



Final Draft of the original manuscript

Morquecho-Marin, D.; Silva-Bermudez, P.; Millan-Ramos, B.;
Victoria-Hernandez, J.; Letzig, D.; Ramirez-Ortega, D.; Depablos-
Rivera, O.; Garcia-Lopez, J.; Barrera-Ortega, C.; Rodil, S.:
Unexpected cytotoxicity of TiO₂-coated magnesium alloys.
In: Materials Letters. Vol. 276 (2020) 128236.

First published online by Elsevier: 02.07.2020

<https://dx.doi.org/10.1016/j.matlet.2020.128236>

Unexpected cytotoxicity of TiO₂-coated magnesium alloys

Daniela Morquecho-Marín^{1,2}, Phaedra Silva-Bermudez^{1,*}, Benjamín Millán^{3,4}, José Victoria-Hernández⁵, Dietmar Letzig⁵, David Ramírez-Ortega³, Osmary Depablos-Rivera³, Julieta García-López¹, Cecilia C. Barrera-Ortega⁶, Sandra E. Rodil^{3,*}

¹Unidad de Ingeniería de Tejidos, Terapia Celular y Medicina Regenerativa; Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra, 14389, CDMX, Mexico

²Posgrado en Ciencias Médicas, Odontológicas y de la Salud, Universidad Nacional Autónoma de México, 04510, CDMX, Mexico

³Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de México, 04510, CDMX, Mexico

⁴Posgrado en Ciencia e Ingeniería de Materiales, Universidad Nacional Autónoma de México, 04510, CDMX, Mexico

⁵Magnesium Innovation Centre MagIC, Institute of Materials Research, Helmholtz-Zentrum Geesthacht, 21502 Geesthacht, Germany

⁶Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, 54090, Edo. de Mexico, Mexico

*Corresponding authors. E-mail addresses: phaedrasilva@yahoo.com (P. Silva-Bermudez) and srodil@unam.mx (S.E. Rodil)

Abstract

Biodegradable Mg-based alloys have attracted increasing attention from orthopedic surgeons and biomaterials scientists since their Young's modulus is close to that of bone. However, control of the degradation rate is still a challenge. Sputtered TiO₂ coatings were deposited on Mg-Zn-Ca alloys aiming to reduce the initial strong reactivity of Mg with biological fluids. However, the degradation tests indicated that the coated samples induced a larger variation in the pH, greater H₂ production and formation of degradation products in comparison to the bare alloys. Consequently, an enhanced cytotoxicity of the coated samples was observed.

Keywords: Magnesium alloys; Sputtering; TiO₂ coatings, cytotoxicity

1.Introduction

The major issue for the clinical applications of Mg-based alloys as biodegradable implants is their rapid corrosion rate [1, 2], which leads to loosening of their mechanical integrity prior to the recovery of bone. Additionally, during the corrosion process there is a significant release of H₂, localized alkalization and increase in the osmolality that affect the cells functions.

Great efforts have been made to reduce the corrosion rate producing Mg-alloys using only bio-inert elements [3, 4]. Nevertheless, there is still need of reducing the immediate reactions in physiological fluids to levels where the corrosion products do not affect the cell attachment and proliferation on the surface of the Mg-based implants [5, 6]. Thin-protective layers are an attractive strategy since the mechanical properties of the bulk can be preserved and the film will eventually breakdown allowing the desirable degradation of Mg. Titanium-based coatings are attractive since Ti has already proven to be biocompatible, osteoconductive and osseointegrable [7]. However, metallic Ti coatings might be problematic due to the large differences in the electrochemical potential between Ti and Mg. An alternative is the use of ceramic TiO₂ coatings that can give similar or even better biological response than commercial Ti surfaces [8]. The idea of using TiO₂ ceramic coatings has been investigated before [9-16], but a careful analysis shows that TiO₂ coatings were usually combined with polymers or ceramics forming multilayers or composite coatings, otherwise, no improvement was obtained (Supplementary file). However, no detail explanation has been given about why to avoid direct TiO₂-Mg contact. In this work, we deposited TiO₂ films directly on polished Mg-alloys surfaces and evaluate the degradation and cytotoxicity.

2.Experimental Details

Fine grained twin-rolled strips of the Mg_{0.7}Zn_{0.6}Ca (ZX11) alloy were hot rolled as described in [17]. For the present work, ZX11 sheets annealed at two recrystallization temperatures: 350 °C (Mg1) and 450 °C (Mg2) were used. These led to coarser grain sizes and less fraction of

precipitates for Mg2 in comparison to Mg1 (Fig. S1). The effect of the microstructure on alloy's properties will be reported elsewhere. Radio frequency magnetron sputtering was used to deposit 80 nm thick TiO₂ coatings on both faces of 10 x 10 x 1 mm³ pieces using a Ti target and a reactive Ar/O₂ atmosphere [8]. The H₂ production and mass-variation during immersion in physiological solutions were evaluated. Cell viability was evaluated by the MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay using indirect cell culture inserts in which the Mg piece was floating and separated by a membrane on top of the cell cultures (human adipose-derived mesenchymal stem cells). In this method, cells are exposed to the degradation effects of the sample without any dilution. The variation of the pH during the first 48 h of alloys culture in DMEM-F12 was evaluated.

3. Results

The coatings were conformal and presented a TiO₂ composition and a Rutile structure with grain sizes below 10 nm (Fig. S2). Degradation of Mg in chloride-rich solutions (Phosphate buffer solution, PBS) and in DMEM-F12 medium occurred via the reactions summarized in Table 1.

Table 1. Mg degradation reactions and degradation products

Anodic reaction	$\text{Mg}_{(s)} \rightarrow \text{Mg}^{2+}_{(aq)} + 2e^{-}$
Cathodic reaction	$2\text{H}_2\text{O} + 2e^{-} \rightarrow \text{H}_2 + 2\text{OH}^{-}$ $2\text{H}_2\text{O} + \text{O}_2 + 4e^{-} \rightarrow 4\text{OH}^{-}$
Product formation	$\text{Mg}^{2+} + 2(\text{OH}^{-}) \rightarrow \text{Mg}(\text{OH})_2$ $\text{Mg}^{2+} + \text{HCO}_3^{-} \rightarrow \text{MgCO}_3 + \text{H}^{+}$
Product Dissolution	$\text{Mg}(\text{OH})_2 + 2\text{Cl}^{-} \rightarrow \text{MgCl}_{2(aq)} + 2\text{OH}^{-}$

During immersion in physiological solutions, the degradation products are accumulated on the surface (Fig. S3 and S4), leading to an increment on the mass that is reported in Fig. 1a. For

both media and Mg substrates, the coated samples presented a larger mass gain indicating a faster degradation. Note that the usual acid chromic acid cleaning was not used since it can damage the TiO₂ film, disturbing the results.

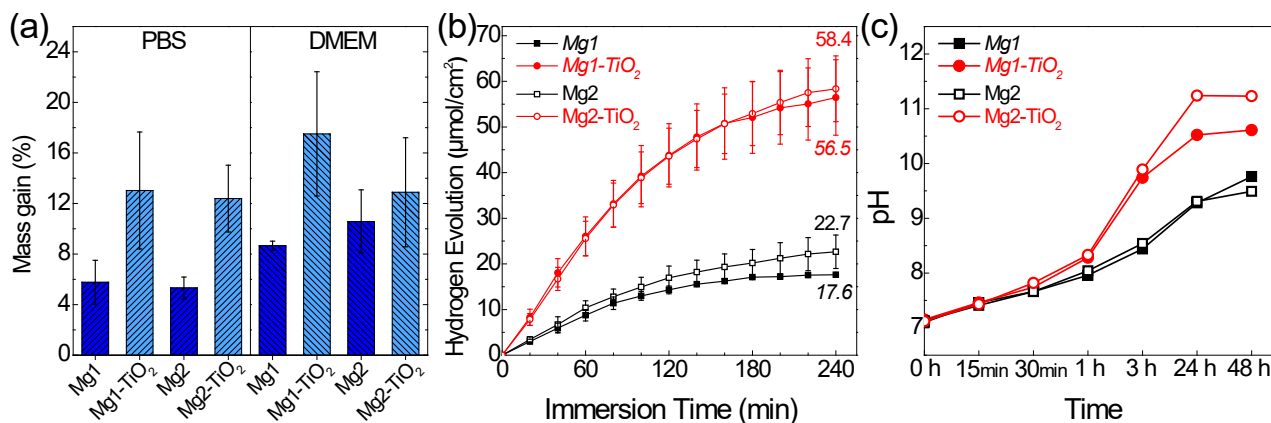


Fig. 1 (a) Mass gain of uncoated and coated Mg1 and Mg2 samples after immersion for 4 days. (b) Hydrogen evolution plot. (c) pH versus time plot.

Moreover, the cathodic reactions involved in the Mg degradation, indicate that H₂ and OH⁻ ions (increase in pH) are released. The H₂ evolution (Fig. 1b) shows that the deposition of TiO₂ nanocoatings on Mg alloys increased 3.2 and 2.6 times the H₂ amount of Mg1 and Mg2, respectively. This is also observed following the pH evolution in the solution (Fig 1c). During the first hour, the pH linearly increased following a similar rate for all samples. However, after one hour, there was a sudden increase in the slope for the coated samples presenting about one unit of pH larger than the bare alloys. The overall results presented in Fig. 1 indicated that the initial degradation of the TiO₂-coated Mg is significantly more severe than that of the Mg alloys, which agrees with the larger cathodic current densities observed in the electrochemical results (Fig. S5).

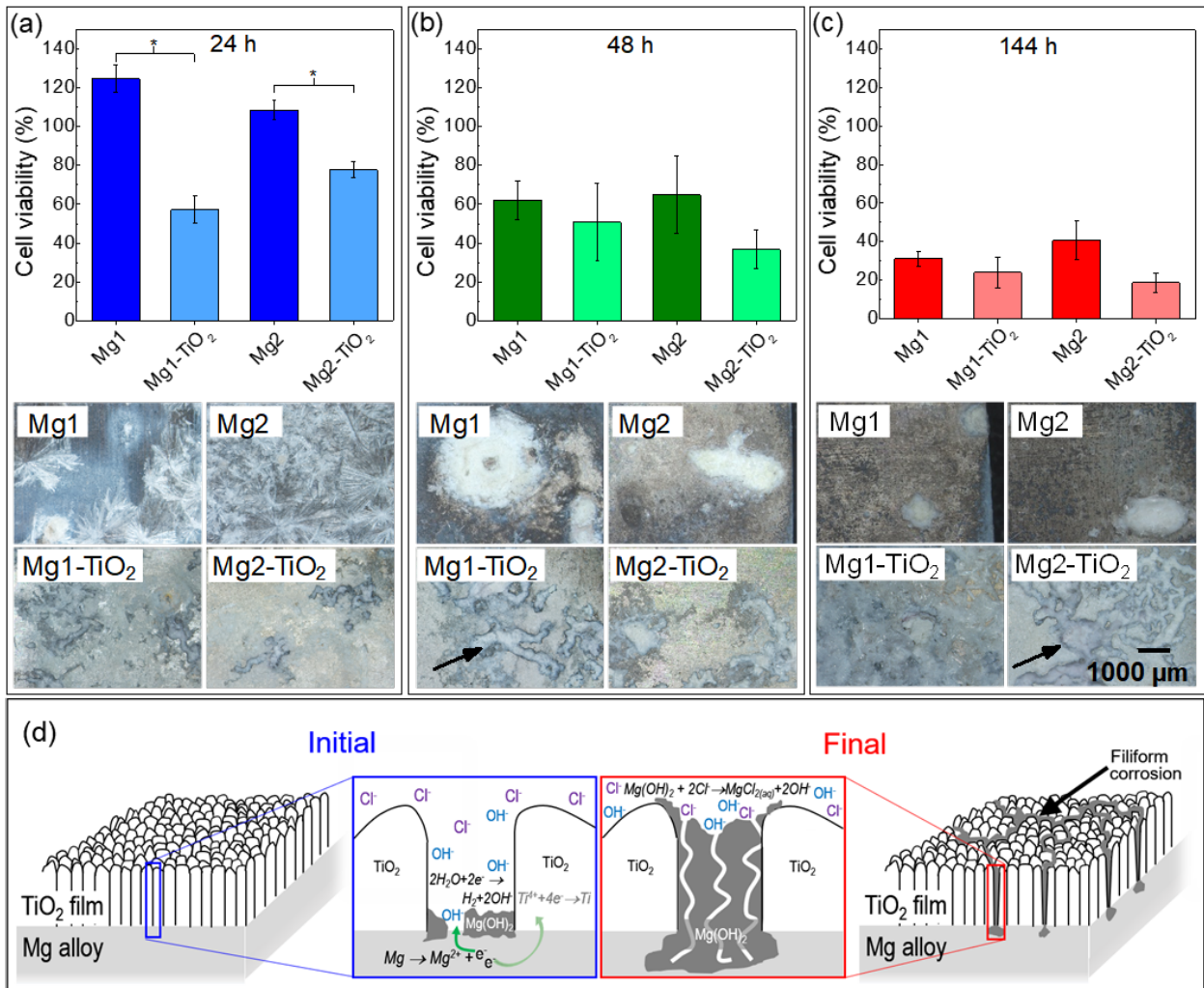


Fig. 2 Cytotoxicity MTT assay results and surface micrographs of uncoated and coated Mg1 and Mg2 samples after immersion during (a) 24, (b) 48 and (c) 144 h in DMEM-F12. * $p \leq 0.05$. Cell viability is reported as considering 100% cell viability that of cells cultured under standard culture conditions (no alloys interaction) at the corresponding culture days. All micrographs have the same scale. (d) Schematic model of the corrosion process in coated samples. Arrows indicate the filiform corrosion morphology.

Figure 2 shows the cytotoxicity evaluated by the MTT test after 24, 48 and 144 h of cells indirect culture. It can be observed that at 24 h, there is no reduction in cell viability for the ZX11-alloys, data which is noteworthy since usually 5X or 10X diluted extracts need to be used to test the cytotoxicity of Mg-based samples [18]. However, for both coated samples, there is a significant reduction, reaching only 41% cell viability for Mg1-TiO₂ and 73% for Mg2-TiO₂. At 48 h and

further, the alloys showed a reduction in cell viability, but the values are always lower for the coated samples.

This unforeseen cytotoxicity observed for the coated samples is difficult to explain but is mainly consequence of the accelerated corrosion, which is evidenced by observing the surface morphology of the samples placed in the culture inserts during the MTT assays. The images show clear evidence of localized corrosion leading to typical filiform patterns on the coated samples (Fig. 2 and S4), meanwhile, the uncoated samples, present accumulation of corrosion products and only small areas of localized corrosion. The increased cytotoxicity was also confirmed by the reduced number of cells, and the morphological changes, observed for the coated samples during the immunocytochemical evaluation of RUNX2 at 6 days of indirect cell culture (Fig. 3).

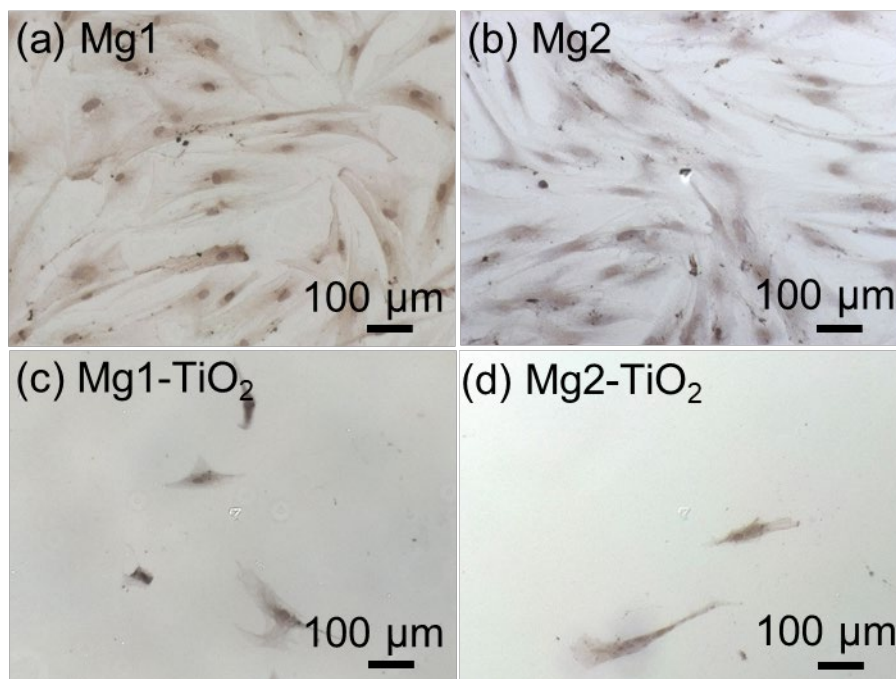


Fig. 3. Immunocytochemical evaluation of RUNX2, as an osteogenic differentiation marker, at 6 days of cells indirect culture (in presence of complete corrosion products of alloys samples) with (a)-(b) uncoated and (c)-(d) coated samples. Similar results were observed in different zones and amplifications (Fig S6).

4. Discussion

Sputtering is a well-established deposition technique, which produces dense thin films that usually provide initial corrosion resistance, but that might contain micro-nano porosity [19]. This film-through porosity allows the electrolyte to encounter both the Mg and the TiO₂ coating on confined localized areas. The corrosion of the Mg alloys is accelerated either for the confinement effect (localized corrosion) or by a possible formation of a micro-galvanic TiO_x-Mg pairs. It is well-known that Mg is a reductor of TiO₂ as indicated by the negative free energy (about -200 KJ/mol) from the Ellingham diagram. The preferential reduction of rutile in P25 TiO₂ powders at any temperature has been observed by Xu et al. [20], while Zu et al. [21] describe the room temperature reduction of TiO₂ by Mg sheets. These papers show partial reduction (Ti(IV) to Ti(III)), but complete reduction could be achieved by the presence of H₂[22] or dissolved chloride species, such as MgCl₂ [23] or TiCl₄ [24], although these reactions usually occur at high temperatures. Analysis of the ZX11-TiO₂ interface after the degradation to corroborate the hypothetical TiO₂ reduction is difficult due to the accumulation of corrosion products. However, the results presented clearly indicated an increase in the corrosion rate and cytotoxicity of the TiO₂ coated Mg samples occurring through localized corrosion. This localized corrosion spreads laterally explaining the filiform shape of the corrosion patterns observed for the coated specimens (Fig 2d) and the larger increase in pH and H₂ evolution.

5. Conclusions

Rutile nanocrystalline coatings deposited on Mg-alloy (ZX11) by magnetron sputtering induced a faster degradation and increased cytotoxicity. No significant differences were observed for the two alloy microstructures, since the major problem was the localized-accelerated corrosion of the alloy occurring at the nano-micro porosity of the TiO₂ film.

Acknowledgements

This work was supported by CONACYT-Frontera-1740, CONACyT-CB-288101, CONACyT-299703, and DGAPA-PAPIIT-IN101419

References

- [1] F. Witte, N. Hort, C. Vogt, S. Cohen, K.U. Kainer, R. Willumeit, F. Feyerabend, *Current Opinion in Solid State and Materials Science* 12(5-6) (2008) 63-72.
- [2] H.-S. Han, S. Loffredo, I. Jun, J. Edwards, Y.-C. Kim, H.-K. Seok, F. Witte, D. Mantovani, S. Glyn-Jones, *Materials Today* 23 (2019) 57-71.
- [3] P. Wan, L. Tan, K. Yang, *Journal of Materials Science & Technology* 32(9) (2016) 827-834.
- [4] M.-S. Song, R.-C. Zeng, Y.-F. Ding, R.W. Li, M. Easton, I. Cole, N. Birbilis, X.-B. Chen, *Journal of Materials Science & Technology* 35(4) (2019) 535-544.
- [5] R. Willumeit, A. Mohring, F. Feyerabend, *Int J Mol Sci* 15(5) (2014) 7639-50.
- [6] R. Amberg, A. Elad, F. Beuer, C. Vogt, J. Bode, F. Witte, *Acta Biomater* 98 (2019) 186-195.
- [7] M. Kaur, K. Singh, *Mater Sci Eng C Mater Biol Appl* 102 (2019) 844-862.
- [8] P. Silva-Bermudez, A. Almaguer-Flores, V.I. Garcia, R. Olivares-Navarrete, S.E. Rodil, *J Biomed Mater Res A* 105(2) (2017) 498-509.
- [9] J. Hu, G. Shaokang, C. Zhang, C. Ren, C. Wen, Z. Zeng, L. Peng, *Surface and Coatings Technology* 203(14) (2009) 2017-2020.
- [10] P. Shi, W.F. Ng, M.H. Wong, F.T. Cheng, *Journal of Alloys and Compounds* 469(1-2) (2009) 286-292.
- [11] H.R. Bakhsheshi-Rad, E. Hamzah, M. Daroonparvar, M. Kasiri-Asgarani, M. Medraj, *Ceramics International* 40(9) (2014) 14009-14018.
- [12] Y. Chen, S. Zhao, M. Chen, W. Zhang, J. Mao, Y. Zhao, M.F. Maitz, N. Huang, G. Wan, *Corrosion Science* 96 (2015) 67-73.
- [13] L. Cordero-Arias, A.R. Boccaccini, S. Virtanen, *Surface and Coatings Technology* 265 (2015) 212-217.
- [14] L.C. Córdoba, M.F. Montemor, T. Coradin, *Corrosion Science* 104 (2016) 152-161.
- [15] X. Zhu, Z. Zhu, C. Chen, N. Bing, Z. Xu, Y. Li, Q. Chen, *Materials Research* 20(2) (2017) 481-486.
- [16] Z. Lin, S. Wu, X. Liu, S. Qian, P.K. Chu, Y. Zheng, K.M.C. Cheung, Y. Zhao, K.W.K. Yeung, *Acta Biomater* 99 (2019) 495-513.
- [17] R. Hou, J. Victoria-Hernandez, P. Jiang, R. Willumeit-Romer, B. Luthringer-Feyerabend, S. Yi, D. Letzig, F. Feyerabend, *Acta Biomater* 97 (2019) 608-622.
- [18] J. Fischer, D. Pröfrock, N. Hort, R. Willumeit, F. Feyerabend, *Materials Science and Engineering: B* 176(20) (2011) 1773-1777.
- [19] K. Taweessup, P. Visuttipitukul, N. Yongvanich, G. Lothongkum, *Surface and Coatings Technology* 358 (2019) 732-740.
- [20] J. Xu, Z. Tian, G. Yin, T. Lin, F. Huang, *Dalton Trans* 46(4) (2017) 1047-1051.
- [21] D. Zu, Z. Xu, A. Zhang, H. Wang, H. Wei, G. Ou, K. Huang, R. Zhang, L. Li, S. Hu, S. Sun, H. Wu, *Chemical Communications* 55(53) (2019) 7675-7678.
- [22] Q. Li, X. Zhu, Y. Zhang, Z.Z. Fang, S. Zheng, P. Sun, Y. Xia, P. Li, Y. Zhang, X. Zou, *Chemical Engineering Science* 195 (2019) 484-493.
- [23] T.H. Okabe, Y. Hamanaka, Y.K. Taninouchi, *Faraday Discuss* 190 (2016) 109-26.
- [24] Y. Zhang, Z.Z. Fang, P. Sun, S. Zheng, Y. Xia, M. Free, *JOM* 69(10) (2017) 1861-1868.