well with the optimized design showing lower mass loss ratio and slower vessel recoil. This was attributed to the larger width of the struts and the lower and more homogeneously distributed stress impeding SCC [62]. Compared to another stent design having a larger mass, the degradation of the optimized stent was slower. This was a result
of the lower maximum stress in the optimized stent, indicating that the degradation rate not only depends on the amount of material but also largely on the design of the stent. The results of these simulations were confirmed by in vitro degradation tests [68].

There is no standard defined for testing the mechanical properties of stents. Grogan et al. developed a virtual test bench to compare different stents with FEM simulation. Different materials including AZ31, WE43 alloys, CoCr (L605) and stainless steel (316L) were implemented along with different stent geometries including the Magic-stent. The Mg stents had a risk of failure significantly higher than BMS. Bending was possible only up to 20% of the bending radius of the CoCr and 316L stents and the longitudinal stiffness was below 50%. The cross-sectional strut areas of the Mg-stents had to be 2.4 times larger than of the CoCr stents for comparable mechanics. Thus, new degradable Mg alloys and suitable stent designs need to be developed, especially regarding higher ductility [58].

As will be shown in the next chapter, another important factor influencing the behavior of BDS is the interaction of the stent with the biological environment at the implantation site. Especially the alterations in fluid dynamics, WSS and cellular response resulting from the stent geometry can have significant effect on the degradation rate of a BDS.

The last step before a medical product is implanted is the sterilization. Depending on the method applied, this can have huge influence on the device properties and thus needs to be studied. Liu et al. analyzed the influence of common sterilization techniques on the surface properties and the biocompatibility of MgCa and pure Mg. Although increasing the hemolysis percentage significantly, Co60 γ-radiation is suggested as the method of choice. Steam autoclave sterilization reduced hemolysis for both materials but changed the surface properties of the MgCa. Glutaraldehyde, ethylene oxide and dry heat sterilization showed hemolysis rates between the previously mentioned methods. The magnitude of the sterilization effects on the material characteristics depended on the Mg alloy they were applied to [69]. Thus, before implantation in humans, every new material has to be tested accordingly.

due to the intrinsic elastic recoil. Depending on the amount of over-dilation, the vessel is injured, which is also the case for balloon angioplasty if carried out prior to stenting. In addition to these initial effects, the vessel is permanently deformed by the stent and deprived of its vasomotoric properties. The resulting vessel geometry, as well as the stent design, alters the blood flow pattern before, in and after the stented segment. The response to such an injury and the mechanical stress caused by the implanted stent is similar to the regular wound healing process and includes platelet activation, thrombus formation, inflammation, cell proliferation and migration followed by remodeling [3].

Endothelial cells (EC) play an important role in suppressing inflammation and thrombosis. They are also important for controlling the vascular tone and function, making the restoration of a healthy endothelium an important goal of vascular therapies [70]. During stent implantation, ECs are crushed or destroyed, thus the production of anti-coagulants at the implantation site is reduced. The mechanical stress induces enhanced SMC proliferation and migration within media and intima. This leads to intimal thickening by SMCs and extracellular matrix (ECM). This NI formation is a general healing response and proportional to the extent of vascular injury [71]. However, it also represents one of three possible mechanisms for restenosis which are elastic recoil, negative remodeling and NI hyperplasia [3].

After revascularization of a stenotic vessel, the immediate effect of a stent is the prevention of vascular recoil by scaffolding the lesion mechanically until it is remodeled for supporting its lumen on its own [6]. Due to their mechanical properties, polymeric stents usually have greater recoil during implantation than metallic stents [8].

In contrast to balloon angioplasty, stents generally prevent early negative remodeling as they span the vessel [9]. This means, in case of a degradable stent, that the radial force must be sufficient for a certain period of time. As mentioned before, NI formation is a regular part of the healing process of injured vessels and is induced for example by BMS. However, excessive NI proliferation can occur as a result of severe inflammation, injury and other adverse effects as well as in response to low endothelial WSS [3].

A simulation of the WSS in a patient who had received an ABSORB polymeric degradable stent (Abbott Vascular) and a 2-year follow-up showed low WSS on the vessel wall directly after implantation. However, WSS was in the physiologic range over the stent struts. Two years later, the WSS was normal everywhere over the former lesion. It is assumed that the low initial WSS contributed to NI

**Interaction of stents with the biological environment**

Besides the effects of the material itself, the deployment of a stent has large influence on its implantation site. During implantation, a balloon-expandable stent is over-dilated
formation, which then helped restoring the geometry, physiologic function and vasomotion by smoothing the luminal surface [72]. The comparison of fluid simulations and calculated WSS of a reconstructed stented porcine vessel segment with histomorphometric NI hyperplasia analysis gave deeper insight in the aforementioned relationship. Low and oscillatory WSS results in great NI response. In contrast to standard simulations utilizing idealized geometries, the employed realistic geometry with proximal overexpansion and asymmetric deployment resulted in a non-uniform WSS-distribution and correlated with non-uniform NI-growth [73]. Another analysis utilizing FEM and animal experiments showed that large flow alterations result in larger thrombus formation as flow disturbances can activate platelet deposition and thrombin and fibrin generation [4]. Thus, the geometry of the stent, its orientation and the implantation site have influence on the change in WSS after implantation. As can be seen in Figure 2, the blood flow pattern can be disturbed by protruding struts, thus altering the WSS [74].

Also, different stents designs yield different flow alterations. The degree of turbulence depends on the strut thickness [4], the strut angle toward the flow, and the degree of expansion of the stent. For least disturbance, the linkages of the stent should be aligned longitudinally to the flow direction [21], the struts should be thin [4], and the stent cells should be fully expanded (i.e., the stent size should fit the vessel, see Figure 3) [73].

Beside the regulation of the tissue response the WSS induced by the blood flow also has direct influence on the degradation of a BDS. Wang et al. analyzed the corrosion of stents (AZ31 alloy, average grain size <30 μm) exposed to different flow rates in vitro. Under static conditions, there was only moderate and uniform corrosion. Under dynamic conditions, the whole surface area showed strong corrosion. It was more pronounced at the surfaces aimed toward the flow. Here the corrosion products could easily be detached resulting in the loss of some struts after 7 days of immersion. The corrosion rates were calculated to 0.37(±0.07) mm/year for the static and 1.21(±0.27) mm/year for the dynamic conditions [75]. These effects are important during the first days and weeks after implantation. However, they lose influence with ongoing NI formation on the stent surface.

The implantation site also plays a crucial role as the initial flow pattern varies depending on the vessel diameter, vessel movement, blood velocity, side branches and others. For deeper analysis, the clinical geometries and vessel properties would have to be taken into account for patient-specific simulations prior to implantation. Also, the intended application alters the boundary conditions. If used as a carrier for heart valves, the stent itself does not interact with the blood and thus does not change the flow pattern. Unfortunately, the inclusion into the analysis of patient specific clinical data is not feasible at the moment as too many surrounding parameters remain unknown [4].

To prevent SMC proliferation and migration, important processes during restenosis, DES have been introduced in clinical practice. These stents release immunosuppressive/anti-proliferative medication, thus reducing excessive NI response. As the medication is not specific to SMCs.
but also has negative effect on EC re-growth, the healing process of the stented lesion becomes delayed. Thus, DES can effectively reduce acute stenosis but are susceptible to late or very late stenosis. DES have reduced the number of re-interventions by more than half compared with BMS. The risk of late (6–12 months) and very late (>12 months) thrombosis is still present [3, 9, 76]. There seems to be a correlation that the more aggressive the therapy against restenosis, the worse the long-term results [77]. A DES with the ability to completely degrade over time might be the solution for this problem. Once no foreign material is present, even with incomplete healing, no chronic inflammation, thrombus formation, malappositioned struts, or other adverse findings should be present [9]. However, it has been reported that even after balloon angioplasty without subsequent implantation of a stent, there is a risk of very late thrombosis [1].

In vivo experience with Mg-stents

Animal and clinical studies

The development and application of degradable coronary stents has been widely discussed in previous reports [1, 2, 78]. Table 1 shows a summary of the Mg-stents used in animal and clinical studies already. The first Mg-stents made from AE21 alloy (Magic stent, Biotronik SE & Co.KG, Bülach, Switzerland) were implanted in pigs in 2003 [28]. After several iterations and animal studies [7, 32], the AMS-1 (absorbable metal stent; WE43 alloy; Biotronik SE & Co.KG, Bülach, Switzerland) has been tested in clinical studies including treatment of critical limb ischemia [6, 13] and coronary diseases (PROGRESS-AMS; non-randomized multicenter trial, n=63). In the initial experiments it was shown that the AMS-1 is generally safe with no stent-related deaths, myocardial infarctions, or stent thrombosis [7, 12, 28, 80]. The PROGRESS-AMS study confirmed these results. After 1 year no in-stent thrombosis was found. As no stent material was left at this time, late adverse effects seem to be very unlikely. Initial mechanical stability and lumen gain were comparable to conventional BMS [8, 79]. However, a high restenosis rate (47.5% of all patients at 4 months follow-up; [8]) was found. The authors attributed this finding to negative remodeling due to the lack of an anti-proliferative drug and too rapid degradation of the stents. Degradation was complete after 4 months, but loss of the mechanical properties might have happened much earlier, resulting in losing the ability to span the vessel wall [8, 79]. The same occurred in an animal study in pigs, where high restenosis rates in the AMS-1 group were observed within the first month after implantation. The authors also identified too-rapid degradation and loss of mechanical stability as the reasons for negative remodeling resulting in restenosis [37].

Table 1: Overview of animal and clinical studies on Mg stents.

<table>
<thead>
<tr>
<th>Stent</th>
<th>Alloy</th>
<th>Study</th>
<th>n</th>
<th>Target vessel</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magic</td>
<td>WE43 (Zr, Y, RE)</td>
<td>Pigs</td>
<td>11</td>
<td>Coronary artery</td>
<td>First Mg-stent in animal</td>
</tr>
<tr>
<td>Magic</td>
<td>Zr, Y, RE</td>
<td>Minipigs</td>
<td>33</td>
<td>Coronary artery</td>
<td></td>
</tr>
<tr>
<td>AMS</td>
<td>Pigs</td>
<td>Animal</td>
<td>17</td>
<td>Coronary artery</td>
<td></td>
</tr>
<tr>
<td>AMS</td>
<td>Zr, Y, RE</td>
<td>Minipigs</td>
<td>50</td>
<td>Coronary artery</td>
<td></td>
</tr>
<tr>
<td>AMS</td>
<td>WE43, refined</td>
<td>Minipigs</td>
<td>11</td>
<td>Coronary artery</td>
<td>First Mg-stent in man</td>
</tr>
<tr>
<td>AMS</td>
<td>WE43, refined</td>
<td>Minipigs</td>
<td>11</td>
<td>Coronary artery</td>
<td>First Mg-stent in man</td>
</tr>
<tr>
<td>AMS-1</td>
<td>PROGRESS-AMS</td>
<td>Adult</td>
<td>5</td>
<td>Coronary artery</td>
<td>Initial evidence for return of vasomotion</td>
</tr>
<tr>
<td>AMS-2</td>
<td>PROGRESS-AMS</td>
<td>Adult</td>
<td>60</td>
<td>Infrapopliteal arteries</td>
<td>Limb ischemia</td>
</tr>
<tr>
<td>AMS</td>
<td>WE43 (Zr, Y, RE)</td>
<td>Preterm baby (26th week)</td>
<td>1</td>
<td>Left pulmonary artery</td>
<td>Age 6 weeks, weight 1.7 kg</td>
</tr>
<tr>
<td>AMS</td>
<td>We43 (Zr, Y, RE)</td>
<td>Coarctation of the aorta</td>
<td>1</td>
<td>Aorta</td>
<td>3-week-old newborn</td>
</tr>
</tbody>
</table>

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Considering these results, Biotronik designed a drug-eluting AMS (DREAMS-1), implementing thicker struts for prolonged degradation and a coating of a paclitaxel-eluting polymer to inhibit negative remodeling. In the BIOSOLVE-I multicenter clinical trial, it showed good safety properties. Compared to its precursor AMS-1 (mean 1.08 mm at 4 months), DREAMS-1 showed better performance with a reduction of in-scaffold late lumen loss at 6 (mean 0.65 mm) and 12 months (mean 0.52 mm). However, these values are still high when compared to conventional DES [67]. This resulted in the development of DREAMS-2, featuring slightly thicker struts. Also, the anti-proliferative drug was changed to sirolimus as it has a larger effect than paclitaxel. Currently, DREAMS-2 is under investigation in the BIOSOLVE-II clinical trial (expected completion date: June 2017) [1, 78].

**Inflammation response**

Inflammation is a normal body reaction to injury and, as described before, stent implantation. However, severe inflammation can be a reason for excessive NI proliferation. Thus, an implant material must not cause exaggerated or chronic inflammation reactions. In animal studies employing pigs, no significant differences in inflammation were found between Magic stents and stainless steel stents [7], or between AMS-1 and BMS [37]. The AMS-1 implanted in the left pulmonary artery of a preterm baby with congenital heart disease also showed no inflammation in the histological analysis about 5 months after implantation [12]. A porcine study was conducted comparing different paclitaxel eluting stents (three test versions of DREAMS-2, TAXUS Libre6 and eucaTAX). After 28 days, the inflammation score of the best version of the AMS-3 was low but still significantly higher than the score of the two control stents (p<0.05). This may be attributed to higher macrophage activity caused by the degradation products. However, this difference was not seen after 90 or 180 days [38].

**NI formation**

NI formation is an important part of the healing process. A thin NI-layer on the implant surface is desirable as it helps in restoring physiological WSS and separates the implant material from the flowing blood. However, as previously described, excessive NI growth can cause restenosis and thereby endanger the success of treatment. The first porcine trials testing the Magic stent showed fast NI formation with an almost complete layer on the stent after 6 days [32] and closed layers at follow-up times of 10 [28] or 28 days [7]. The AMS explanted from a preterm baby after 5 months showed a complete NI layer up to 100 μm around the former stent struts with cells infiltrating the remains of the Mg alloy [12]. The degraded parts of the former struts were replaced by fibrotic tissue [12, 32]. The PROGRESS study confirmed fast reendothelialization with complete ingrowth of the stent, stating actual corrosion going on inside the vessel wall [8]. Compared to BMS, the AMS showed less NI formation with no new NI growth after 4 months. This is attributed to the degradation process eliminating the stimulus for NI proliferation over time [79]. In the BIOSOLVE-I study with DREAMS-1, the addition of paclitaxel resulted in prolonged NI formation up to 6 months, then remaining constant until 12 month follow-up [67].

**Safety**

One concern about the safety of degradable stents is the loss of metallic strut parts, especially when an anti-proliferative drug is incorporated in a coating. The Magic stent was still intact after 3 days when reendothelialization had already started. At 28 day follow-up, NI formed a dense layer on the stented area thus eliminating the risk of losing stent particles. NI volume was significantly smaller in the Mg-stented lesions than in the stainless steel reference (Lekton Motion) [7]. The prolonged NI formation period in the DREAMS-1 stent did not result in particle loss as it was shown in the BIOSOLVE-I study in 46 patients [67].

**Restoration of vasomotion**

After degradation of a stent, vessel growth and restored vasomotion is expected [7, 8]. Zartner et al. found some vessel growth in a preterm baby with the stent having been implanted at a diameter of 3 mm, while the lumen diameter of the explanted vessel after 5 months was 3.7 mm. The vessel showed good elasticity except for some fibrotic strands. No remaining struts or solid remains were found, thus no circular structures limiting growth were present [12]. The PROGRESS-AMS study indicated the possibility of normal vessel physiology with active vasomotion after degradation of the stent [77]. A progressive increase in lumen area was found in a porcine study employing the everolimus-eluting ABSORB stent made from degradable polylactide (Abbott Vascular). In addition, vasomotion (here defined as the difference of end-diastolic and
end-systolic mid-lumen areas) was restored between 12 and 24 months (see Figure 4) [82].

The same stent was tested in the Cohort A and B clinical trials. The return of vasomotion as response to acetylcholine and methylergonovine was demonstrated as a function of the reduction of polymer strut echogenicity, which in the case of a polymer is equivalent to the degradation progress (12 and 24 months follow-up) [83].

**Correlation between in vitro and in vivo experiments**

Besides confirming the safety of an implant, its performance has to be assessed during in vitro tests. Every stent needs sufficient radial strength and long-term integrity, low recoil and low risk of embolization. For degradable implants, the main parameter is the degradation rate. However, no strong correlation between in vitro and in vivo corrosion rate is yet available [84]. The degradation is usually much faster in vitro than in vivo [35, 85]. There are several reasons for this divergence. Different studies showed that static systems with rather simple corrosive media like simulated body fluids (SBF) cannot represent the much more complex human body. The alloying elements seem to have different influence on the corrosion rates depending on the corrosive medium [86, 87]. Also, there are various testing methods available like mass loss or \( \text{H}_2 \) evolution (unpolarized tests) and potentiodynamic polarization and electrochemical impedance spectroscopy (polarized tests) [84]. The transferability to in vivo experiments is not straightforward as these tests can usually not be applied in a living animal. Another problem is the assessment of the in vivo conditions, i.e., what needs to be simulated. The exact flow conditions, influences of proteins, macrophages and other blood components, time to ingrowth by Ni-formation, and so on are not fully understood yet. Maeng et al. implanted eleven AMS (Biotronik) in the coronary arteries of pigs. They found too rapid degradation of the stent which was complete after 2 months. However, they were not able to determine the time of loss of the mechanical integrity [37]. The exact in vivo degradation kinetics remain to be studied in detail [79]. EC loss and re-endothelialization after stent implantation are not fully understood yet. Also, the influence of strain on EC function or the combination of WSS and strain are not well understood [70].

Mechanical analysis of stents is usually carried out in homogenous, straight geometries. Compared to the intended implantation sites, these idealized geometries are unrealistic and bias the results. In pre-clinical testing the implants may withstand tests without fracture. When implanted in patients, the crooked geometry induces multiaxial stresses yielding strut fracture after a relatively short time [88]. Also, the distribution and absolute value of the WSS is different when a stent is implanted asymmetrically [73]. In the future, patient-specific and/or lesion-specific FEM simulation of mechanics and blood flow patterns may help in designing more reliable implants.

Another problem is the comparability of animal and clinical studies. Beside the differences in anatomy, animals usually have faster healing responses than men. Also, their vessels are healthy when receiving a stent. In humans, atherosclerosis or other diseases are present when a stent is implanted [3].

In order to prepare new stents with altered properties, fast and reliable in vitro pre-screening systems are needed [56] as the test setup, corrosive medium and test conditions can change the results dramatically [47]. Some suggestions for better correlation of in vitro and in vivo results have been made [29, 85, 89]. Martinez Sanchez et al. recently set up a corrosion factor for Mg alloys used for bone implants. Although it was a retrospective approach, it was stated that reliable prediction seems possible if the corrosive media and the parameters of the in vitro tests are considered [35]. To overcome the problems of in vitro tests, some authors recommend using whole human blood and dynamic test systems like Chandler-loops [86, 87].
Bowen et al. applied a fibrin coating on Mg and Fe wires before placing them in corrosive medium [89]. During an animal trial they implanted pure Mg and Fe wires into the wall of the abdominal aorta of rats applying the technique mentioned above. For quantification of degradation they exercised tensile tests on these wires and on some degraded in vitro [85]. This approach seems very promising as it measures the most important parameters of degradation, which are the remaining strength and ductility. Pierson et al. suggested an easy method for in vivo testing of potential degradable stent materials. A sharpened wire is put into the prepared vessel by puncturing, then advanced along the vessel wall and led out again by a second puncture [29].

As mentioned before, not only the alloy composition but the design and every step of the manufacturing process can alter the degradation properties of magnesium stents. Thus, for reliable comparison of in vitro and in vivo results, the exact same material has to be used. This requires either the employment of finished stents for in vitro studies or the implantation of test material like rods, wires or alike in vivo. As the production of stents is rather expensive, the implantation of wires seems much more convenient. However, the surgical approach described before has some shortcomings [29, 85]. It is a surgical intervention with two lacerations of the vessel. This may yield false results as, for example, the NI-formation is proportional to the amount of injury of the vessel [71]. Also, the reproducibility is rather low, as the exact position of the wire along the vessel wall cannot be assessed. Therefore, a new method was developed where wires are sutured on an inert polymeric carrier stent (see Figure 5). Each stent can be equipped with several wires which, after minimally invasive implantation, are aligned along the vessel wall with high reproducibility [90].

**Conclusion**

In general, it can be stated that Mg-stents are safe. In several studies, no major adverse cardiac events or stent thrombosis occurred. The performance of the stents is not yet equal to existing stents but the results are promising. The research focus has mostly been on coronary stents, thus there is great need for research on other applications including larger stent sizes and stents as scaffold for advanced implants like tissue engineered heart valves and pediatric applications. In these cases, the surrounding parameters can differ widely from those in the coronaries. The crimping ratio (i.e., the stent diameter implanted in relation to the diameter during implantation) may be higher, requiring more ductile materials. The biological responses to the stent can differ when implanted in larger vessels as these have different blood flow patterns. Also, if the stent is completely embedded in tissue, there is no direct contact with the blood thus changing the environment substantially. Considering all the different possibilities, adjustable stent properties may be required, adjustable degradation rates in particular. This can be achieved by varying alloys, surface treatments and coatings or by combining different materials. As described above, there are many possibilities for changing the properties of a stent. As more biodegradable materials are coming closer to clinical practice, new standards for in vitro testing need to be established. This is important for comparability of results as well as for legal certainty for patients, hospitals and manufacturers. A possible first step toward such standards would be to gain a better understanding of the ongoing processes at the implantation site as a function of time. Even for coronary application, the required degradation time separated into complete mechanical strength, gradually loss of scaffold capability and loss of circumferential integrity are not well known.

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References


