Magnesium-based biodegradable alloys: Degradation, application, and alloying elements

MAKSYM POGORIELOV*, EUGENIA HUSAK, ALEXANDR SOLODIVNIK, SERGII ZHDANOV

Medical Institute, Sumy State University, Sumy, Ukraine

*Corresponding author: Maksym Pogorielov; Medical Institute, Sumy State University, 31 Sanatornaya Street, Sumy 40018, Ukraine; Phone/Fax: +380 669005448; E-mail: m.pogorielov@gmail.com

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Abstract: In recent years, the paradigm about the metal with improved corrosion resistance for application in surgery and orthopedy was broken. The new class of biodegradable metal emerges as an alternative for biomedical implants. These metals corrode gradually with an appropriate host response and release of corrosion products. And it is absolutely necessary to use essential metals metabolized by hosting organism with local and general nontoxic effect. Magnesium serves this aim best; it plays the essential role in body metabolism and should be completely excreted within a few days after degradation. This review summarizes data from Mg discovery and its first experimental and clinical application of modern concept of Mg alloy development. We focused on biodegradable metal application in general surgery and orthopedic practice and showed the advantages and disadvantages Mg alloys offer. We focused on methods of *in vitro* and *in vivo* investigation of degradable Mg alloys and correlation between these methods. Based on the observed data, a better way for new alloy pre-clinical investigation is suggested. This review analyzes possible alloying elements that improve corrosion rate, mechanical properties, and gives the appropriate host response.

Keywords: biodegradable metals, magnesium, corrosion, surgery, orthopedy

Introduction

In the last decades, the paradigm establishing that implants must be inert and corrosion resistant has been displaced by the advent of a new class of metallic biomaterials: biodegradable metallic materials [1]. Compared with other materials, these metals have high impact strength, high wear resistance, high ductility, and toughness [2]. Hence, these metals are used in orthopedy, general, and cardiovascular surgeries because of their appropriate mechanical and corrosion properties after providing structural support for a certain period to complete both the regeneration and the healing processes.

Iron (Fe), zinc (Zn), and magnesium (Mg) are considered as the basic biodegradable materials for medical application. Mechanical parameters of these three pure metals are shown in *Table I*.

Among these metals, Fe is an interesting candidate for biodegradable materials in terms of its mechanical properties. Because of its higher elastic modulus, Fe has a high radial strength, which is helpful in making materials with thinner struts. It has also high ductility, which is helpful during the implantation when the material is plastically deformed [3]. The first biodegradable metallic stent was fabricated from Armco® iron (Fe > 99.8%) and implanted in descending aorta of New Zealand white rabbits in 2001 [4]. The results from the implantation of the first Fe stent showed no significant evidence of either an inflammatory response or neointimal proliferation, and organ examination did not reveal any systemic toxicity. However, the slow degradation rate (0.16 mm year^{-1}) and the ferromagnetic nature of pure Fe led to problems when these materials were used as implantable devices [5]. The addition of manganese (Mn) increases the degradation rate up to 0.44 mm year^{-1}, but still it does not have wide application.

Zn-based alloys may also be promising candidates for biodegradable implants. The advantages of Zn-based

Table I Mechanical parameters and degradation rate of pure Fe, Zn, and Mg used for medical applications (compare the stainless steel)

	Yield strength (MPa)	Tensile strength (MPa)	Elongation (%)	*In vitro* degradation rate (mm year^{-1})
316L SS: annealed	190	490	40	–
Pure Fe: annealed	150	200	40	0.16
Pure Zn: as cast	17	20	0.2	0.2
Pure Mg: as cast	20	86	13	407

alloys are its low melting point and low reactivity in molten state. Therefore, they can be prepared by simple melting, gravity, or die casting in air atmosphere and hot forming [6]. Zn alloys do not show local or general toxicity or other biological compatibility [7]. However, one drawback of pure Zn as potential biodegradable metal lies in that pure Zn has quite low strength and plasticity.

Mg and its alloys are biocompatible materials with appropriate biomechanical parameters that can corrode completely in biological media. These properties make them promising candidates for biomedical applications [8]. Degradation of Mg under the physiological conditions avoids reoperation to remove bone implant. In recent century, Mg alloys were extensively investigated, but still they are not used as the optimal material for controlling biodegradation and tailoring alloy composition and microstructure depending on texture, grain size, manufacturing method, and postprocessing techniques [9–11].

This review summarize the discovery of Mg and its alloys for biomedical application. Furthermore, it also summarizes the different fields of Mg alloy application and mechanisms of Mg degradation (both *in vitro* and *in vivo*).

Mg Discovery and Its First Biomedical Application

Mg was first identified by Sir Humphry Davy in 1808 *(Fig. 1)*, and in 1833, it was first extracted by Michael Faraday using electrolysis [12]. In the mid-19th century, it was produced by small companies in Germany, the USA, and the UK for pyrotechnical and photographical applications.

The first medical application of Mg alloy was reported in 1878, when Huse used wire as ligature to stop bleeding from radial artery (2 cases) and during the operation for varicocele [12]. In all cases, the application of Mg ligature was successful, but the further researches on this application were not carried out until Payr reported other possible Mg applications in 1900 [13, 14].

Mg in Surgery

Payr was a pioneer in medical application of Mg; he carried out both human and animal testings. In his first

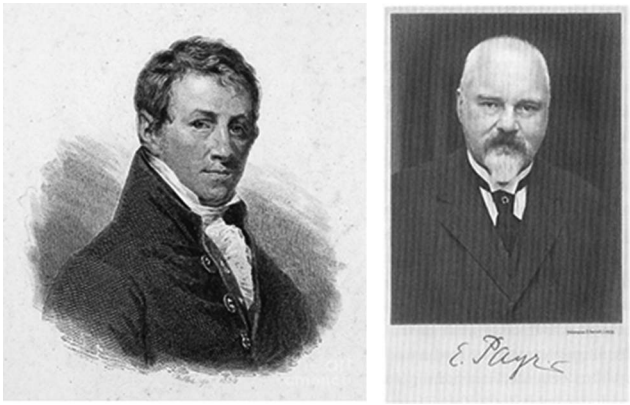
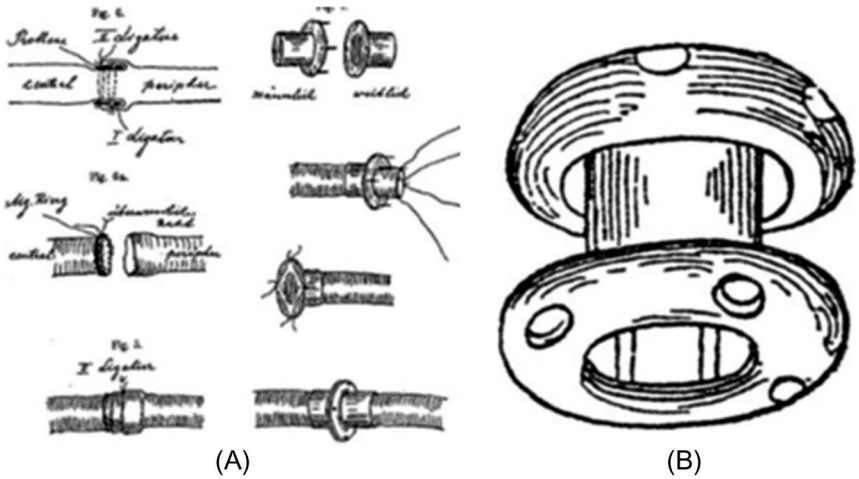


Fig. 1. Left: Sir Humphry Davy (17 December 1778 – 29 May 1829), English chemist, who discovered Mg in 1808; Right: Dr. Erwin Payr (17 February 1871 – 6 April 1946). Austrian–German surgeon, a pioneer in medical application of Mg

experiment in 1900, he used Mg vessel connectors in animal femoral artery [13, 14]. The original image from the Payr article is shown in *Fig. 2A*. He proved that the connection of the vessel ends became solid after 8 days and observed a severely thickened intima layer at the anastomosis, with a fibrous ring on the outer side at that point. In the same study, he also suggested that only the intravascularly placed Mg tubes exhibited thrombotic blood clotting at the end of the tubes, which, however, never closed the remaining lumen. Also, no thrombosis was observed with extravascularly placed Mg tubes. He recommended using Mg plates and sheets for well-vascularized organ suturing and treatment of cavernous hemangioma and large vessel aneurysms [17–19]. He proved that hemostasis was effective after the partial liver excision on animal model using Mg sheet, and then, he successfully applied this method in a human case. In the animal model, he suggested that the resorption time of Mg plates after hemostasis varied from 50% resorption after 3 weeks to minor corrosion after 5 weeks. Two years later, in 1905, Payr and Martina showed that hemostatic effect after Mg sheets application was because of the tamponade effect of hydrogen gas [17]. In 1900, he carried out his first successful treatment of hemangioma in a 14-year-old girl. Few years later, he suggested that treatment with Mg arrow was only beneficial for treating



(A) (B)

Fig. 2. (A) Mg vessel connectors (extravasal Mg rings – left column, two-part connectors – right column) designed by Payr [15]. (B) Mg connector for intestine anastomosis, designed by Chlumsky in 1900 [16]

subcutaneous cavernous hemangioma [19]. In 1914, Sonntag confirmed Payr's results in several clinical cases [20]. Finally, Payr suggested the use Mg tubes for sutures of nerves and used this technique both in animal experiments and in human cases (7 nerves) [14]. During the experiments, he observed several round cells and granular tissue formation around the corroding Mg [14]. The granular tissue was highly vascularized and contained giant cells with metallic particles and leucocytes. He also found that the strong activation of blood clotting was because of the corroding Mg implant [17].

At the same time, Chlumsky suggested the use of Mg tubes as connectors for intestine anastomosis, but he used high-purity Mg, which corroded homogeneously *(Fig. 2B)*. The corroding rate of the connectors was between 2 and 4 weeks, depending on their anatomical localization [21]. Also, Chlumsky interposed 0.1–0.8-mm-thick Mg sheets between the freshly separated bone surfaces in the knee joints of dogs and rabbits, proving the complete corrosion after 18 days. Both in animal and human cases, Chlumsky prevented joint stiffness and restored the joint motion [16]. But later observation showed that all neo-joints became stiff over the years.

In 1903, Hopfner used Mg cylinders for vessel anastomosis and observed thrombosis in vessels with diameter <3 mm. He suggested that thrombosis was because of the extensive intima lesion during the operation and recommended to use Mg for anastomosing of large vessels [22].

In 1910, Lespinasse used Mg metallic ring plates with punched holes for extravasal sutures [23]. The Mg ring plates were found to maintain their original shape for about 30 days before they began to break down and completely degrade within 80–100 days. The Mg rings were tied firmly together, but not so tight to cut the intima and cause vessel necrosis. Lespinasse did not observe any thrombosis or secondary vessels constriction.

In 1917, Andrews used the absorbable Mg clips and staples for successful hemostasis in the brain, deep wounds, and intestinal anastomosis [24]. Unlike the previous researches, he used both pure metals and Mg alloys for different applications. He made alloys with aluminum (Al), cadmium (Cd), and Zn, but soon discovered that all these alloys were too hard and brittle and could not be used for cardiovascular application. In 1924, Seelig found that the available Mg wires on the market were too brittle. So, he used pure Mg produced by distillation in vacuum to obtain more ductile Mg wires. Also, noble metals, such as gold and silver (Ag), were alloyed with Mg to increase its ductility. But after some experiments, he suggested that these wires had a low tensile strength and were not sufficiently pliable [25]. Two years later, Glass had unsuccessful results from two hemangioma treatments using Mg alloy. The additional animal experiments showed that Mg was not beneficial for large and purely cavernous hemangioma [26]. The same inappropriate results were obtained in 1981 after Mg arrow treatment of 27 hemangioma patients [27]. But in 1928, Hoffheinz and Dimitroff found that Mg corroded fast in well cavernous hemangioma with its transformation in connective tissue in rabbit model [28].

In 1951, Stone and Lord used thrombogenic materials for successful intravascular clotting in aortic aneurysms. They used pure Mg wires (0.025 inch diameter) and Mg–Al wires (0.03 inch diameter) in dogs' aortas as double-coiled wires. They found that Mg wires were twice as thrombogenic as stainless steel and that the thrombogenic potential of Al-alloyed Mg wires was as much as three times higher than that of stainless steel. The pure Mg wire was very brittle, while the addition of 2% Al allowed bending and clinical application. Stone and Lord stated that both wires were suitable for the intended application, while the Mg–2% Al exhibited higher thrombogenic potential than commercially pure Mg [29].

Mg in Orthopedic Practice

Mg and its alloys have several advantages for orthopedic surgery. Materials currently used for this purpose, including stainless steel, titanium, cobalt–chromium, and zirconium (Zr) alloys, have limitation because of the possible release of toxic ions during corrosion or wear processes and noncompliance with elastic module of natural bone [30, 31]. Also, metallic materials for permanent fixtures should be removed after a few months that lead to possible complication and the increase of treatment cost [32]. In contrast to other metals, Mg has density and elastic module close to natural bone [33]. During the degradation, Mg alloy releases nontoxic MgO that is most completely excreted in the urine [34]. Also, there are some evidences that Mg has stimulatory effect on the new bone tissue growth [35–37].

Possible Mg implants, such as pins, nails, wires, and plates, were developed by Payr in 1900 [13]. But the first practical application of Mg alloy in orthopedy was carried out by Lambotte in 1906 [38]. After the clinical failure of performing metal osteosynthesis of fractured tibia for 17-year-old child, he used Mg plates with six steel screws. But after the operation, the extensive subcutaneous gas cavities formed were treated by removing the fragments of Mg plates on the eighth day. So, the electrochemically developed Mg, which degraded extensively between the Mg plate and the steel screws. After some animal experiments, Lambotte and Verbrugge found total Mg resorption between 7 and 10 months after implantation. Later clinical investigations of pure Mg without steel screw showed successful results with children suffering from bone fracture *(Fig. 3)*. Thus, they recommended to use Mg implants in Bennett fractures, scaphoid fractures, foot surgery, clavicular fractures, carpus fractures, phalanx and metacarpal fractures, radius epiphyseal fractures, lower arm diaphyseal fractures, supra and condylar fractures in children, humeral head fractures, malleolus fractures, oblique tibial fractures, and pertrochanteric fractures [39].

But, in 1913, Groves investigated Mg as intramedullary peg and suggested that it cannot be used for orthopedy because of the formation of abscess cavities and quick degradation before fracture healing [40]. Later, Zierold proved the stimulation of connective tissue production and acceleration of new bone growth during alloy application [41]. In 1920, Verbrugge investigated Mg alloy with 8 wt. % of Al in animal experiments and clinical cases and found the resorption of Mg after 6–8 months with no signs of inflammation and tissue irritation. Thus, concluding that gas formation was not damaging any tissue. He demonstrated callus formation in 21 clinical cases, in which there was no reaction of skin, soft tissues, bone, and joints to corroded Mg products [42].

After some animal trials, in 1938, McBrigge reported that pure Mg plates were not suitable for bone reconstruction because of its fast degradation time. But he observed that Mg screw was more resistant to corrosion compared with the plates and should be used for bone surgery [43]. Later, McBride used Mg–Al–Mn alloy for bone grafting and fracture fixation in 20 patients *(Fig. 4)*. He did not observe any inflammatory or systemic reaction and slow degradation rate of new alloy. McBride reported that Mg had positive effect on periosteal tissue and callus deposition [44]. Two years later, Maier reported about two positive cases of fracture healing using spindle-shaped Mg sheets. Subcutaneous implantation of Mg implants in rabbit showed corrosion and formation of gas cavities with strong periosteal reaction. Maier [45] suggested that MgO, as a product of corrosion, had irritant effect on bone cells and stimulated periosteal reaction.

Troitskii and Tsitrin in 1944 used Mg alloy with small amount of Cd for treatment of 34 patients with different bone fractures. Of the 34 patients, only 9 were unsuccessful because of infection. In other cases, complete bone healing with no inflammatory reaction around implants was observed; also, they did not find any correlation between Mg degradation and concentration in blood serum. Corrosion process was slow and

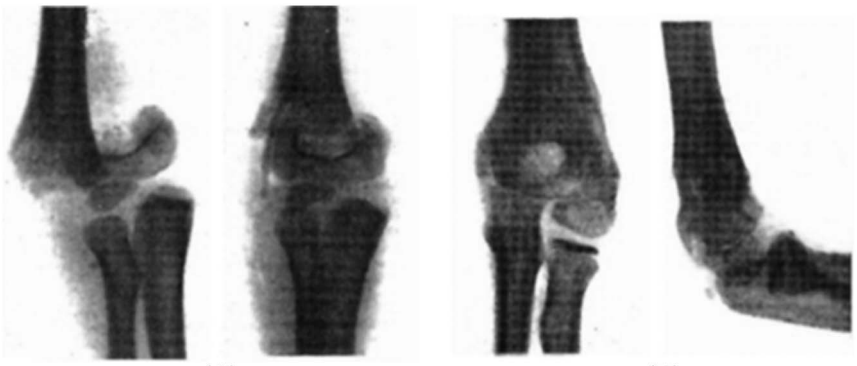


Fig. 3. Supracondylar humerus fracture of a child, fixated with Mg nail by Lambotte (A) and results after several months with total Mg nail corrosion (no gas cavities observed) (B) [38]

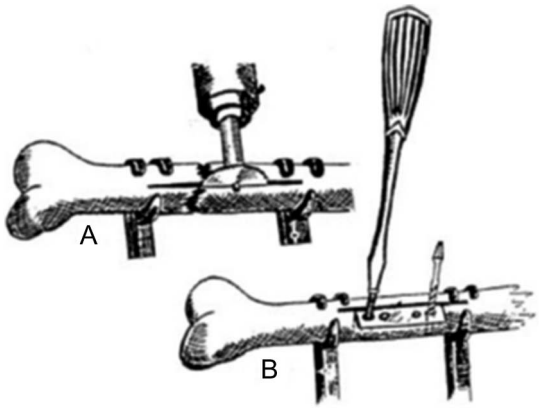


Fig. 4. Rotation-resistant osteosynthesis with Mg–Mn metal plate, provided by McBride [44]

complete within 10–12 months. At the same time, they also reported that some implants were resorbed in 3–5 weeks because of incensement of acidity level in the fracture zone [46]. In 1945, similar positive result of 2 fracture healing was reported by Znamenskii. He used Mg with 10 wt. % of Al, and implants were not detected in the fracture zone in 6 months after grafting [47].

In 1972, Borodkin et al. used Mg alloy with rare earth elements. The alloy was composed of 0.4–4 wt. % rare earth metal, 0.05–1.2 wt. % Cd, 0.05–1.0 wt. % calcium (Ca) or Al, and variable, trace (0.8%) levels of Mn, Ag, Zr, or silicon (Si). They showed the slow degradation of complex alloy in 5–10 months *in vivo,* but they did not report about trace element distribution and any complications [48].

From 2001 to 2005, Witte et al. studied *in vivo* degradation of 4 Mg alloys – with Al and Zn (3 wt. % Al + 1 wt. % Zn and 9 wt. % Al + 1 wt. % Zn) and with rare earth elements (4 wt. % of yttrium (Y) + 3 wt. % of neodymium, cerium, and dysprosium and 4 wt. % of lithium (Li) + 4 wt. % of Al + 2 wt. % of cerium, lanthanum, neodymium, and praseodymium). Microtomography showed alloy degradation in 18 weeks after operation with significant increase of bone formation compared with the control group (polylactide rod). They proved the slowest corrosion rate in Li–Al-rare elements alloy. The rare elements were detected in the corrosion layer in the presence of amorphous $Ca_3(PO_2)_4$, but not in the surrounding bone tissue [49].

In recent years, several researchers have investigated different Mg alloys for increasing its degradation stability, mechanical properties, and biological response. Trincă et al. (2015) suggested the use of an alloy based on Mg with addition of 0.4% Ca and 0.5% Si and carrying out an Si concentration gradient of 0.25 mm depth from the sample surface to inside. In the case of the tibiae implant, the variation of the main biochemical and histological parameters sustained normal evolution of the bone fracture with a short resorption stage on the background of a relatively constant bone formation rate. The specific histological stains showed the intense and active bone formation after 2 weeks of implantation, whereas after the 4th week, the bone remodeling process had already started. X-Ray and computed tomography (CT) registered the presence of experimentally created defect in the tibia and revealed some of the bone-specific recovery stages in relation with the biodegradation process of the implant sample [50].

In 2015, Wang implanted Mg–Zn–Zr alloy cylinders into the femoral condyles of Japanese big-eared white rabbits. In the 24th week, the implant was more obscure and the density of the surrounding cancellous bone increased. Micro-CT confirmed that new bone tissue on the surface of the residual alloy implant increased between 12th and 24th week. On 12th week, many cavities in the cancellous bone tissue around the implant were noted with a CT value, similar to a gas value, and increased by the 24th week. The histological examination of hard tissue slices showed that bone tissue was visibly attached to the alloy in the femoral condyle at the 12th week. The trabecular bone tissues became more intact and dense, and the cavities were filled with soft tissue at 24th week. In general, gas produced by the degradation of the Mg–Zn–Zr alloy can cause cavitation within cancellous bone, which does not affect osteogenesis around Mg alloy [51].

Pan et al. developed the new wrought Mg–2Sn–1Ca wt. % (TX21) and Mg–2Sn–1Ca–2Zn wt. % (TXZ212) alloys with high strength and ductility, simultaneously produced by conventional casting, homogenization, and indirect extrusion. They proved that the high strengths were because of the high number density of nano-Mg–Sn–Ca phases, G.P. zones, and ultra-fine grain size (~0.8 μm) [52]. In the same year, fine-grained Mg–1.8Gd–1Zn–0.1Zr (at.%) alloy with long-period stacking ordered (LPSO) phase was obtained via solid solution (SS) treatment plus multipass equal-channel angular pressing (ECAP). The effects on post-ECAP rolling on microstructure changes and deformation characteristics of the Mg alloy were investigated. The results showed that the fine-grained alloy after 16 ECAP at 658 K had yield strength of 334.4 MPa with an elongation of 22.5%. The grain refinement with LPSO formed and improved the strength and ductility of the ECAPed alloy simultaneously, indicating good plastic formability [53]. But the *in vivo* experiment was not performed, and the biodegradation rate was not studied.

Yang Liu added 2×10^{17} ions cm^{-2} of Ag, Fe, and Y to the Mg–1Ca alloy using metal vapor vacuum arc technique. Y-ion implantation induced an Mg/Ca-deficient outer oxidized layer, and the distribution of Y along with depth was more homogeneous. Both electrochemical and immersion tests revealed the accelerated corrosion rate of Ag-implanted Mg–1Ca and Fe-implanted Mg–1Ca, whereas Y-ion implantation showed a short period of protection as enhanced corrosion resistance was obtained by electrochemical test, but accelerated corrosion rate was

found by long-period immersion test. Indirect cytotoxicity assay indicated good cytocompatibility of Y-implanted Mg–1Ca [54].

Hofstetter investigated the effect of trace impurity elements on the degradation behavior of high-strength Mg alloys of type ZX50 (Mg–5Zn–0.3Ca). It is shown that trace impurity elements increased the degradation rate, predominantly in the initial period of the tests, and also increase the material's susceptibility to localize corrosion attack. These effects are explained on the basis of the corrosion potential of the intermetallic phases presented in the alloys [15].

The recent study of different statuses of Mg–strontium (Sr) showed that the as-cast Mg–Sr alloy exhibited a rapid degradation rate compared with the as-extruded alloy because of the intergranular distribution of the second phase and micro-galvanic corrosion. However, the initial degradation could be tailored by the coating protection, which was proved to be cytocompatible and also suitable for bone repair observed by in vivo implantation. The integrated fracture calluses formed and bridged the fracture gap without gas bubble accumulation, meanwhile the substitutes simultaneously degraded. In conclusion, the as-cast Mg–Sr alloy with coating is potential to be used for bone substitute alternative [55].

Zhou et al. developed extruded Mg–1Mn–2Zn–*x*Nd alloys ($x = 0.5$, 1.0, 1.5 mass%). The experimental results indicated that all extruded Mg–1Mn–2Zn–*x*Nd alloys show good ductility and much higher mechanical strength than that of cast pure Mg and natural bone. The tensile strength and elongation of the extruded alloys increase with an increase in neodymium content. Their compressive strength does not change significantly with an increase in neodymium content. The extruded alloys show good biocompatibility and much higher corrosion resistance than that of cast pure Mg [56].

In summary, an ideal Mg alloy (for degradation rate, *in vivo* response, and mechanical strength) is still not found.

Mg Alloy Degradation

The great challenge is to tailor implant degradation in a manner that is suitable for a biological environment [8]. The best way for Mg alloy production for orthopedic and surgery application is still investigated. The fast resorption can lead to mechanical instability before complete bone healing, but the low degradation can lead to the inappropriate host response. The basic conditions that determine the corrosion rate are alloy compound and environment around implant. In aqueous media, Mg alloy degrades during the electrochemical reaction known as a corrosion and produces magnesium hydroxide ($Mg(OH)_2$) and hydrogen gas. $Mg(OH)_2$ is not soluble and forms a protective layer on alloy surface. When the chloride concentration is above 30 mmol/l, it converts into the soluble $MgCl_2$. Chloride content in body fluids is about 150 mmol/l, and degradation of Mg alloy starts just after their insertion to the organism [57].

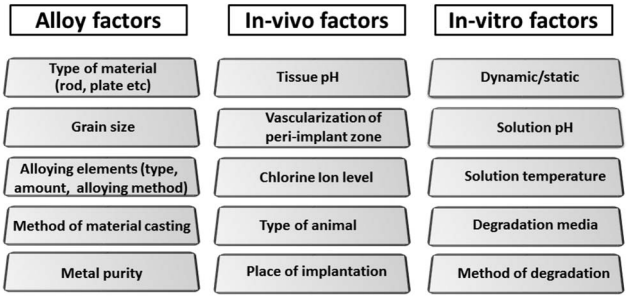


Fig. 5. Factors that can change Mg and Mg alloy degradation

The degradation rate of Mg depends on several factors (*Fig. 5*). Sanchez et al. reviewed more than 100 papers for *in vitro* (23 alloys) and *in vivo* (20 alloys) degradation, and pointed on different time of degradation and the absence of correlation data between *in vitro* and *in vivo* experiments [8].

The methods of *in vitro* degradation include electrochemical tests, hydrogen evolution, and mass/volume loss after immersion test [8]. Electrochemical test is simple and reproducible, but it leads to acceleration of corrosion that does not correlate with *in vivo* degradation [58]. For example, degradation rate using immersion method for pure Mg reported by Zhang was 0.26 mm $year^{-1}$ [59], whereas degradation rate using electrochemical method was 2.52 mm $year^{-1}$ [60]. The methods of mass or volume loss are similar to *in vivo* conditions but have some limitations. For example, mass loss without removing the corrosion products can lead to negative degradation rate [61].

The main challenge during the corrosion test is choosing the media for experiment. For this test, solution that simulates *in vivo* environment should be used. The best test media for this purpose are Hank's solution, simulated body fluid (SBF), Earle's balanced salt solution (EBSS), or minimum essential medium (MEM). The ion concentration in SBF is very similar to blood plasma, but MEM contains glucose, amino acid, and vitamins [8]. MEM and EBSS contain a slightly lower amount of Ca and Mg compared with blood [62] (*Table II*). Using different media, it is possible to obtain different valuable results. For the pure Mg, corrosion rate measured by immersion in EBSS and reported by Walker was 0.39 mm $year^{-1}$ [63], whereas in SBF and Hank's solution, it was 1.39 [64] and 2.05 mm $year^{-1}$, [65] respectively. It is also likely that with alloying Mg – degradation rate for Ca–P coating – an increase from 0.25 mm $year^{-1}$ in Hanks solution [66] to 1.88 mm $year^{-1}$ in SBF [67]. It should be noted that not only methods but also solution can influence the degradation rate.

Table II The ions and glucose level in blood and experimental media for testing of biodegradability

Ions and organic composition (mmol/l)	Blood plasma	SBF	MEM	EBSS	Hank's solution
Na	135.0–145.0	142.0	143.0	144.0	142.0
K	3.5–5.3	5.8	5.4	5.4	5.8
Mg	1.5–2.3	0.8	0.4	0.4	0.8
Cl	103.0	145.0	125.0	125.0	145.0
Ca	2.1–2.8	2.5	1.8	1.8	2.5
HPO_4	0.8–1.5	0.4	0.9	1.0	0.4
SO_4	0.4–0.6	0.8	0.4	0.4	0.8
HCO_3	18.0–23.0	4.2	26.0	26.0	4.2
Glucose	3.5–5.5	–	5.6	5.6	–

Experimental temperature can significantly influence degradation of Mg alloy *in vitro*. Pure Mg degrades two times faster at 37 °C compared with 20 °C. The same authors demonstrated that the temperature increases up to 40 °C and accelerates corrosion rate by 50% compared with 37 °C [68]. This finding shows potential risk of extensive Mg alloy corrosion after implantation, especially during the inflammatory processes.

The influence of pH solution on Mg corrosion was described in several publications [63, 69–71]. They showed that using buffering system for keeping constant pH around material was very important for the appropriate experiment results. The nonbuffered solution leads to pH increase, formation of protective layer on alloy surface, and decrease of corrosion rate. The best buffering solution that mimics *in vivo* environment is $NaHCO_3$/CO_2 buffers. It maintains pH in neutral regimen, and corrosion will not be stopped [8].

To mimic *in vivo* environment, we need to use a dynamic test. In static immersion test, protective layer on Mg alloy should be formed because we did not remove degradation products. The last can lead to changes in solution environment and stop corrosion. Shi et al. showed that degradation rate of AZ31 Mg alloy during the static condition (0.3 mm $year^{-1}$) was five times lower compared with the dynamic condition (1.5 mm $year^{-1}$). Moreover, dynamic experiment significantly correlates with *in vivo* experiment (1 mm $year^{-1}$) [72].

All these data show that the ideal conditions for *in vitro* Mg and its alloy corrosion test are still investigated, and the main parameters of this research are solution compound, temperature, and pH.

In vivo Mg resorption was completed from a few weeks to more than 1 year, depending on type of Mg alloy and host tissue environment. The first Mg plate application reported a fast degradation because of electrochemical reaction with stainless steel screws [38]. Recent research showed slower degradation *in vivo*, but all factors that determine Mg alloy corrosion in animal and human body are still not found.

Sanchez in her review summarized data of more than 50 papers for Mg alloy degradation [8]. She did not find any correlation between alloy compound and degradation rate. Pure Mg corrosion rate varies from 0.33 mm $year^{-1}$ after subcutaneous implantation [63] to 0.86 mm $year^{-1}$ in femur implantation model [73]. Mg–Al–Zn alloy degrades faster in rabbit compared with the rats during the bone no-intramedullary implantation: 1.64 mm $year^{-1}$ [49] compared with 0.168 mm $year^{-1}$ [74]. In the same case, intramedullary implantation in femur of Mg–Zn–Ca–Mn alloy corrodes three times faster after the intramuscular implantation insertion [65, 75].

Based on enumerated data, we cannot choose the main causes that influence Mg corrosion *in vivo*. The first is animal model that involves the experiment and anatomical region of implantation. Animals have different water contents and blood flow that can influence the removing of degradation products. Water content in human bone tissue is 43.9% and it significantly increases in rabbit bone tissue up to 58.1%. For example, blood flow increases from rat (2.3 ml/min/100 g) to rabbit (19.1 ml/min/100 g) and human (120 ml/min/100 g). Water content in skin is significantly higher in all animal models as well as in human, but blood flow in rabbit skin decreases to 12.7 ml/min/100 g. The amount of water and blood flow can affect the removal of degradation product and prevent the formation of protective layer on alloy surface [57].

Chloride-contained environment can transform Mg $(OH)_2$ into soluble $MgCl_2$ that accelerates corrosion. But the level of chlorine ion depends not only on the species but even on the tissue and body condition. This factor can significantly decrease corrosion rate and is probably the main factor that determines the difference between *in vitro* and *in vivo* experiments [49, 76].

As mentioned previously, pH is one of the factors that determine degradation rate *in vitro*. After the metal implantation, tissue response such as inflammation or foreign body reaction can be observed. It can lead to formation of stable corrosion layer in first term after the surgical procedure [77]. Body fluid ion concentration as

well as temperature can also change the rate of Mg corrosion [78].

By comparing the data for *in vitro* and *in vivo* degradation, it can be observed that different corrosion rates depend on numerous factors. *In vitro* test gives only general information about alloy degradation, and this information should be used for planning *in vitro* investigation. But the last aspect does not answer the key question—how does alloy will corrode in clinical case? Only the systematic analysis of clinical trials and *in vivo* animal studies with different conditions can give complete information about the relations between the degradable Mg alloy and the hosting organ.

Mg Alloying Elements

Pure Mg implants have low corrosion resistance and unsatisfactory properties, and Mg fast degradation and distribution over the body may cause clinical complications. The main purpose of developing a alloy is to improve the mechanical properties, corrosion resistance, and the production cost [57]. The basic elements used for Mg alloying are Al, Ca, copper, Fe, Li, Mn, nickel, Sr, Y, zinc, Zr, and rare earth elements [49, 77, 79–82]. But the alloy properties also depend on intermetallic compound and microstructural effect based on the processing route.

Witte [57] classified Mg alloys into three groups: (1) pure Mg with trace of other elements, (2) Al-containing Mg alloy, and (3) Al-free Mg alloys. The most used Al-contained alloys are Al–Zn, Al-rare elements, Al–Ca, Li–Al, and Al–Li-rare elements [83, 84]. The most typical Al-free composites are Mg–Mn–rare elements, Mg–Mn–Zn, Mg–Yt–Zn, and Mg–Ca [85]. Nontoxic alloying elements should be used for humans.

As a widely used element for Mg alloying with maximum solubility of 12.7 wt. %, Al can provide both solid strengthening solution and precipitation. Witte et al. reported that increase of the Al content lowers the liquidus and solidus temperature lines and enhances the castability of alloys with high Al solidus [57]. The Al addition of Mg alloys leads to the improvement of strength and the small increase of density (the density of Al is close to that of Mg), but it causes the decrease of elongation [86]. Insoluble Al_2O_3 will be formed in the corrosion products layer in alloys containing Al and Mg during corrosion [87]. Al should be used carefully because of its possible biological complications such as risk factor in Alzheimer's disease, muscle damage, and decrease of activities of osteoclasts [88–90].

Zn is a nontoxic element that plays significant role in human metabolism as a co-factor for some enzymes, and it is essential for immune system [91, 92]. The consumption of Zn in amounts higher than the upper limit (40 mg/day) is generally considered relatively nontoxic, and amounts approaching 100 mg/day can be tolerated for a few days [93]. Zn is an important alloying element with a relatively high solubility in Mg—up to 6.2 wt. %. Zn content that is up to 4 wt. % significantly increases the ultimate tensile strength and elongation of as-cast Mg–Zn alloys, but any higher percentage of Zn would lead to the reduction of both properties and decrease the corrosion resistance of the alloy [94]. But it was shown that amorphous Mg–Zn-based alloys containing about 5.0 wt. % of Zn had excellent strength, high corrosion resistance, low hydrogen evolution rate, and good biocompatibility in animals; therefore, these are promising candidates for biodegradable bone implants. But problems arise from a quite difficult preparation of metallic glasses and especially forming them to a final product. The common processes involved rapid solidification of melt that limited the maximum thickness of amorphous alloys to hundreds of micrometers [95]. In amount <2 wt. % Zn contributes to strength because of SS strengthening, but larger amount leads to embrittlement in combination with Al [83]. The mechanical and degradation properties being the main concerns, the Mg–Zn alloys with low Zn content (<4 wt. %) were further alloyed by adding the third alloying elements, including Ca [96], Mn [97], Sr, Y, and Zr [98].

Ca is the most abundant mineral in the human body, and it is strictly regulated by homeostasis of skeletal, renal, and intestinal mechanisms. It plays a significant role in bone function, vascular, and heart physiology [99]. The solubility of Ca in Mg is about 1.34 wt. %, and under the equilibrium conditions, Ca contributes to solid strengthening solution and precipitation. It also acts to some extent as a grain-refining agent and additionally contributes to grain boundary strengthening. In binary Mg–Ca alloys, the Laves phase Mg_2Ca is formed, whereas in Al-containing alloys, the Laves phase Al_2Ca is formed first. Both phases improve creep resistance because of SS strengthening, precipitation strengthening, and grain boundary pinning. Mg_2Ca intermetallic phase is brittle, which can act as potential sources for cracking to occur and indicates a negative effect on the ductility of Mg, and it also accelerates the degradation because of galvanic corrosion. In the Mg–Ca system, Mg_2Ca is the only second phase besides α-Mg and distributes around grain boundaries. Wan et al. [100] reported that 0.6 wt. % of Ca addition could improve the bending and compressive strength of pure Mg, whereas a higher Ca addition deteriorated these properties. Large amounts of Ca (>1 wt. %) can lead to problems during casting like hot tearing or sticking [57]. Zn, Y, and Sr were introduced in the Mg–Ca binary alloys to optimize their mechanical and degradation properties [101]. The introduction of Zn (2.31 wt. %) into the as-cast Mg–3Ca alloy can improve the strength and ductility of the alloy, and its presence supports the formation of the eutectic phase (α-Mg + Mg_2Ca + $Ca_2Mg_6Zn_3$) that leads to a decreased degradation rate of the alloy [102]. Ca-contained alloy

positively influences the cell viability and proliferation rate [103].

Mn is an essential element that plays important role in metabolic cycle of lipids, amino acids, and carbohydrates. It also influences the function of immune system, bone growth, and blood clotting [104]. In Mg alloy, Mn is mainly used to enhance ductility. More important is the formation of Al–Mn intermetallic phases in Al-containing Mg alloys. These phases can pick-up Fe and can therefore be used to control the corrosion of Mg alloys because of the detrimental effect of Fe on the corrosion behavior [57]. Several researches show nontoxic influence of Mn during cell culturing, but its poisonous effect from Mg alloys on the cell viability and the proliferation has also been observed [57].

Zr is a powerful grain refiner for Mg alloys; it is usually used in alloys containing Zn, RE, Y, and thorium, and it cannot be used together with Al and Mn as they form stable compounds with Zr [105]. Recently, the Mg–Zr alloys had attracted considerable attention because of their high-specific damping capacity (around 80%), which may help to suppress the vibrations generated during movement and stress at the implant/bone interface [106]. It was indicated that 1 wt. % of Zr addition in Mg resulted in significant improvement of the strength and ductility of the metal and reduced the degradation rate by 50%, and co-addition of Sr and Sn could effectively reduce the degradation of as-cast Mg–Zr–Ca alloy [107]. Some authors investigated alloys with a wide range of Zr content 1–5 wt. % and showed that the degradation rate increased with increasing Zr content [108].

Rare earth elements are used both in Al-contained and Al-free alloys for changing final alloy mechanical properties, resorption rate, and biological response. We need to balance between possible toxicity and benefit. For Mg alloy processing, several rare elements have been used, such as cerium (Ce), lanthanum (La), neodymium (Nd) and praseodymium (Pr), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu) [57, 109]. Usually, they are used in combination with other alloying elements, but currently, there are some Mg alloys containing only rare earth elements.

Alloying elements can significantly improve mechanical properties, control corrosion rate, and influence biological response on Mg alloy. But it is difficult to choose the best alloy, and to do this, we need some more *in vitro* and *in vivo* experiments and clinical investigation.

Conclusion

Since 1900, after the first experimental application, Mg and its alloys were applied during different clinical cases, but they were not widely used because of uncontrolled corrosion and excessive hydrogen formation. It led to implant failure and clinical complications. There are two ways of biodegradable implants improvement – alloying elements addition and new methods of alloy casting to change intermetallic phases and grain. There are many bi- and poly-phase alloys created with improvement properties, but their biological response and long-term clinical results are still not completely clear.

In vitro and *in vivo* studies have been used for evaluation of degradation rate and host response. There is no correlation between these methods and should be used together for better alloy assessment. The best method for *in vitro* degradation is emersion in medium that simulates body environment, such as SBF, MEM, or EBSS. Electrochemical method and emersion in saline solution do not give reliable results. The results of *in vivo* research depend on animal species, implant anatomical location, and some physiological parameters, such as pH, blood flow speed, and chloride ion concentration. All these parameters could significantly change corrosion rate and host response.

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