

Original article

Magnesium alloy composite coatings: In vitro study of cytotoxicity, adhesion, cellular structure and function

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Received 14 June 2024, Revised 12 September 2024, Accepted 17 October 2024

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Abstract: *Background* — Synthesis of novel grafts and biomaterial substitutes for bone reconstruction and study of their properties confirmed that the ideal material for this purpose has not yet been developed. Further efforts are needed to create contemporary biocompatible implants. It was shown that degradable biomaterials such as magnesium (Mg) alloys have potential clinical applications for bone reconstruction. However, poor resistance of Mg alloys to corrosion is a serious obstacle to their clinical use. This study aimed at investigating the biocompatibility and potential application of novel multifunctional self-healing coatings on Mg alloys to ensure stable performance of Mg implants in clinical settings.

Methods — We studied a magnesium-based Mg MA8 alloy samples both with and without coatings containing hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. The latter were obtained by plasma electrolytic oxidation (PEO) and included cerium (Ce) and polycaprolactone (PCL). Toxicokinetic properties, cell adhesion, cytocompatibility and cellular enzyme activity were assessed after contact with the composite coating covering Mg.

Results — The highest numbers of viable fibroblasts and tumor cells from the HeLa cell culture were observed after contact with Mg+PEO. Moderate cytotoxicity was found for the hydroxyapatite hybrid coating including cerium oxide (CeO_2) and PCL. The variability of the parameters for these samples was similar to the variability of the parameters for intact cells and cells in contact with titanium, which showed minimal cytotoxic effects. Compared with copper, which had high cytotoxic activity, the values for the studied samples were significantly lower. After 30 minutes of exposure to Mg, a large number of agglomerates of rounded cells with numerous folded and filiform pseudopodia were observed. Cells on Mg+PEO surfaces were less aggregated and predominantly had fibrillar pseudopodia, also known as filopodia. Numerous rounded folds were observed on the surface of the Mg+PEO+ CeO_2 +PCL hybrid coating, and cell groups were found in close contact resembling a syncytium. The number of stimulated cells on Mg+PEO decreased over time compared to the PEO-based hybrid coating on Mg with CeO_2 +PCL.

Conclusion — The results of this study imply that the bioactive PEO composite coating with PCL and CeO_2 modifies the Mg alloy surface by providing porosity that promotes strong cell attachment. These findings provide an alternative approach to develop multifunctional Mg-based implants and expand the biomedical applications of these materials. The data support the need for further investigation of the osteoinductive properties of coatings on biodegradable Mg implants.

Keywords: biodegradable implants, magnesium alloys, plasma electrolytic oxidation, hydroxyapatite, cytotoxicity, proliferation, inflammation.

Cite as Plekhova NG, Novikova PA, Chernenko IN, Stepanyugina AK, Shevchenko OV, Marchenko VS, Gnedenkov AS, Sinebryukhov SL, Gnedenkov SV, Shumatov VB. Magnesium alloy composite coatings: In vitro study of cytotoxicity, adhesion, cellular structure and function. *Russian Open Medical Journal* 2025; 14: e0203.

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Introduction

Bone defects in affected individuals represent a major health problem that can lead to diminished productivity, increased financial costs and limited mobility. Road traffic accidents and skeletal disorders are the leading causes of orthopedic fractures. It is estimated that by 2040, approximately 316 million people worldwide will suffer from osteoporotic fractures, and currently approximately 3 million people suffer from hip injuries annually

[1, 2]. The mechanisms of injury, including oblique, transverse and comminuted fractures, complex locations in the spine, head and limbs, and injury types are important factors in determining the appropriate treatment. Regeneration is a complex physiological process that involves continuous remodeling of bone tissue throughout life. Bone defects can be divided into two types: cavitary defects, in which bone loss does not significantly affect the biomechanics of the limbs, and segmental defects, which have a significant impact on bone stability and motion [3]. Metallic

implants are commonly used for stable and functional osteosynthesis in orthopedics and traumatology to treat bone injuries.

Depending on the expected duration of use, implants can be divided into two categories: permanent and temporary. Permanent implants are made of materials such as titanium or stainless steel. These materials are known for their strength, durability, and biocompatibility. Permanent implants do not react with surrounding tissues, cannot be absorbed by the body, and are often used for long-term stability and durability. Despite the mechanical strength of permanent implant structures, the chemical composition of these metals can change under the influence of environmental factors and the length of time they spend in the body [4-6]. Repeated surgical interventions to remove permanent metal structures after fracture consolidation can have a negative impact on the body. Temporary implants are made of various materials, including polymers, ceramics, and metals. Bioresorbable materials such as magnesium (Mg) and its alloys received considerable attention [7]. The latter are used as fixing devices for orthopedic and craniofacial surgeries [8].

Mg, a lightweight metal, has a density similar to that of human bone. Approximately 50% of it is found in bone tissue, and its corrosion products, Mg^{2+} and OH^- , are nontoxic. The latter are efficiently excreted via the kidneys [9]. This metal is biocompatible and does not cause immune reactions or other adverse reactions, making it safe for medical use [10]. Mg has unique mechanical properties similar to those of natural bone, including a low elastic modulus, which reduces the risk of implant rejection. When properly designed, Mg implants can conform to the shape and structure of bone [11-13]. This material also has osteoconductive properties, promoting osseointegration, a key factor in successful implantation [14, 15]. The tendency of Mg ions to actively promote the proliferation and differentiation of bone-forming cells, thereby stimulating the formation of new bone tissue, was pointed out in previous studies [16]. However, there are concerns regarding the use of this material as an implant due to its potential for rapid corrosion in the aggressive physiological environment of the body, which may lead to premature failure of the metallic structure [17]. To address these issues, researchers are currently investigating new Mg-based alloys and surface treatment methods that may slow down the corrosion rate and better match the tissue regeneration process [16, 17].

A detailed analysis of the mechanical properties of Mg implants during degradation was described in some studies, including in vitro and in vivo models [14, 18]. A number of Mg-based implants have been designed, including plates, screws, intramedullary rods, Herbert screws, spinal cages, ring splints for joint treatment, and regenerative scaffolds. These implants vary in manufacturing technology, geometric dimensions, and shapes [13, 18-20]. Also, the surface of Mg-based implants requires appropriate treatment or alloying with other chemical elements to enhance their corrosion resistance in physiological environments. The main approaches to improving the properties of Mg alloys are surface modification using physical vapor deposition [21], electrochemical deposition [22], conversion coating formation [23], anodic oxidation [24], and plasma spraying [25]. These approaches are not environmentally friendly and are insufficient to improve corrosion resistance and adhesion strength [26, 27].

Plasma electrolytic oxidation (PEO), also known as microarc oxidation, is a process that involves treating a metal or alloy,

typically Mg, aluminum, or titanium, as an anode at a high voltage. This creates a ceramic-like surface on the material [28, 29]. The process uses a cathode, typically graphite or steel. The ideal biodegradable Mg coating should have good corrosion resistance and biocompatibility, as well as high adhesion to the Mg substrate. It should also have biological functions such as antibacterial properties and the ability to promote bone formation, while preventing implant degradation and hydrogen evolution [19, 27]. The porous oxide coating created by PEO exhibits high adhesion, thereby minimizing the risk of defects during implant placement. Its coarse porous structure promotes cell adhesion [29]. However, when using the conventional PEO process, due to the occurrence of plasma microdischarges and gas evolution, many pores and microcracks appear on the coating surface, which may affect the corrosion resistance of Mg. Through these formed pores, the aggressive environment can penetrate into the alloy substrate, reducing the corrosion resistance of the material [29-31]. Therefore, it is important to change the pretreatment and modification conditions for the PEO coating on biodegradable Mg alloys. Several technologies were proposed to resolve this issue, including the formation of additional protective layers such as calcium phosphate (Ca-P-PEO) [30-32], layered double hydroxide (LDH-PEO) coatings [33, 34], ZrO_2 -containing coatings [35], and the incorporation of antibacterial ingredients or drugs into the coatings. In addition, composite coatings based on some polymer (Pol) and self-healing coatings were also investigated as potential solutions [41-44].

In summary, Mg-based orthopedic implants serve multiple biological purposes, including bactericidal function, osteogenesis, and angiogenesis. These functions make them useful for the treatment of large bone defects [45]. One way to create self-healing coatings is to incorporate bifunctional agents such as drugs, metal ions, or peptides [46]. However, the formation of such coatings is complicated by the difficult-to-control drug release kinetics [47]. Therefore, it is imperative to develop novel multifunctional coatings for Mg implants that meet clinical requirements. In our previous study, we prepared a self-healing coating on Mg alloy (MA8) using PEO in a silica-fluoride electrolyte [48, 49]. We showed that the protective properties of the PEO coating can be improved by impregnating it with a corrosion inhibitor, 8-hydroxyquinoline. We also proposed a self-healing mechanism for this coating. This study proposes a method for creating a self-healing coating and provides biocompatibility data for a multifunctional protective layer on Mg-based alloy. The objective of this study was to investigate the biocompatibility and potential application of this novel multifunctional autonomous self-healing coating on biomedical Mg alloys for long-term use of Mg implants in clinical practice.

Material and methods

Formation of magnesium-based coatings. Plates of MA8 Mg alloy (15×20×1.5 mm) were obtained and investigated. Alloy composition in wt.% was as follows: 1.3-2.2% of Mn, 0.15-0.35% of Ce, and the rest (up to 100%) was Mg. The initial surface of the samples was homogenized by mechanical grinding on fine-grained silicon carbide (SiC) grinding paper ([Figure 1](#)). The grain size was gradually reduced to P1000, with an abrasive grain size of 14-20 μm . After grinding, the samples were washed in isopropyl alcohol and dried in an oven at a temperature of 40 °C. The formation of protective PEO layers was carried out using a special installation

(Figure 1A). An aqueous solution of calcium glycerophosphate, sodium fluoride and sodium orthosilicate was used as an electrolyte to form the coating.

The bipolar oxidation mode was employed. The anodic component was maintained at 400 V in a potentiostatic mode, while the cathodic component ranges from 1.3 to 0.85 A/cm² in a galvanodynamic mode at a rate of 4 mA/cm². The total oxidation time was 110 seconds, and the duty cycle was 1 [50]

The PEO-coated samples were rinsed with deionized water and then placed in an oven at 40 °C. The formation of inhibitor-containing layers was achieved by immersion in a medium containing Ce³⁺ ions and polycaprolactone (PCL) [-(CH₂)₅-CO-O-]_n for 60 minutes (Figure 1B). The samples were then removed and placed in a drying oven at 50 °C for 42 hours. The experiment was repeated twice using the coating types listed in Table 1.

Cell cultures

To investigate the properties of the coatings, we used a monolayer cell culture of FetMSC cells (Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia). These are mesenchymal stem cells obtained from the bone marrow of a 5-6-week-old human embryo, with fibroblast-like morphology. The cells were cultured in αMEM medium (BioloT LLC, Russia)

containing fetal calf serum (Biowest, France) and gentamicin K (Merck, USA) at a concentration of 0.004%. The cells had a diploid chromosome number of 46, with a modal number of 97.0% (±1.7%). The karyotype was normal, with 46 chromosomes (XY), and there were 3% polyploid cells. DNA profiling (STR) revealed the following results: amelogenin (X, Y), CSF1PO (9, 12), D13S317 (11, 12), D16S539 (11, 11), D5S818 (12, 13), D7S820 (10, 12), THO1 (7, 8), TPOX (8, 11), and vWA (14, 15). On average, 33.5 hours were required for doubling of the cell population, and the active (logarithmic) growth phase lasted 48 hours at the sixth passage. Expression of surface antigens characteristic of mesenchymal stem cells on the cells: CD44 (95%), CD73 (99.6%), CD90 (87.4%), CD105 (95.4%). No expression of CD34 and HLA-DR antigens was observed. A monolayer tumor cell line Hela-v (Research Institute of Virology, Russian Academy of Medical Sciences, BioloT LLC, Russia) obtained from human cervical carcinoma was also used. It had an epithelial-like morphology and was cultured in medium 199 containing 10% fetal calf serum and 0.004% gentamicin K. Karyological species identity and lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase isoenzymes were monitored. Test samples were distributed into wells of a culture plate after sterilization with ultraviolet rays. Then 5 mL of the medium with cells at a concentration of 106 cells/mL were added and incubated at 37 °C and 4.2% CO₂ for 3.7 days and 14 days.

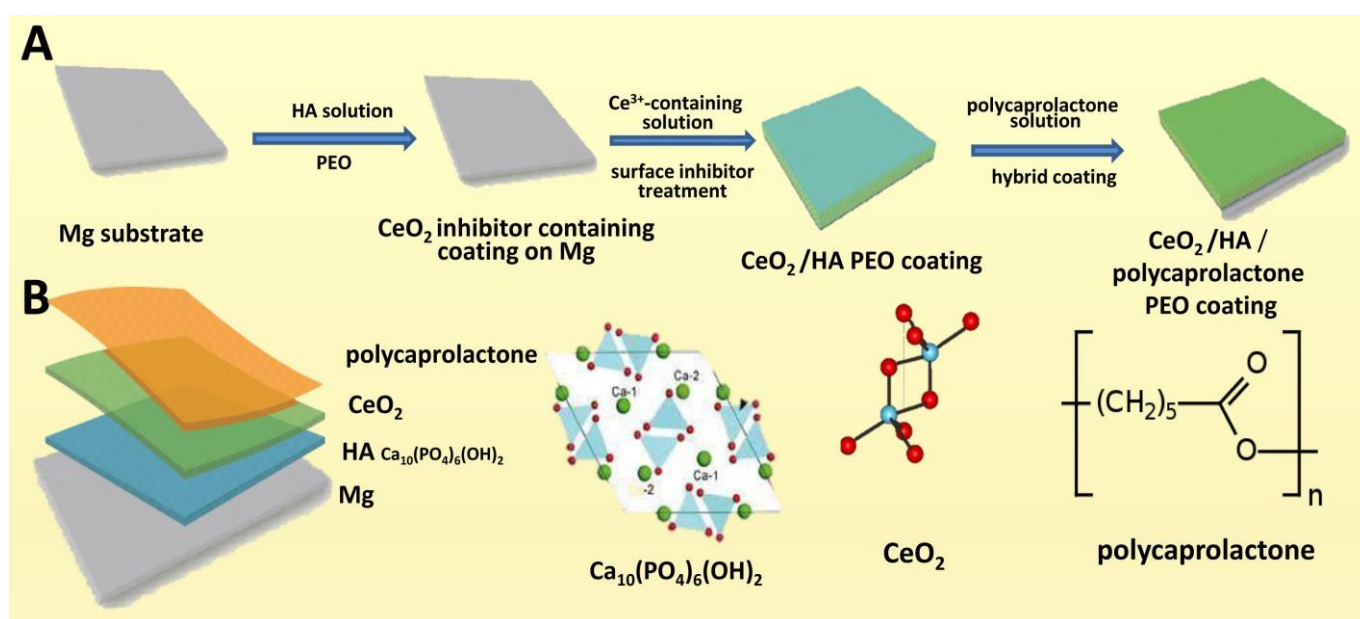


Figure 1. Overview of experimental process and coating performances. (A) Schematic representation of the fabrication procedure of hybrid PEO coating: hydroxyapatite (HA), CeO₂, polycaprolactone (PCL), HA/CeO₂/Pol on Mg alloy, which is constructed by combining PEO and layer-by-layer assembly techniques. (B) Architecture of multilayered coating (HA/CeO₂/Pol) on Mg substrate and molecular structures of HA, CeO₂, and Pol.

Table 1. Characteristics of samples

N°	Abbreviation	Compound
1	MA08	uncoated magnesium alloy: Mg, MgO
2	MA08 PEO	magnesium coated by plasma electrolytic oxidation Coating composition: MgO, Mg ₂ SiO ₄ , Ca ₁₀ (PO ₄) ₆ (OH) ₂ (hydroxyapatite)
3	Ti	titanium
4	Cu	copper
5	CompCe	magnesium with a composite coating, after treating the PEO layer with a solution of a corrosion inhibitor (based on a cerium-containing compound): MgO, Mg ₂ SiO ₄ , Ca ₁₀ (PO ₄) ₆ (OH) ₂ (hydroxyapatite), CeO ₂
6	HybCePol	magnesium with the hybrid composite coating, after treating the PEO layer with a solution of a corrosion inhibitor (based on a cerium-containing compound) and polyester solution MgO, Mg ₂ SiO ₄ , Ca ₁₀ (PO ₄) ₆ (OH) ₂ (hydroxyapatite), CeO ₂ , [-(CH ₂) ₅ -CO-O-] _n (polycaprolactone)

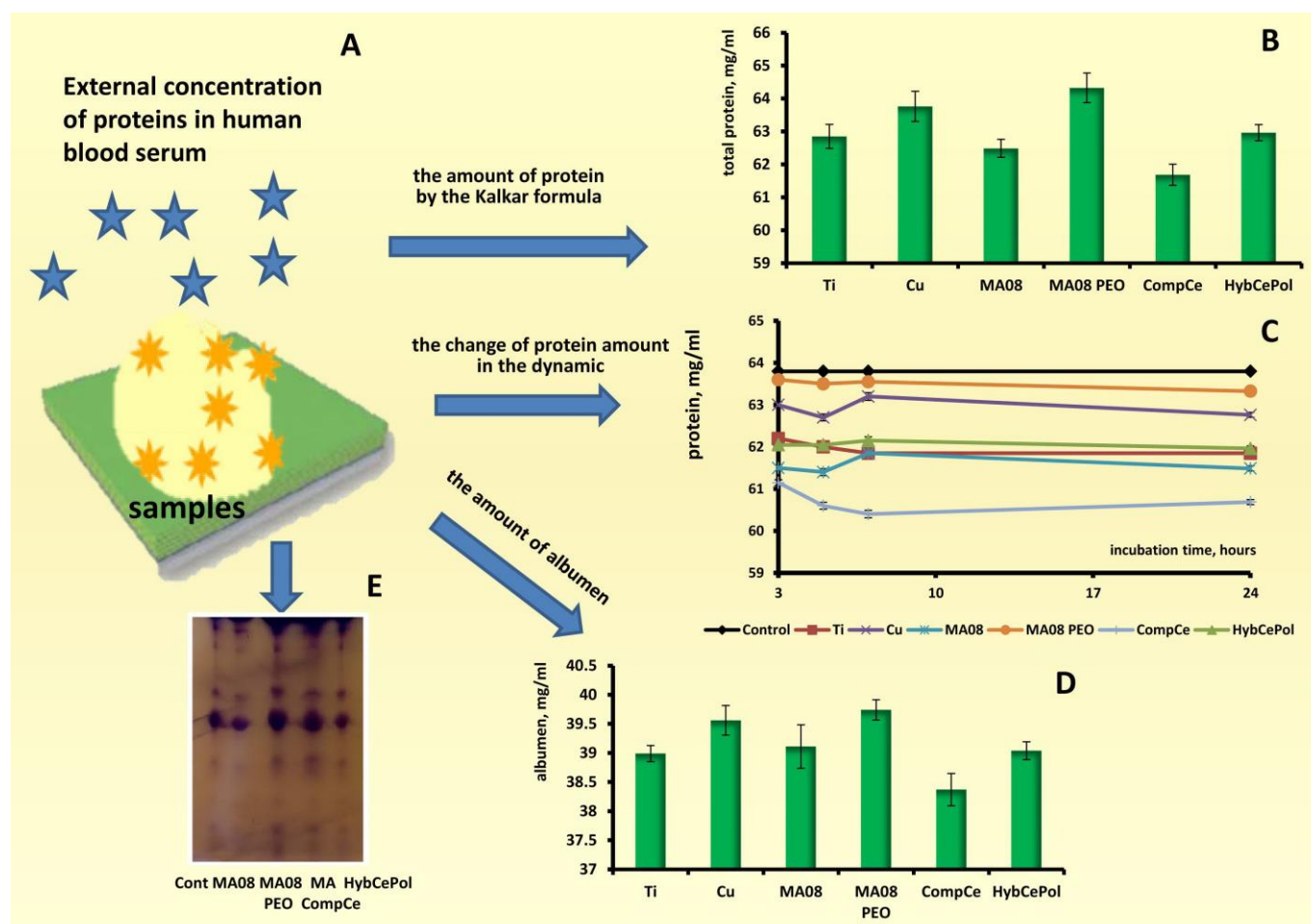


Figure 2. Studying toxicokinetic properties of coatings on magnesium. (A) Schematic representation of the experimental setup. (B) Measuring the concentration of total protein in the supernatant by spectrophotometry after 24-hour contact. (C) Measuring the concentration of total protein over time by a biochemical method. (D) Measuring the concentration of albumin in the supernatant by a biochemical method after 24-hour contact. (E) Electrophoresis-based analysis of protein fractions in the supernatant obtained after 24-hour incubation of serum with samples.

Toxicokinetic properties of magnesium-based coatings

We examined protein adsorption by incubating samples with defibrinated donor plasma (blood type B-) for 1, 3, 5, 7, and 24 hours at 37 °C in a thermostat (Figure 2A). Protein content in plasma before and after sample incubation was determined using the formula based on optical density measurements of the solution (protein content = $1.45 \times \lambda_{280} - 0.74 \times \lambda_{260}$ mg/mL) using a FlexA-200 NT spectrophotometer (Alsheng, China) at $\lambda=280$ and $\lambda=260$ nm wavelengths. Additionally, the total protein (biuret assay) and albumin concentrations (bromocresol green assay) were determined using a Mindray BS-120 biochemistry analyzer (Mindray, China). Protein fractions of plasma (α -1, α -2, β -1, β -2, and γ -globulin) and albumin were identified by two-dimensional polyacrylamide gel electrophoresis on a Compact S/XS system (Biotetra, Germany), followed by Coomassie Brilliant staining R250 (ApplChem, Germany).

Testing cell functions

After contact with the samples, the concentration of viable cells was determined by staining with a 0.4% trypan blue solution using a TC20 counter (Bio-Rad, USA). The cytotoxicity of the samples was detected using the MTS test. A phosphate buffer (pH 7.2) containing 0.4% MnCl₂ and 2 mg/mL of 4,5-dimethyl-2-

thiazolyl)2,5-diphenyl-2H-tetrazolium bromide (MTS) was added to a cell monolayer at an initial concentration of 600 cells per 200 μ L. The cells were then destroyed by adding isopropyl alcohol acidified with 0.04 M HCl to the substrate. The optical density of the substrate was measured on a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, USA) at $\lambda=540$ nm. The results were expressed as a stimulation index (T, %) calculated as the ratio of the difference in the mean optical density between solutions containing the reaction products from cells in contact with samples and a plastic (control) and the mean optical density of a control solution.

Determination of LDH and creatine kinase (CK) in the supernatant was performed on a Mindray BS120 analyzer manufactured by Mindray (China) using Mindray enzyme activity determination reagents from the same manufacturer. The kits provided a linear range of enzyme activity from 20 to 1000 units U/L with a deviation from linearity of no more than 5%, a sensitivity of no more than 15 U/L, and a coefficient of variation of the determination results of no more than 5%.

Scanning electron microscopy was used to study the surface architecture of cells adhered to the samples. For this purpose, the cells were fixed in a complex fixative consisting of 0.2 molar cacodylate buffer with pH 7.4 and containing 3% formaldehyde

and 0.02% picric acid. They were then postfixed in 1% osmium tetroxide and dehydrated in acetone, after which they were coated with carbon by thermal evaporation in a vacuum. Images were obtained using an Ultra 55 electron microscope by Carl Zeiss (Germany) operating at an accelerating voltage of 0.8 to 1 kV.

Statistical analyses

The compliance of collected data with the normal distribution was verified using the χ^2 method, and the significance of differences between the experimental and control groups was evaluated using the Student's t-test and the nonparametric Mann-Whitney U test at a confidence level of $\geq 95\%$ ($p < 0.05$), using Statistica 8.0 software (StatSoft, Inc., USA).

Results

Toxicokinetic properties of magnesium-based coatings

In plasma, three main proteins are responsible for binding to biologically active components such as albumin, α -1 acid glycoprotein, and lipoproteins. Not only individual proteins but also their fractions can bind to the implant surface. When studying

the total protein content in blood plasma using the biuret method, it was established that the lowest amount of protein was absorbed by copper and Mg alloy samples with a PEO layer, and the highest amount of protein was absorbed by the PEO coating containing cerium oxide (CeO_2). [Figure 2B](#) shows this information. Albumin accounts for approximately 60% of the total amount of proteins in blood plasma. It is a biologically active component that also binds to endogenous substances such as fatty acids and bilirubin. It was shown that the main binding protein for basic or cationic molecules is α -1 glycoprotein [51]. It was discovered that albumin is adsorbed on the coatings in minimal quantities, indicating the absence of specific interaction of this protein with the surface of the samples. The total amount of blood plasma protein in our study was 64.8 g/L, and after contact with plastic for 1, 3, 5, 7 and 24 hours, its concentration in the supernatant decreased by 1.25 ± 0.1 g/L. When studying the amount of bound protein in blood plasma using the biuret method, we confirmed that titanium and Mg samples without a protective coating and with a PEO coating containing PCL and cerium adsorbed more than 1 g/L, with the latter adsorbing up to 2.35 ± 0.2 g/L in the largest amount ([Figure 2C](#)).

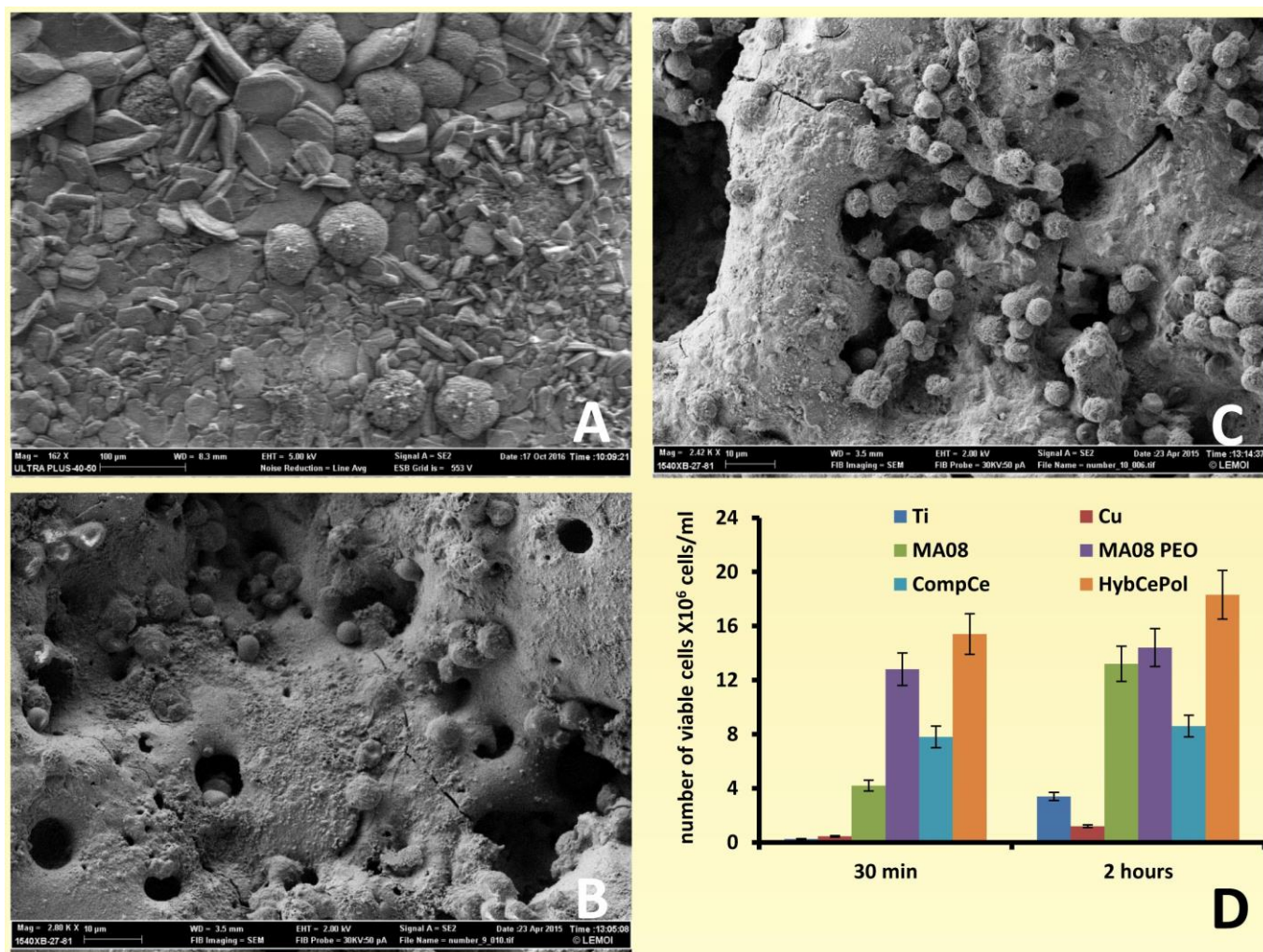


Figure 3. Fibroblasts on the surface of magnesium (A), PEO coating with hydroxyapatite (B), and a hybrid coating with cerium oxide and polycaprolactone (C), as seen in scanning electron microscopy after 30 minutes of incubation. (D) Number of cells adhered to the surface per 1 mm².

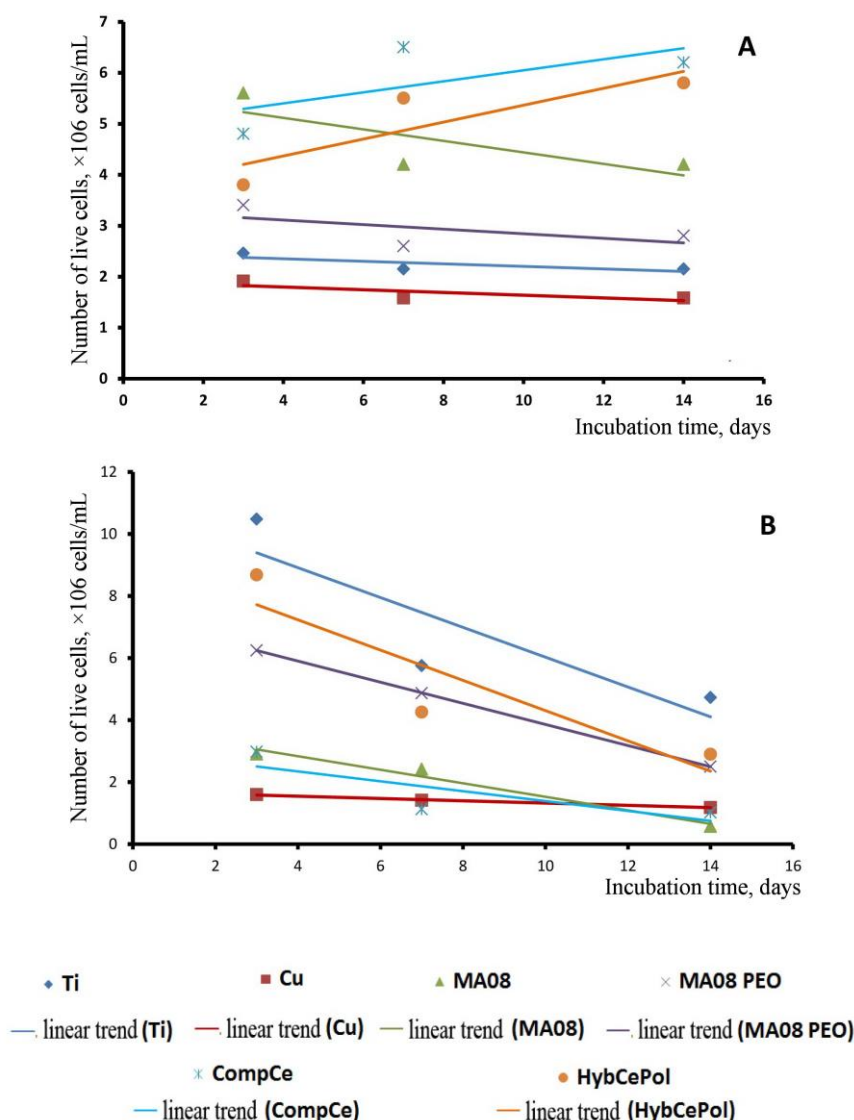


Figure 4. Number of viable fibroblasts and tumorigenic HeLa cells after contact with titanium, copper and magnesium both with and without various protective coatings.

Table 2. Indicators of the model for predicting the viability of fibroblasts after incubation with samples

Samples	Trend equation	Approximation reliability coefficient, R^2
Ti	$y = -0.4366x + 6.81$	0.4653
Cu	$y = -0.3955x + 6.392$	0.2568
MA08	$y = -0.3545x + 8.13$	0.6107
MA08 PEO	$y = -2.24x + 10.3$	0.6637
CompCe	$y = -1.1664x + 7.87$	0.2076
HybCePol	$y = -0.11773x + 7.34$	0.3166

After the subjects' exposure to blood plasma for 7 hours, electrophoresis did not reveal any protein fractions with molecular weights different from those of the plasma protein reference ranges (Figure 2E). Based on the quantitative data, the mass content of protein fractions did not differ from physiological norms. Overall, these results indicate that the samples did not affect the structure of plasma proteins.

Cell adhesion to magnesium coatings

Cell morphology analysis using scanning electron microscopy confirmed that fibroblasts adhered to the Mg coatings. After 30 minutes of contact, the highest cell adhesion was found for the hybrid PEO coating containing CeO₂ and PCL ($22.1 \pm 1.5 \times 10^3$ cells/mm², Figure 3). The minimum number of cells was observed on the surface of the magnesium alloy ($4.2 \pm 0.4 \times 10^3$ cells/mm²) and the PEO coating ($12.8 \pm 1.3 \times 10^3$ cells/mm²). The difference between the cell adhesion values after contact with different samples was statistically significant ($p < 0.05$), with the highest number on the surface observed after 2 hours (Figure 3D). The cells attached to the Mg alloy had a typical rounded shape, without close contact with the surface (Figure 3A). On the PEO coating with hydroxyapatite (HA), a flattened shape with a small number of pseudopodia along the perimeter was observed (Figure 3B). After contact with the hybrid PEO coating, the cells had a different morphology (Figure 4). For instance, numerous folds and filiform structures were observed on the surface of the flattened

cells. Fibroblasts were mostly in close contact with each other (Figure 3G).

Cytocompatibility of magnesium coatings

As shown in Figure 4, the number of viable fibroblasts decreased until the end of the observation period (14 days). Copper had a significant inhibitory effect, while Mg had a stimulatory effect, as indicated by the approximation coefficient values. All tested samples had a supporting effect on the cells, but at a level slightly lower than that of the Mg alloy without a protective layer (Figure 4A), with the exception of the PEO-coated sample. Mg alloy samples with a composite coating containing CeO₂ and a hybrid coating (including an inhibitor and a Pol) had a stimulatory effect, maintaining cell viability (Figure 4A). A trypan blue assay to assess the state of proliferating HeLa cells revealed no cytotoxic effect.

To predict the trends and long-term consequences of the studied samples, we applied a linear trend model to the time series of viable cell counts. Based on the trend equations, we analyzed the sequence of values to determine the x-intercept (b) and the value by which the next time series value increases or decreases. This analysis led to the conclusion that there is a

general trend towards a decrease in the viable cell count (Table 2). A significant slope coefficient above 0.1 for the trend was noted for the Mg alloy samples with a composite PEO coating containing PCL and a cerium-containing compound. This coefficient indicated that the straight line matched the observed data on similar dynamics (Table 2). The slope coefficient values allowed us identifying to what extent the incubation time affects the viable cell count. The intercept (y at $x=0$) provided information on the initial value of the dependent variable.

The approximation reliability coefficient (R^2) shows the degree of fit of the trend model to the initial data. It ranges from 0 to 1, with higher values indicating a better fit. The R^2 values for each sample indicate the level of reliability of the linear trend model for the values of the viable cell count.

As shown in Table 2, the R^2 values were close to 1 for the Mg alloy samples without a protective layer, with a PEO coating, a composite coating containing PCL, and a hybrid coating containing a corrosion inhibitor. This implies that the model accurately describes the data for these samples, suggesting that there are no cytotoxic effects from the MA8 Mg alloy samples with composite PEO coatings containing PCL and CeO₂ over time.

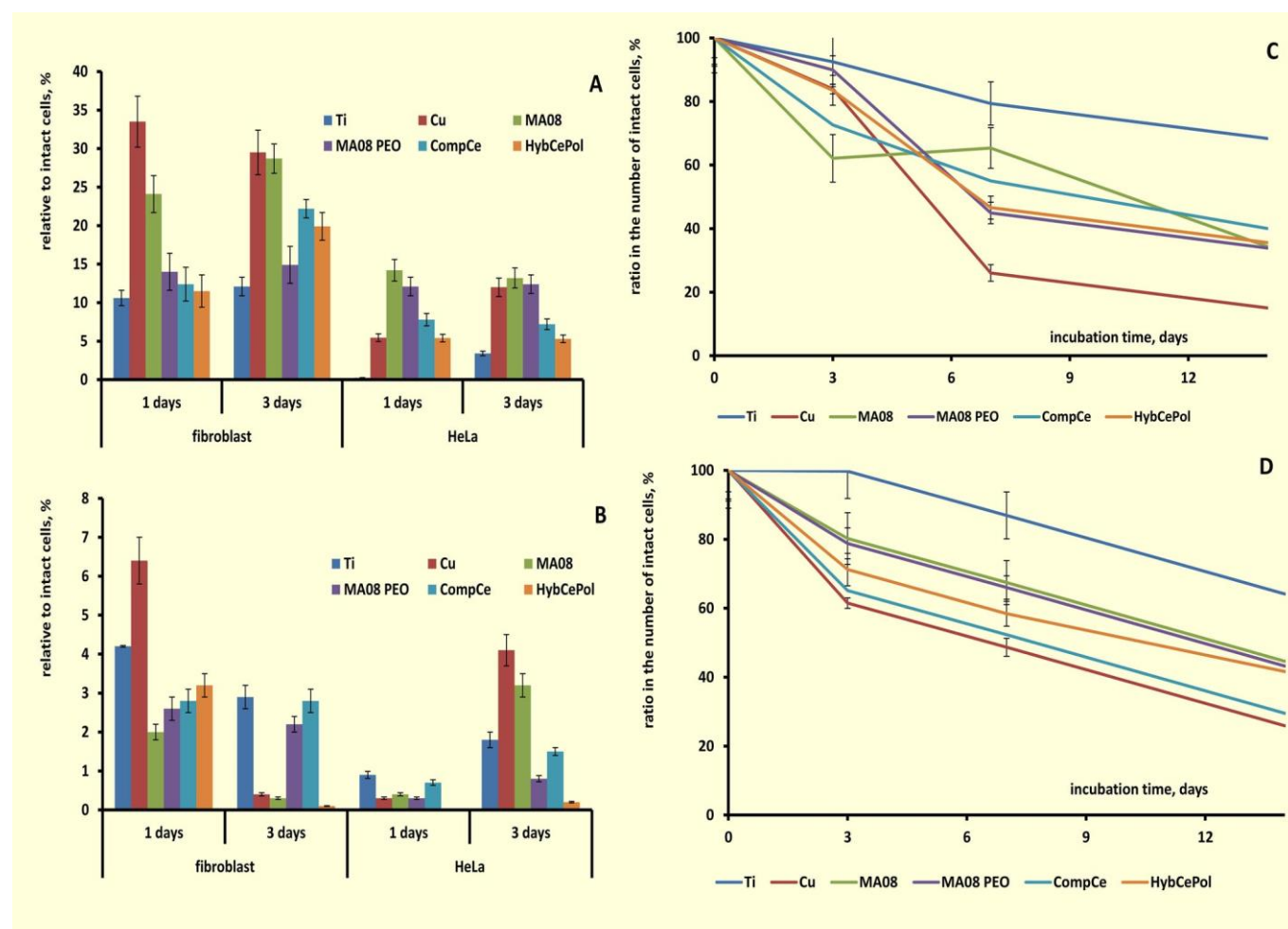


Figure 5. Intracellular content of lactate dehydrogenase (A), creatine kinase (B) and succinate dehydrogenase (C) after co-incubation of fibroblasts and tumor-forming HeLa cells (D) with titanium, copper and magnesium alloy both with and without various protective coatings (after 14 days).

Activity of cellular enzymes after contact with magnesium coatings

LDH is an intracellular enzyme that plays a role in the oxidation of glucose and the conversion of lactate (lactic acid) to pyruvate (pyruvic acid). It belongs to the class of oxidoreductases and catalyzes the removal of hydrogen atoms from lactate, resulting in the formation of pyruvate. LDH is ubiquitously expressed in human cells, meaning that it can be found in virtually all tissues. It is normally present in small amounts in the blood. The highest LDH concentration was observed in the supernatant after fibroblasts were exposed to copper or Mg without any protective coating, which suggested a cytotoxic effect. However, no significant differences in enzyme levels were found after cells were exposed to protective coatings on Mg alloy, thereby indicating a minimal effect on cell damage. Increased LDH levels in the supernatant were observed after HeLa tumor cells were exposed to copper, Mg alloy without a protective coating, and with PEO coating ([Figure 5A](#)). It is worth noting that overall LDH levels for these cells were significantly lower than for fibroblasts, which suggested their lower cytotoxicity towards tumor cells. No significant differences in values were detected depending on the coating composition.

CK catalyzes the reaction that transfers a phosphoryl group from adenosine triphosphate (ATP) to creatine, forming creatine phosphate and ADP. ATP is the molecule that provides energy for biochemical reactions in the human body. CK can be found in the mitochondria and/or cytoplasm of cells. Its increased activity indicates damage or destruction of cells containing the enzyme. It is not specific to any particular type of cell damage, as the enzyme is released when any cell is damaged. When CK levels were studied in the supernatant after exposure of fibroblast cells to various substances, its reduced levels were found in case of samples exposed to titanium, indicating a lack of stimulation ([Figure 5B](#)). Samples exposed to copper had the highest content of this enzyme, suggesting the strongest cytotoxic effect. The lowest cytotoxicity was demonstrated by the samples with MG alloy and hybrid PEO coating with corrosion inhibitor and Pol. No significant coating composition-based differences between the levels after contact with other samples were observed. CK was detected in small amounts in the tumor cell supernatant after contact with Mg coatings. The highest cytolysis rates were observed after co-incubation of cells with copper-Mg alloy ([Figure 5B](#)).

Succinate dehydrogenase (SDH) or succinate-ubiquinone oxidoreductase is an enzyme of the oxidoreductase class that plays an active role in the mitochondrial respiratory chain and is involved in oxidation reactions. Our results show that titanium has a stimulating effect on fibroblast activity, while copper inhibits its activity ([Figure 5C](#)). Regarding the effect of Mg alloys with and without protective coatings, we observed that Mg alloys without protective coating have a moderate inhibitory effect on SDH activity, which decreased with increasing incubation time. After three days of incubation, the number of active cells based on SDH content ranged from 62.2% for Mg alloy-exposed cells to 92.5% for titanium-exposed cells ([Figure 5C](#)). The number of formazan-containing cells decreased over time, and after 14 days it was 15.1% for Mg-exposed cells and 68% for titanium-exposed cells. The levels of high-SDH fibroblasts co-cultured with different coatings differed significantly after three days. The greatest effect was observed with PEO and hybrid MG coating, as shown in [Figure 5C](#). The numbers of these cells decreased over time, and the values were independent of the coating type. When exposed

to HeLa tumor cell samples, titanium, PEO-coated Mg alloy, and a hybrid coating containing CeO₂ and PCL demonstrated the lowest cytotoxicity, as seen in [Figure 5D](#). No significant differences in the number of cells with active SDH were observed depending on the coating type.

Discussion

Various types of modern materials are used in implant surgery. Modification of the surface properties in these materials affects the bone tissue regeneration, including protein adsorption and cell proliferation. Among these materials, the most promising are metals and alloys with calcium phosphate coatings, since they have chemical and phase compositions close to the mineral components of bone tissue [52, 53]. The PEO method is widely used to treat the surface of Mg alloys. This method creates anticorrosive coatings that provide biocompatibility and allow the implant to integrate with bone tissue. The purpose of protecting resorbable low-alloy Mg alloys is to create a protective layer with anticorrosive properties and biological activity. In this study, protective coatings were obtained on Mg alloy (MA8) using the PEO method. The composition of the formed anticorrosive layers includes calcium phosphate compounds, which are close to HA in stoichiometric composition. Intensive electrolyte thermolysis carried out during the PEO process allows the synthesis of a coating containing HA with a developed porous surface, which significantly reduces the corrosion rate of Mg alloys [54]. However, to further improve the corrosion resistance, it is necessary to add other components to the coating. We included CeO₂ in the coating composition using a modifier. This compound attracts considerable interest on the part of researchers due to its ability to mediate antibacterial activity through oxidative stress [55]. The known ability of cerium to replace calcium in the body is considered the main mechanism of its beneficial effects. As another component, we used biocompatible Pol based on ϵ -caprolactone. This Pol has the ability to stimulate the growth of fibrous tissue and promote the replenishment of its volume due to its unique properties [56].

Biodegradable Mg and its alloys attract much attention due to their ability to decompose in the body. This material is used in implants for the reconstruction of large bone defects and in cardiovascular stents [57]. For practical applications, it is important to improve the corrosion resistance of Mg alloys. For this purpose, Mg alloys are enriched with various elements such as Ca and Zn. Some studies showed that in the biological environment of the body, these alloys effectively promote bone healing and regeneration. Their biodegradability means that they do not require re-operation, minimizing trauma for patients [58]. To slow down corrosion and coordinate bone regeneration in fractures, Mg alloys are coated. High heparinization levels in a poly(L-lactic acid) (PLLA) polyethylene oxide (PEO) composite coating (PEO/PLLA) on AZ31 biodegradable Mg alloy were achieved by incorporating polydopamine from mussels. This composite coating showed no obvious cytotoxicity against human umbilical vein endothelial cells (HUVECs) and arterial smooth muscle cells, and after surface heparinization, it became more suitable for HUVEC growth while inhibiting the growth of a second culture [59]. Cytotoxicity, cell adhesion, live/dead staining, and proliferation data of rat bone marrow stem cells demonstrated that the bilayer PEO coating with Mg and aluminum hydroxide significantly improved the cytocompatibility of the substrate,

suggesting potential use in orthopedic surgery [37]. PEO coating with HA is better for osteoblast adhesion and proliferation and has higher cytocompatibility than uncoated Mg alloy. Our data showed that sealing the pores of the PEO coating with Pol material significantly reduced the amount of inhibitor that diffused into the aggressive environment and slowed down the degradation of the Mg alloy [46-48]. High protein adsorption, especially serum albumin, was observed on the PEO-coated Mg alloy, while it was significantly lower for the CeO₂-containing coating ($p < 0.05$). When the coating was supplemented with PCL, protein adsorption increased. These results indicate that the material has high adsorption properties due to the decrease in the coating porosity. Determination of the adsorption rate of blood plasma proteins showed that at an albumin content of 2.35 ± 0.2 g/L in the supernatant, no significant differences in the content of the protein fraction were found for the coating containing PCL and CeO₂ (PEO layer) vs. the intact samples. This implies that this coating meets the requirements for medical devices used in clinical practice.

Self-healing coatings, due to their unique properties, have a wide range of applications in protecting Mg alloys from corrosion [60]. However, there remains the problem of creating an internal self-healing structure capable of simultaneously restoring the damaged surface of the coating and preserving its protective properties, while remaining biocompatible [61]. There are two main mechanisms of self-healing in protective layers. One of them is due to the formation of corrosion products that block the access of the aggressive environment to the active centers in the Mg alloy. The other is due to the activation of organic or inorganic corrosion inhibitors that are present in the original coating composition and react with environmental components [62]. Technology used for creating modified PEO coatings with improved morphology, corrosion resistance, wear resistance and biocompatibility, as well as the possibility of their impregnation with antibacterial and medical agents are considered in some studies [29, 63-65]. One of the versatile approaches is the bottom-up coating method that uses multilayer polyelectrolytes. These are weak polyelectrolyte polymers that can adjust the molecular weight and pH of the materials [66]. The self-healing ability of these coatings is due to the high mobility of Pol chains, which is initiated by an aqueous environment. There are two main methods for creating these multilayer coatings. The first method involves the formation of a layer including corrosion inhibitors. These inhibitors are released as a result of damage such as scratches or cracks, forming a barrier layer via physical adsorption and chemical complexation [67]. The scratch healing mechanism is explained by the formation of a complex between inhibitors and metals, but achieving cyclic healing is difficult due to irreversible reactions with the substrate material [68]. To create a multifunctional internal self-healing Pol coating, we used the second method, which provides healing through the rearrangement of molecular chains and dynamically reversible chemical bonds. These include disulfide bonds, hydrogen bonds, amide bonds, and electrostatic interactions. Such reversible reactions in scratched or damaged areas trigger a self-healing process in the protective layer, theoretically allowing it to repair damage multiple times [69].

The interaction between the implant surface and the surrounding tissue is critical in *in vivo* models. Mammalian cells typically undergo a process of cell adhesion that involves attachment to the substrate, spreading on it, cytoskeletal

development, survival, and subsequent proliferation. Cells on the PEO coating containing PCL and CeO₂ were found to have a high number of filopodia, which is in good agreement with the scanning electron microscopy results in [Figure 3](#). After 24 hours of culture, fibroblasts were better distributed on the PEO coatings than on the MA8 alloy. The use of PCL as an outer layer in the composite coating provides better affinity for the cells; their surface has a polygonal shape and a large number of filopodia and lamellipodia. Thus, the results demonstrate good cell adhesion, which is the first step in the integration of the implant with the surrounding tissue. Cytotoxicity studies of biomaterials use cell cultures to measure cell lysis, retardation of their growth and analyze other types of effects caused by contact with medical devices and materials [70, 71]. The composite coating with CeO₂ and Pol had a supporting effect without a cytotoxic effect on fibroblasts throughout the entire observation period (14 days). During cell lysis, enzymes are released into the intercellular space and their activity increases in the supernatant, which accompanies cell destruction. After contact with protective coatings on magnesium alloy, LDH and CK levels are lower than after contact with copper alloy, indicating a minimal effect on cell death. PEO and hybrid coatings had a significant effect on SDH activity, with the number of stimulated cells decreasing over time. SDH is a protein complex located in the inner mitochondrial membrane that plays an important role in cellular energy metabolism. It catalyzes the oxidation of succinate to fumarate, which produces ATP, a key molecule in energy production. Mammalian SDH also plays a role in cellular oxygen regulation and tumor suppression [53, 55]. Our data suggest that PEO coatings stimulate oxidative metabolism and structural changes in adherent cells, which may be associated with increased SDH activity.

Study limitations

There are certain limitations to our study regarding the biocompatibility of the coatings we synthesized for clinical use. From an empirical point of view, it would be necessary to investigate how the coating components stimulate the functional activity of mesenchymal stem cells to differentiate into osteocytes. Besides that, it is important to determine the degree of proinflammatory activity of these coatings, which requires further experiments both *in vitro* and *in vivo*. In addition, it is important to examine how biological fluids can affect the multifunctional coatings and the Mg base of the implants over time. Although encouraging preliminary results have been achieved in our study of coatings on biodegradable Mg, we plan to continue our research to ensure validity, representativeness and reliability of data.

Conclusion

The synthesis of novel grafts and biomaterial substitutes for bone reconstruction, as well as the study of their properties, demonstrated that the ideal material has not yet been developed, and further efforts are needed to create modern biocompatible implants. Under cell contact conditions, surface roughness and chemical composition are the most significant parameters of hybrid coatings, determining their effect on osteogenic differentiation at both cellular and tissue levels. The results of this study imply that a bioactive composite coating based on PEO with PCL and CeO₂ modifies the surface of Mg alloy, providing porosity that promotes strong cellular adhesion. Our results represent a

potential alternative for the development of multifunctional Mg-based implants and expanding the biomedical application of these materials.

Conflict of interest

No conflicts of interest were declared by the authors.

Funding

The study was supported by the Government Procurement from the Russian Federation Ministry of Healthcare (project no. 056-000-55-24-00). The protective coatings were financially supported the Government Procurement from the Ministry of Science and Higher Education of the Russian Federation (project no. FWFN-2024-0001).

References

- Oden A, McCloskey EV, Kanis JA, Harvey NC, Johansson H. Burden of high fracture probability worldwide: Secular increases 2010-2040. *Osteoporos Int* 2015; 26(9): 2243-2248. <https://doi.org/10.1007/s00198-015-3154-6>.
- Agarwal-Harding KJ, Meara JG, Greenberg SLM, Hagander LE, Zurakowski D, Dyer GSM. Estimating the global incidence of femoral fracture from road traffic collisions: A literature review. *J Bone Joint Surg Am* 2015; 97(6): e31. <https://doi.org/10.1007/s00198-015-3154-6>.
- Schierjott RA, Hettich G, Graichen H, Jansson V, Rudert M, Traina F, et al. Quantitative assessment of acetabular bone defects: A study of 50 computed tomography data sets. *PLoS One* 2019; 14(10): e0222511. <https://doi.org/10.1371/journal.pone.0222511>.
- Sheikh Z, Najeeb S, Khurshid Z, Verma V, Rashid H, Glogauer M. Biodegradable materials for bone repair and tissue engineering applications. *Materials (Basel)* 2015; 8(9): 5744-5794. <https://doi.org/10.3390/ma8095273>.
- Li F, Jiang X, Shao Z, Zhu D, Luo Z. Research progress regarding interfacial characteristics and the strengthening mechanisms of titanium alloy/hydroxyapatite composites. *Materials (Basel)* 2018; 11(8): 1391. <https://doi.org/10.3390/ma11081391>.
- Kumar A, Biswas K, Basu B. Hydroxyapatite-titanium bulk composites for bone tissue engineering applications. *J Biomed Mater Res A* 2015; 103(2): 791-806. <https://doi.org/10.1002/jbm.a.35198>.
- Seetharaman S, Sankaranarayanan D, Gupta M. Magnesium-based temporary implants: Potential, current status, applications, and challenges. *J Funct Biomater* 2023; 14(6): 324. <https://doi.org/10.3390/jfb14060324>.
- Antoniac I, Manescu Paltanea V, Antoniac A, Paltanea G. Magnesium-based alloys with adapted interfaces for bone implants and tissue engineering. *Regen Biomater* 2023; 10: rbad095. <https://doi.org/10.1093/rb/rbad095>.
- Iskandar ME, Aslani A, Tian Q, Liu H. Nanostructured calcium phosphate coatings on magnesium alloys: Characterization and cytocompatibility with mesenchymal stem cells. *J Mater Sci Mater Med* 2015; 26(5): 189. <https://doi.org/10.1007/s10856-015-5512-5>.
- Makkar P, Sarkar SK, Padalhin AR, Moon BG, Lee YS, Lee BT. In vitro and in vivo assessment of biomedical Mg-Ca alloys for bone implant applications. *J Appl Biomater Funct Mater* 2018; 16(3): 126-136. <https://doi.org/10.1177/2280800017750359>.
- Chakraborty Banerjee P, Al-Saadi S, Choudhary L, Harandi SE, Singh R. Magnesium implants: Prospects and challenges. *Materials (Basel)* 2019; 12(1): 136. <https://doi.org/10.3390/ma12010136>.
- Savio D, Bagno A. When the total hip replacement fails: A review on the stress-shielding effect. *Processes* 2022; 10(3): 612. <https://doi.org/10.3390/pr10030612>.
- Seetharaman S, Sankaranarayanan D, Gupta M. Magnesium-based temporary implants: Potential, current status, applications, and challenges. *J Funct Biomater* 2023; 14(6): 324. <https://doi.org/10.3390/jfb14060324>.
- Drobyshev A, Komissarov A, Redko N, Gurganchova Z, Statnik ES, Bazhenov V, et al. Bone remodeling interaction with magnesium alloy implants studied by SEM and EDX. *Materials (Basel)* 2022; 15(21): 7529. <https://doi.org/10.3390/ma15217529>.
- Zhou H, Liang B, Jiang H, Deng Z, Yu K. Magnesium-based biomaterials as emerging agents for bone repair and regeneration: From mechanism to application. *J Magnes Alloy* 2021; 9(3): 779-804. <https://doi.org/10.1016/j.jma.2021.03.004>.
- Liu Y, Zhang Y, Wang YL, Tian YQ, Chen LS. Research progress on surface protective coatings of biomedical degradable magnesium alloys. *J Alloys Compd* 2021; 885: 161001. <https://doi.org/10.1016/j.jallcom.2021.161001>.
- Agarwal S, Curtin J, Duffy B, Jaiswal S. Biodegradable magnesium alloys for orthopaedic applications: A review on corrosion, biocompatibility and surface modifications. *Mater Sci Eng C Mater Biol Appl* 2016; 68: 948-963. <https://doi.org/10.1016/j.msec.2016.06.020>.
- Makkar P, Sarkar SK, Padalhin AR, Moon BG, Lee YS, Lee BT. In vitro and in vivo assessment of biomedical Mg-Ca alloys for bone implant applications. *J Appl Biomater Funct Mater* 2018; 16(3): 126-136. <https://doi.org/10.1177/2280800017750359>.
- Kocijan A, Kovač J, Junkar I, Resnik M, Kononenko V, Conradi M. The influence of plasma treatment on the corrosion and biocompatibility of magnesium. *Materials (Basel)* 2022; 15(20): 7405. <https://doi.org/10.3390/ma15207405>.
- Posada VM, Ramírez J, Civantos A, Fernández-Morales P, Allain JP. Ion-bombardment-driven surface modification of porous magnesium scaffolds: Enhancing biocompatibility and osteoimmunomodulation. *Colloids Surf B Biointerfaces* 2024; 234: 113717. <https://doi.org/10.1016/j.colsurfb.2023.113717>.
- Sivapragash M, Kumaradhas P, Vettivel SC, Retnam BSJ. Optimization of PVD process parameter for coating AZ91D magnesium alloy by Taguchi grey approach. *J Magnesium Alloys* 2018; 6(2): 171-179. <https://doi.org/10.1016/j.jma.2018.02.004>.
- Chen XB, Easton MA, Biribilis N, Yang HY, Abbott TB. Corrosion-resistant coatings for magnesium (Mg) alloys. In: Song GL, Ed. Handbook. Corrosion prevention of magnesium alloys. Woodhead Publishing Limited, 2013: 282-312. <https://doi.org/10.1533/9780857098962.2.282>.
- Saji VS. Organic conversion coatings for magnesium and its alloys. *J of Industrial and Engin Chem* 2019; 75: 20-37. <https://doi.org/10.1016/j.jiec.2019.03.018>.
- Zhang Y, Tang S, Hu J, Lin T. Formation mechanism and corrosion resistance of the hydrophobic coating on anodized magnesium. *Corrosion Science* 2016; 111: 334-343. <https://doi.org/10.1016/j.corsci.2016.05.023>.
- Gurgenc T. Microstructure, mechanical properties and ELM based wear loss prediction of plasma sprayed ZrO₂-MgO coatings on a magnesium alloy. *Materials Testing* 2019; 61(8): 787-796. <https://doi.org/10.3139/120.111387>.
- Czerwinski F, Ed. Magnesium alloys: design, processing and properties. IntechOpen. 2011; 540 p. <https://doi.org/10.5772/560>.
- Hornberger H, Virtanen S, Boccaccini AR. Biomedical coatings on magnesium alloys – a review. *Acta Biomater* 2012; 8(7): 2442-2455. <https://doi.org/10.1016/j.actbio.2012.04.012>.
- Gnedenkova AS, Sinebryukhov SL, Filonina VS, Gnedenkova SV. Hydroxyapatite-containing PEO-coating design for biodegradable Mg-0.8Ca alloy: Formation and corrosion behavior. *J Magnes Alloy* 2023; 11(12): 4468-4484. <https://doi.org/10.1016/j.jma.2022.12.002>.
- Gnedenkova AS, Kononenko YI, Sinebryukhov SL, Filonina VS, Vyalii IE, Nomerovskii AD, et al. The Effect of Smart PEO-Coatings Impregnated with Corrosion Inhibitors on the Protective Properties of AlMg3 Aluminum Alloy. *Materials (Basel)* 2023; 16(6): 2215. <https://doi.org/10.3390/ma16062215>.

30. Gnedenkov AS, Sinebryukhov SL, Mashtalyar DV, Gnedenkov SV. Microscale morphology and properties of the PEO-coating surface. *Physics Procedia* 2012; 23: 98-101. <https://doi.org/10.1016/j.phpro.2012.01.025>.
31. Gnedenkov A.S., Mashtalyar D.V., Filonina V.S., Gnedenkov S.V. Features of the Magnesium Alloys Corrosion in the Chloride-Containing Media. *Solid State Phenomena* 2014; 213: 143-148. <https://doi.org/10.4028/www.scientific.net/SSP.213.143>.
32. Gnedenkov A.S., Sinebryukhov S.L., Nomerovskii A.D., Filonina V.S., Ustinov A.Yu., Gnedenkov S.V. Design of self-healing PEO-based protective layers containing in-situ grown LDH loaded with inhibitor on the MA8 magnesium alloy. *J Magnes Alloy* 2023; 11(10): 3688-3709. <https://doi.org/10.1016/j.jma.2023.07.016>.
33. Chen J, Liang S, Fu D, Fan W, Lin W, Ren W, et al. Design and in situ prepare a novel composite coating on Mg alloy for active anti-corrosion protection. *J Alloys Compd* 2020; 831: 154580. <https://doi.org/10.1016/j.jallcom.2020.154580>.
34. Li CY, Gao L, Fan XL, Zeng RC, Chen DC, Zhi KQ. In vitro degradation and cytocompatibility of a low temperature in-situ grown self-healing Mg-Al LDH coating on MAO-coated magnesium alloy AZ31. *Bioact Mater* 2020; 5(2): 364-376. <https://doi.org/10.1016/j.bioactmat.2020.02.008>.
35. Wang L, Fu W, Yi G, Chen Z, Gao Z, Pan Q. Discharge Characteristics, Plasma Electrolytic Oxidation Mechanism and Properties of ZrO₂ Membranes in K₂ZrF₆ Electrolyte. *Membranes (Basel)* 2022; 12(5): 516. <https://doi.org/10.3390/membranes12050516>.
36. Chaharmahali R, Fattah-alhosseini A, Nouri M, Babaei K. Improving surface characteristics of PEO coatings of Mg and its alloys with zirconia nanoparticles: A review. *Applied Surface Science Advances* 2021; 6: 100131. <https://doi.org/10.1016/j.apsadv.2021.100131>.
37. Zhang D, Han Q, Yu K, Lu X, Liu Y, Lu Z, et al. Antibacterial activities against Porphyromonas gingivalis and biological characteristics of copper-bearing PEO coatings on magnesium. *Journal Materials Science & Technology* 2021; 61: 33-45. <https://doi.org/10.1016/j.jmst.2020.05.025>.
38. Liang DY, Liang PC, Yi QQ, Sha S, Shi JF, Chang Q. Copper coating formed by micro-arc oxidation on pure Mg improved antibacterial activity, osteogenesis, and angiogenesis in vivo and in vitro. *Biomed Microdevices* 2021; 23(3): 39. <https://doi.org/10.1007/s10544-021-00573-0>.
39. Xue K, Tan PH, Zhao ZH, Cui LY, Kannan MB, Li SQ, et al. In vitro degradation and multi-antibacterial mechanisms of β -cyclodextrin@curcumin embodied Mg (OH)₂/MAO coating on AZ31 magnesium alloy. *Journal Materials Science & Technology* 2023; 132: 179-192. <https://doi.org/10.1016/j.jmst.2022.04.053>.
40. Bordbar-Khiabani A, Yarmand B, Sharifi-Asl S, Mozafari M. Improved corrosion performance of biodegradable magnesium in simulated inflammatory condition via drug-loaded plasma electrolytic oxidation coatings. *Materials Chemistry and Physics* 2020; 239: 122003. <https://doi.org/10.1016/j.matchemphys.2019.122003>.
41. Fattah-Alhosseini A, Chaharmahali R, Babaei K, Nouri M, Keshavarz MK, Kaseem M. A review of effective strides in amelioration of the biocompatibility of PEO coatings on Mg alloys. *J Magnes Alloy* 2022; 10(9): 2354-2383. <https://doi.org/10.1016/j.jma.2022.09.002>.
42. Gnedenkov AS, Filonina VS, Sinebryukhov SL, Gnedenkov SV. A Superior Corrosion Protection of Mg Alloy via Smart Nontoxic Hybrid Inhibitor-Containing Coatings. *Molecules* 2023; 28(6): 2538. <https://doi.org/10.3390/molecules28062538>.
43. Gnedenkov AS, Sinebryukhov SL, Filonina VS, Ustinov AY, Sukhoverkhov SV, Gnedenkov SV. New Polycaprolactone-Containing Self-Healing Coating Design for Enhance Corrosion Resistance of the Magnesium and Its Alloys. *Polymers (Basel)* 2022; 15(1): 202. <https://doi.org/10.3390/polym15010202>.
44. Gnedenkov AS, Sinebryukhov SL, Filonina VS, Ustinov AY, Gnedenkov SV. Hybrid Coatings for Active Protection against Corrosion of Mg and Its Alloys. *Polymers (Basel)* 2023; 15(14): 3035. <https://doi.org/10.3390/polym15143035>.
45. Li W, Thian ES, Wang M, Wang Z, Ren L. Surface design for antibacterial materials: from fundamentals to advanced strategies. *Adv Sci (Weinh)* 2021; 8(19): e2100368. <https://doi.org/10.1002/adv.202100368>.
46. Wang D, Tan J, Zhu H, Mei Y, Liu X. Biomedical Implants with Charge-Transfer Monitoring and Regulating Abilities. *Adv Sci (Weinh)* 2021; 8(16): e2004393. <https://doi.org/10.1002/adv.202004393>.
47. Gnedenkov AS, Sinebryukhov SL, Mashtalyar DV, Gnedenkov SV, Sergienko VI. Corrosion Monitoring of PEO-pretreated Magnesium Alloys. *Corros Sci Technol* 2017; 16(3): 151-159. <https://doi.org/10.14773/cst.2017.16.3.151>.
48. Gnedenkov AS, Sinebryukhov SL, Filonina VS, Plekhova NG, Gnedenkov SV. Smart composite antibacterial coatings with active corrosion protection of magnesium alloys. *J Magnes Alloy* 2022; 10(12): 3589-3611. <https://doi.org/10.1016/j.jma.2022.05.002>.
49. Zhou P, Gong HR. Phase stability, mechanical property, and electronic structure of an Mg-Ca system. *J Mech Behav Biomed Mater* 2012; 8: 154-164. <https://doi.org/10.1016/j.jmbbm.2011.12.001>.
50. Keerthiga G, Prasad MJNV, Vijayshankar D, Singh Raman RK. Polymeric coatings for magnesium alloys for biodegradable implant application: a review. *Materials (Basel)* 2023; 16(13): 4700. <https://doi.org/10.3390/ma16134700>.
51. Zhang C, Schumacher KN, Dodds ED, Hage DS. Glycoprotein analysis using lectin microcolumns and capillary electrophoresis: Characterization of alpha1-acid glycoprotein by combined separation methods. *J Chromatogr B Analyt Technol Biomed Life Sci* 2021; 1179: 122855. <https://doi.org/10.1016/j.jchromb.2021.122855>.
52. Keerthiga G, Prasad MJNV, Vijayshankar D, Singh Raman RK. Polymeric coatings for magnesium alloys for biodegradable implant application: a review. *Materials (Basel)* 2023; 16(13): 4700. <https://doi.org/10.3390/ma16134700>.
53. Razavi M, Fathi M, Savabi O, Vashae D, Tayebi L. In vivo assessments of bioabsorbable AZ91 magnesium implants coated with nanostructured fluoridated hydroxyapatite by MAO/EPD technique for biomedical applications. *Mater Sci Eng C Mater Biol Appl* 2015; 48: 21-27. <https://doi.org/10.1016/j.msec.2014.11.020>.
54. Ding Y, Wen C, Hodgson P, Li Y. Effects of alloying elements on the corrosion behavior and biocompatibility of biodegradable magnesium alloys: a review. *J Mater Chem B* 2014; 2(14): 1912-1933. <https://doi.org/10.1039/c3tb21746a>.
55. Barker E, Shepherd J, Asencio IO. The use of cerium compounds as antimicrobials for biomedical applications. *Molecules* 2022; 27(9): 2678. <https://doi.org/10.3390/molecules27092678>.
56. Busuioc C, Alecu AE, Costea CC, Beregoi M, Bacalum M, Raileanu M, et al. Composite fibers based on polycaprolactone and calcium magnesium silicate powders for tissue engineering applications. *Polymers (Basel)* 2022; 14(21): 4611. <https://doi.org/10.3390/polym14214611>.
57. Shi B, Li YR, Xu J, Zou J, Zhou Z, Jia Q, et al. Advances in amelioration of plasma electrolytic oxidation coatings on biodegradable magnesium and alloys. *Heliyon* 2024; 10(4): e24348. <https://doi.org/10.1016/j.heliyon.2024.e24348>.
58. Wei Z, Tian P, Liu X, Zhou B. Hemocompatibility and selective cell fate of polydopamine-assisted heparinized PEO/PLA composite coating on biodegradable AZ31 alloy. *Colloids Surf B Biointerfaces* 2014; 121: 451-460. <https://doi.org/10.1016/j.colsurfb.2014.06.036>.
59. Przygoda RT. Safety assessment and global regulatory requirements for genetic toxicity evaluations of medical devices. *Environ Mol Mutagen* 2017; 58(5): 375-379. <https://doi.org/10.1002/em.22102>.
60. Li B, Xue S, Mu P, Li J. Robust self-healing graphene oxide-based superhydrophobic coatings for efficient corrosion protection of magnesium alloys. *ACS Appl Mater Interfaces* 2022; 14(26): 30192-30204. <https://doi.org/10.1021/acsami.2c06447>.
61. Xiong P, Yan J, Wang P, Jia Z, Zhou W, Yuan W, et al. A pH-sensitive self-healing coating for biodegradable magnesium implants. *Acta*

- Biomater* 2019; 98: 160-173.
<https://doi.org/10.1016/j.actbio.2019.04.045>.
62. Atrens A, Johnston S, Shi Z, Dargusch MS. Viewpoint – Understanding Mg corrosion in the body for biodegradable medical implants. *Scr Mater* 2018; 154: 92-100.
<https://doi.org/10.1016/j.SCRIPMAT.2018.05.021>.
 63. Shi Z, Yang F, Hu Y, Pang Q, Shi L, Du T, et al. An oxidized dextran-composite self-healing coated magnesium scaffold reduces apoptosis to induce bone regeneration. *Carbohydr Polym* 2024; 327: 121666.
<https://doi.org/10.1016/j.carbpol.2023.121666>.
 64. Zhao Y, He P, Yao J, Li M, Bai J, Xue F, et al. Self-assembled multilayered coatings with multiple cyclic self-healing capability, bacteria killing, osteogenesis, and angiogenesis properties on magnesium alloys. *Adv Healthc Mater* 2024; 13(9): e2302519.
<https://doi.org/10.1002/adhm.202302519>.
 65. Dong Q, Zhou X, Feng Y, Qian K, Liu H, Lu M, et al. Insights into self-healing behavior and mechanism of dicalcium phosphate dihydrate coating on biomedical Mg. *Bioact Mater* 2020; 6(1): 158-168.
<https://doi.org/10.1016/j.bioactmat.2020.07.019>.
 66. Xiong P, Jia Z, Zhou W, Yan J, Wang P, Yuan W, et al. Osteogenic and pH stimuli-responsive self-healing coating on biomedical Mg-1Ca alloy. *Acta Biomater* 2019; 92: 336-350.
<https://doi.org/10.1016/j.actbio.2019.05.027>.
 67. Alkekhaia D, Hammond PT, Shukla A. Layer-by-Layer Biomaterials for Drug Delivery. *Annu Rev Biomed Eng* 2020; 22: 1-24.
<https://doi.org/10.1146/annurev-bioeng-060418-052350>.
 68. Liu S, Li Z, Yu Q, Qi Y, Peng Z, Liang J. Dual self-healing composite coating on magnesium alloys for corrosion protection. *Chem Eng J* 2021; 424: 1305512021. <https://doi.org/10.1016/j.cej.2021.130551>.
 69. Chen G, Wen S, Ma J, Sun Z, Lin C, Yue Z, et al. Optimization of intrinsic self-healing silicone coatings by benzotriazole loaded mesoporous silica. *Surf Coat Tech* 2021; 421: 127388.
<https://doi.org/10.1016/j.surfcoat.2021.127388>.
 70. Qian B, Zheng Z, Michailidis M, Fleck N, Bilton M, Song Y, et al. Mussel-Inspired Self-Healing Coatings Based on Polydopamine-Coated Nanocontainers for Corrosion Protection. *ACS Appl Mater Interfaces* 2019; 11(10): 10283-10291. <https://doi.org/10.1021/acsami.8b21197>.
 71. Zhang D, Cheng S, Tan J, Xie J, Zhang Y, Chen S, et al. Black Mn-containing layered double hydroxide coated magnesium alloy for osteosarcoma therapy, bacteria killing, and bone regeneration. *Bioact Mater* 2022; 17: 394-405.
<https://doi.org/10.1016/j.bioactmat.2022.01.032>.

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