Slovak University of Technology in Bratislava
Faculty of Materials Science and Technology in Trnava

Ahmed Mohamed Hassan Ibrahim, M.Sc.

Offprint of Dissertation Thesis

Titanium-magnesium composite for dental implants (BIACOM)

for being awarded the academic degree (Doctor ("philosophiae doctor", abbrev. as "PhD.")

in PhD. study programme: Advanced Materials and Material Design

in study branch: Mechanical Engineering

form of study: Full-time

Trnava 2021

STU

Dissertation Thesis was elaborated at: Institute of Materials and Machine Mechanics of the Slovak Academy of Sciences

Submitted by: **Ahmed Mohamed Hassan Ibrahim, M.Sc.**
Institute of Materials and Machine Mechanics of the Slovak Academy of Sciences
Dúbravská cesta 9, 845 13 Bratislava

Supervisor: **Ing. Martin Balog, PhD.**
Institute of Materials and Machine Mechanics of the Slovak Academy of Sciences
Dúbravská cesta 9, 845 13 Bratislava

Reviewers:

Offprint was sent on:

Defence of Dissertation Thesis will be on **at** **o´clock**.

at Faculty of Materials Science and Technology in Trnava Jána Bottu 2744/24, 917 24 Trnava Slovakia

prof. Ing. Miloš Čambál, CSc.
Faculty Dean

ABSTRACT

Metallic implant materials are biomaterials that have experienced major development over the last fifty years, yet some demands posed to them have not been addressed. For the osseointegration process and the outcome of endosseous implantation, it is crucial to reduce the stress shielding effect and achieve sufficient biocompatibility. Powder metallurgy (PM), as a well-known cost-effective and widely used approach, was utilized in this study to fabricate a new type of Ti+Mg composite to enable stress-shielding reduction and obtain better biocompatibility compared with that of the traditional Ti and Ti alloys used for dental implants. Ti+(0–24) vol% Mg composites were fabricated from two different types of Ti powders (hydride–dehydride (HDH) Ti Grade 4 and plasma atomized (PA) Ti Grade 1) and gas atomized (GA) Mg powders. The relation between the microstructure, mechanical properties, fatigue endurance, degradation behavior, and in-vitro biological response of the Ti+Mg composites was investigated with respect to the purity and particle morphology of Ti powders, and the Mg content. Concisely, the Ti(PA)+17 vol% Mg composite showed the best trade-off of properties among all fabricated Ti+Mg composites and therefore was chosen as the promising composition for further investigations. The results revealed that the as-processed Ti(PA)+17 vol% Mg composite exhibited an attractive combination of an ultimate tensile strength (UTS) of 450 ± 1 MPa, 0.2% strain offset yield stress ($YS_{0.2}$) of 331 ± 5 MPa, and elongation (ε) of 8.9 ± 1%, in addition to a reduced Young`s elastic modulus (E) of 88 ± 0.1 GPa. Moreover, the as-processed Ti(PA)+17 vol% Mg composite showed a reasonable fatigue life, while it reached a fatigue tensile stress of 260 MPa at $1.5 \cdot 10^7$ cycles. The fatigue stress of the Ti(PA)+17 vol% Mg dental implants, after corrosion in the HBSS for 6 days, was 180 N at $5 \cdot 10^6$ cycles and still remained at a reasonable level compared to the one obtained for the as-processed Ti Grade 4 dental implants of identical design (i.e. 300 N). The advantageous mechanical properties of the Ti(PA)+17 vol% Mg composite, superior to those of the Ti(HDH)+Mg counterpart, resulted from a specific ultrafine Ti grain structure. The degradation of Mg enabled a further decrease of E down to 84.3 ± 0.3 GPa, while the tensile strengths remained unaffected, and ε and fatigue stress decreased. Nevertheless, the mechanical performance of the corroded Ti(PA)+17 vol% Mg remained appropriate for a specific application of dental implants with the addition of porosity induced osseointegration. Owing to a refined Mg grain structure, in-vitro corrosion tests confirmed a reduced degradation rate (DR) of the Mg component from the Ti(PA)+17 vol% Mg composite when compared with that of the Ti(HDH)+Mg counterpart. The accomplished

results of the in-vitro evaluation provided strong evidence for a desirable in-vitro response of the pre-washed Ti(PA)+17 vol% Mg (referred as Ti17Mg) in the HBSS on cell viability, adhesion, and proliferation without any induced adverse effects, such as DNA damage or oxidative stress. Moreover, the surface modification by grinding and polishing, as an alternative way, crucially influenced the degradation response and material/cell interaction. The results revealed that the surface-modified Ti17Mg composite (Ti17Mg-P) had a pronounced response represented by an acceptable DR and a distinct in-vitro biological behavior. The smooth and less strained surface of the Ti17Mg-P composite eliminates the need for pre-washing or further surface treatments and concurrently maintains the bioactive surface nature when compared with the pre-washed Ti17Mg composite or conventional Ti-based materials. The preliminary in-vivo study of the Ti+Mg implant-like samples after 12 weeks revealed complete bone healing, and no signs of gaseous enclosures or any undesired effects were determined. The study confirmed that the Ti17Mg-P composite is an immensely promising material for applications in endosseous dental implants subjected to intense and cyclic loading.

ABSTRAKT

Kovové biomateriály pre implantáty prešli za posledných päťdesiat rokov intenzívnym vývojom. Aj napriek tomu stále ostávajú v nedostatočnej miere doriešené niektoré požiadavky, ktoré sú na ne kladené. Pre dokonalý výsledok osseointegrácie a endoséznej implantácie je žiaduce znížiť tzv. stress-shielding efekt a dosiahnuť dostatočnú biokompatibilitu a bioaktivitu. V tejto práci bola využitá prášková metalurgia (PM) na výrobu nového typu čiastočne biodegradovateľného titánového (Ti)+horčíkového (Mg) kompozitu pre aplikácie intenzívne mechanicky a cyklicky zaťažovaných trvalých implantátov; typicky zubné implantáty. Nový Ti+Mg kompozit znižuje stress-shielding efekt a navyšuje bioaktivitu v porovnaní s tradičnými materiálmi na báze Ti, ktoré sú referenčnými pre výrobu trvalých implantátov. Ti+Mg kompozit v tejto práci bol vyrobený konvenčnými, ekonomickými a dostupnými postupmi PM. Kompozity Ti+(0–24) obj.% Mg boli pripravené z dvoch rôznych typov Ti práškov: i) hydrid–dehydrid (HDH) Ti triedy 4 a ii) v plazme atomizovaný (PA) Ti triedy 1) a v plyne atomizovanom (GA) Mg 99,8 hm.% prášku. Bol detailne skúmaný vzťah medzi mikroštruktúrou, mechanickými vlastnosťami, únavovou životnosťou, degradačným koróznym správaním a in-vitro biologickou reakciou Ti+Mg kompozitov s ohľadom na parametre vstupných Ti a Mg práškov, obsah biodegradovateľnej Mg zložky a technologické parametre PM prípravy kompozitov. Potvrdilo sa, že kompozit Ti(PA)+17 obj.% Mg vykazoval najvýhodnejšiu kombináciu vlastností z pomedzi všetkých vyrobených materiálov, a preto bol vybraný ako sľubný materiál pre ďalší detailný výskum. Popri želanom znížením Youngovom module (E) 88 ± 0,1 GPa (~20% pokles oproti Ti triedy 5) Ti(PA)+17 obj.% Mg kompozit dosahoval dostatočnú medzu pevnosti v ťahu (UTS) 450 ± 1 MPa, zmluvnú medzu klzu v ťahu ($YS_{0,2}$) 331 ± 5 MPa a ťažnosť (ε) 8,9 ± 1%. Navyše bola potvrdená primeraná únavová životnosť, t.j. medzná únavová pevnosť v ťahu 260 MPa pri $1{,}5 \cdot 10^7$ cykloch. Vystavenie Ti(PA)+17 obj.% Mg kompozitu koróznemu prostrediu, t.j. simulovanému fyziologickému Hankovmu roztoku (HBSS), viedlo ku rozpúšťaniu biologicky odbúrateľnej Mg zložky a následnej tvorbe pórovitosti. Rozpúšťanie Mg bolo mimoriadne rýchle v počiatočnej fáze pričom neskôr po 5 dňoch expozície dochádza ku stabilizácii degradácii Mg. Tvorba povrchovej pórovitosti zabezpečila ďalšie, želané zníženie E až na 84,3 ± 0,3 GPa, zatiaľ čo UTS a $YS_{0,2}$ zostali zachované, a došlo len k malému poklesu ε a medznej únavovej pevnosti. Výhodné mechanické vlastnosti Ti(PA)+17 obj.% Mg boli dosahované vďaka špecifickej ultrajemnozrnnej (UFG) Ti štruktúre. Mechanické vlastnosti Ti(PA)+17 obj.% Mg, v stave po výrobe ako aj v korodovanom stave, sú porovnateľné

s požadovanými hodnotami pre biomedicínsky materiál typu 4 podľa ISO 22674 štandardu, ktorý zahŕňa aj vysoko a cyklicky zaťažované implantáty. Navyše bola nameraná primeraná medzná únavová sila 180 N pri $5 \cdot 10^6$ cykloch zubných implantátov vyrobených z Ti(PA)+17 obj.% Mg (po korózii) stanovená podľa normy pre zubné implantáty, ktorá bola mierne pod úrovňou únavovej životnosti identických zubných implantátov Ti triedy 4. Rýchle počiatočne rozpúšťanie Mg bolo sprevádzané s neakceptovateľne intenzívnym vývojom H_2 a nárastom pH s toxickým vplyvom na okolité tkanivo. K nadlimitnej miere korózii dochádzalo aj napriek jemnozrnnej rovnoosej subštruktúre Mg zložky, ktorá účinne redukuje rýchlosť korózie Mg. Aby sa predišlo negatívnemu vplyv hypermagnesémie musel byť povrch Ti(PA)+17 obj.% Mg stabilizovaný. Boli optimalizované dva rozličné spôsoby stabilizácie: i) stabilizácia v HBSS po dobu 20 h pri pomere objemu elektrolytu a povrchu vzorky 20 $ml.cm^{-2}$ a ii) stabilizácia pomocou modifikácie povrchu vhodným trieskovým opracovaním. Oba spôsoby stabilizácie vzoriek a zubných implantátov viedli ku negácii cytotoxického účinku (nepriama a priama MTT štúdia) pri reakcii s bunkovou líniou myších fibroblastov (L929) a ľudského osteosarkómu (Saos-2) s dostatočnou mierou životaschopnosti, adhézie a proliferácie buniek. Dosiahnuté výsledky in-vitro interakčných COMET a plazmidovej DNA analýz spolu s detekciou oxidačného stresu DNA pomocou metódy ROS potvrdili negatívnu genotoxickú reakciu buniek v extrakčných médiách stabilizovaného Ti(PA)+17 obj.% Mg kompozitu. Pozitívne výsledky predbežnej implantačnej štúdie Ti+Mg vzoriek implantovaných do holennej kosti 4 oviec potvrdili graduálny priebeh osseointegrácie na rozhraní kosť – implantát, úplné prerastenie kostného tkaniva v blízkosti závitov vzoriek a tvorba zrelých osteoidov 12 týždňov po chirurgickom zákroku, pričom sa nepreukázali žiadne nežiaduce zápalové reakcie a tvorba pľuzgierov. Mg zložka pôsobila ako prospešný modulátor na vytváranie osseoaktívneho povrchu spontánnym odbúravaním v prostredí tela ovce a stimulátor tvorby nového kostného tkaniva. Práca potvrdila, že Ti(PA)+17 obj.% Mg so stabilizovaným povrchom je sľubným materiálom pre aplikáciu endoséznych implantátov vystavených intenzívnemu a cyklickému zaťaženiu.

PREFACE

Increase in human life expectancy has led to increasing demand for different types of bone implantation surgical procedures in diverse biomedical areas. Namely in dentistry, the implants are an increasingly attractive economic option, where no substitute for dental implants is expected to emerge in the next years and high single-digit growth is considered most likely. Ti and its alloys are the most widespread metallic biomaterials used in prosthodontic surgery because of their good mechanical properties and biocompatibility. However, the well-established and commonly utilized Ti-based materials may suffer from insufficient surface bioactivity aside from the potential release of toxic constituents through corrosion or wear processes. This may adversely affect their biocompatibility, in particular in the case of long-term applications. Furthermore, Young`s elastic modulus (E) of currently utilized Ti-based biomaterials is not analogous to that of the natural bone. This leads to the stress shielding effect. Due to such drawbacks of Ti-based materials, the main concern of this systematic study is to develop novel bioactive and biocompatible Ti-based materials composed of non-toxic constituents that possess E values that are close to those of bones.

1. RESEARCH AIM AND APPROACH

The aim of this study is to produce a two-phase Ti+Mg composite material, which is named BIACOM (bioactive composite metal), and the finished device (i.e. dental implant) that would selectively exploit the advantages of both biometals. While Ti would serve as the matrix material providing the implant with strength, Mg would bring about the reduction of E thus minimizing the stress shielding effect during loading, and moreover, gradient porosity would form with time as the result of selective Mg degradation into the surface and volume of the implant. The degradation of the Mg phase should improve the osseointegration process thanks to the porosity, which acts as nucleating sites for new bone ingrowth (**Fig. 1**). **Fig. 2** shows a flowchart of the systematic work plan.

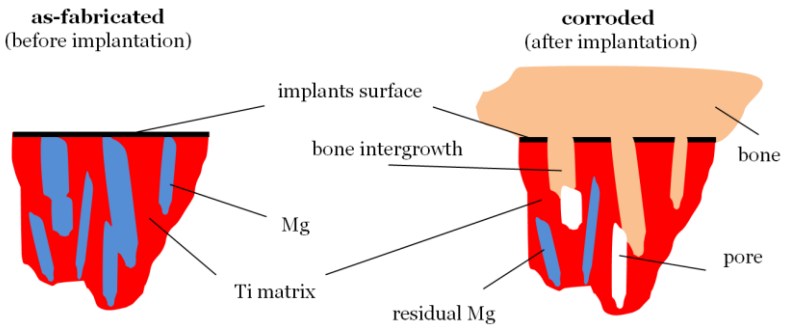


Fig. 1. *A systematic configuration of the solution approach.*

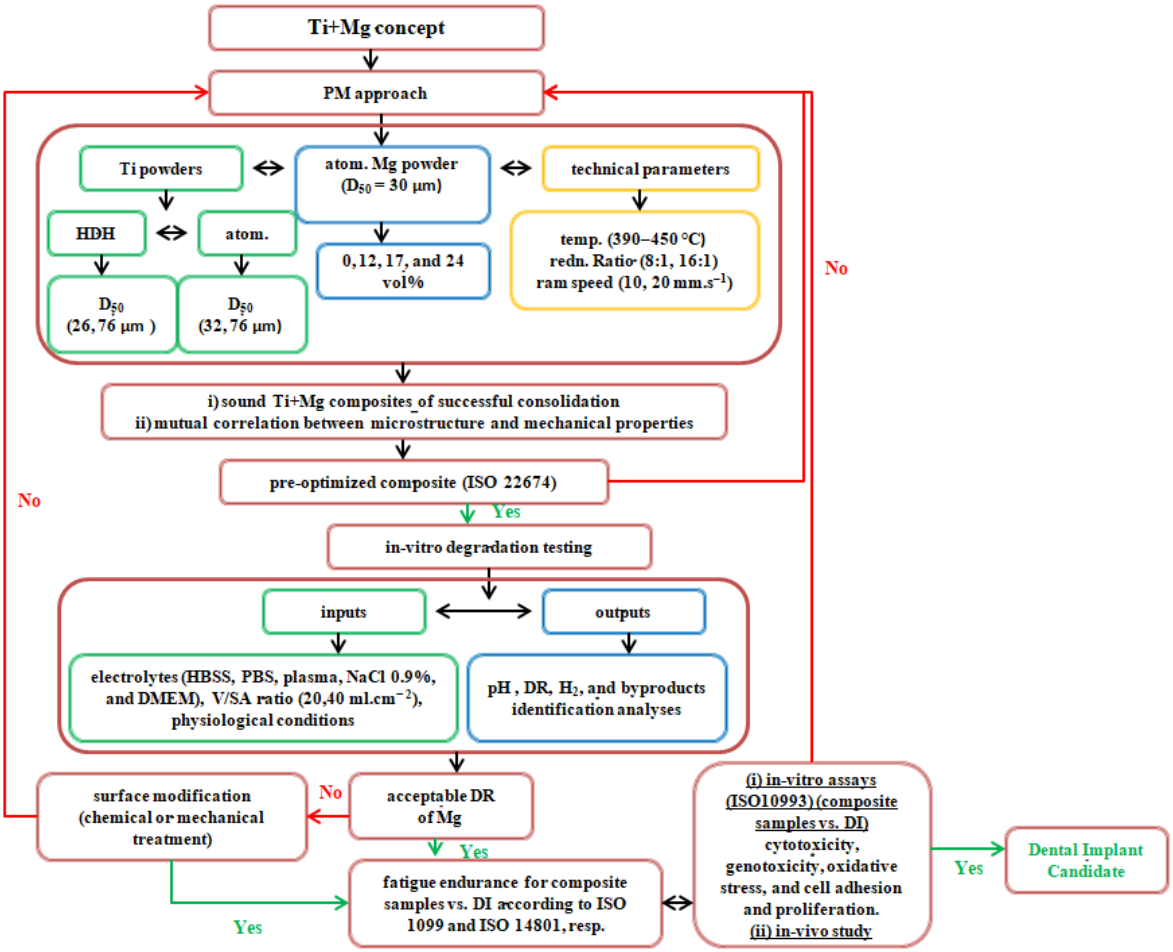


Fig. 2. *Flowchart of the work plan.*

2. EXPERIMENTAL

The precursors utilized in this study were two size fractions of hydride–dehydride (HDH) Ti Grade 4, labeled as coarse Ti(C) and fine Ti(F), plasma atomized (PA) Ti Grade 1, and gas atomized (GA) Mg powders (**Table 1**). The powders were homogeneously mixed according to the nominal compositions of Ti+xMg (x = 0, 12, 17, and 24 vol%). The powder mixtures were pre-compacted by cold isostatic pressing (CIP). After that, the CIP blanks were instantly pressed by uniaxial hot vacuum pressing (HVP). Eventually, the HVP blanks were fully consolidated and sheared by direct extrusion (DE) into rod bars with a diameter of 7.5 mm by a flat face die at a reduction ratio of 16:1 and a ram speed of 10 $mm.s^{-1}$. The schematic of the fabrication process is illustrated in **Fig. 3**.

Table 1 *Characteristics of the Ti(HDH,F), Ti(HDH,C), Ti(PA), and Mg(GA) powders.*

	Ti(HDH,F)	**Ti(HDH,C)**	**Ti(PA)**	**Mg(GA)**
Fabrication process	Hydride–dehydride	Hydride–dehydride	Plasma atomization	Gas atomization
Purity (%)	99.4	99.4	99.8	99.8
Morphology	Irregular	Irregular	Spherical	Spherical
Size fraction (µm)	20–40	63–150	45–106	20–40

D_{50} (µm)	26	76	76	30
O (wt%)	0.477 ± 0.003	0.182 ± 0.008	0.08 ± 0.004	-
H (wt%)	0.0006	0.0011 ± 0.0005	0.002 ± 0.0005	-
N (wt%)	0.02 ± 0.001	0.011 ± 0.004	0.015 ± 0.001	-

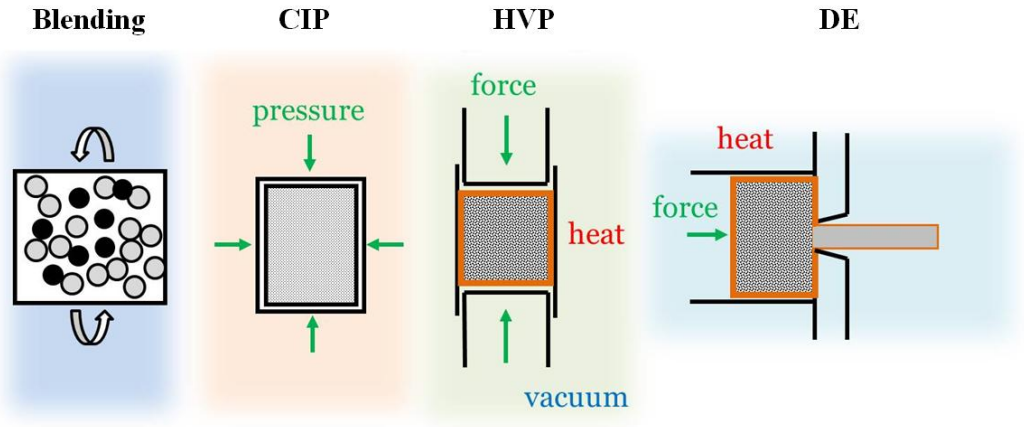


Fig. 3. *Schematic of the fabrication process.*

The microstructure of the powders and composite materials was examined by a scanning electron microscope (SEM) equipped with an energy dispersive X-ray spectrometer (EDS) and an electron back-scattering diffraction system (EBSD) and by a scanning transmission electron microscope (STEM) equipped with an EDS detector. Tensile tests were performed by means of a Zwick Roell 1474 machine in accordance with the ASTM E8 standard. The E of the as-extruded Ti and Ti+Mg, and corroded Ti+Mg specimens was investigated through a three-point bending test by dynamic mechanical analysis (DMA). The fatigue tests were performed by means of an axial force-controlled method according to the ISO 1099 procedure. To evaluate the mechanical integrity of the Ti+Mg composites after Mg degradation, the E, tensile properties, and fatigue life were assessed after the as-machined specimens for DMA, tensile, and fatigue testing were corroded in an Hank's Balanced Salt Solution (HBSS) for various time periods. Furthermore, the fatigue life of the Ti(PA)+17 vol% Mg dental implants was tested after corrosion in the HBSS for 6 days in accordance with the conditions defined by the ISO 14801 standard, and the results were compared to those of the as-processed Ti Grade 4 dental implant of identical design. The Ti(PA)+17 vol% Mg dental implant specimens were a commercial design MV4.5-10 implants with a thread diameter of 4.5 mm and a length of 10 mm (**Fig. 4**). The implants were produced by MARTIKAN s.r.o. by means of a common CNC machining approach.

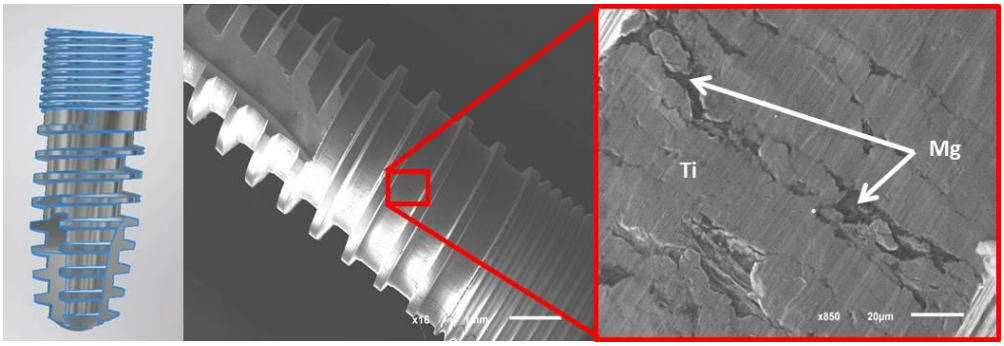
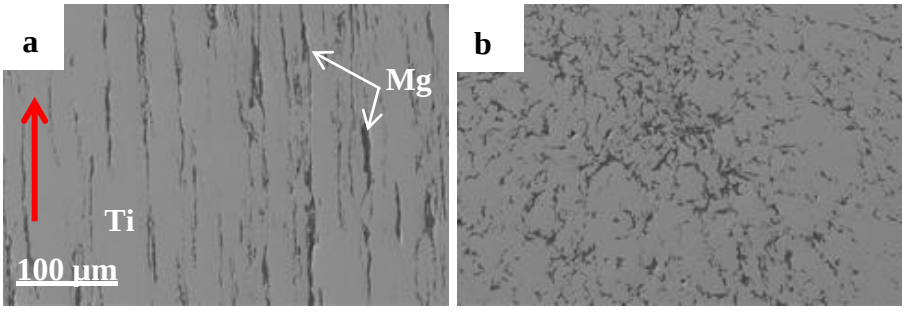


Fig. 4. *MV3.6-10 dental implant fabricated from the Ti(PA)+17 vol% Mg composite material (from the left: the implant drawing, scanning electron image of the implant, and a detail of the thread surface area).*

The DR of Mg was evaluated either by the weight loss (WL) measurement or the H_2 evolution volumetric method. The specimens of the Ti+Mg composites were exposed to the HBSS for various time periods extended up to 21 days. The surface of corroded specimens was characterized by SEM and EDS. The corrosion by-product was evaluated by X-ray diffraction (XRD, Philips X'Pert machine). To verify the biocompatibility of the optimized Ti(PA)+17 vol% Mg material (composite samples *vs.* DI) and to assess the effects of surface modification either by chemical or mechanical treatment, the extent of the degradation behavior of Mg along with the in-vitro biological evaluation, including cytotoxicity, genotoxicity, oxidative stress, cell adhesion, and proliferation following the ISO 10993 guidelines were investigated. The obtained results were directly compared with those of a Ti Grade 4 control material.

3. RESULTS AND DISCUSSION

A new type of Ti+(0–24) vol% Mg composites were fabricated from the Ti(HDH) and Mg(GA) powders by the PM approach. The microstructure of the composites was formed by a biodegradable Mg component with a low E in the form of filaments homogeneously dispersed within a permanent Ti matrix. With the increase of the Mg content, discrete filaments became interconnected with each other thus forming a continuous Mg network embedded in the Ti matrix (**Fig. 5)**.



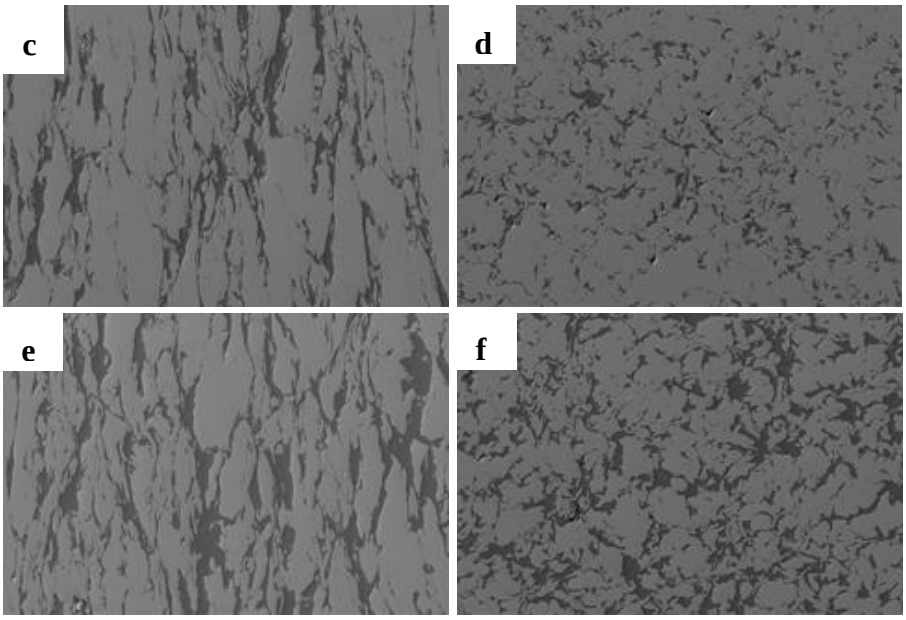


Fig. 5. *SEM micrographs of the as-extruded Ti(HDH,C)+12 vol% Mg composite in the (a) longitudinal and (b) transversal directions, the Ti(HDH,C)+17 vol% Mg composite in the (c) longitudinal and (d) transversal directions, and the Ti(HDH,C)+24 vol% Mg composite in the (e) longitudinal and (f) transversal directions. The red arrow indicates the extrusion direction.*

The Ti(HDH,C)+12 vol% Mg composite showed the best trade-off of properties, with E reduced by ~ 15%, a reasonably high $YS_{0.2}$, and sufficient ε after Mg degradation from the sample surface. Although the Ti(HDH,C)+Mg composites with the Mg content above 12 vol% resulted in materials that exhibited a further reduction in the E (which was desired), their ε dropped noticeably below the specification defined by the ISO 22674 standard. Furthermore, the degradation behavior in simulated body fluids and the mechanical performance of the Ti+Mg composites with corroded surface have not been addressed thoroughly. Therefore, it was indispensable to develop further the Ti+Mg composite material.

Sound Ti+Mg composites were fabricated from the Ti(PA) powders by the PM consolidation approach. The Ti(PA)+17 vol% Mg composite showed superior performance compared with the Ti(HDH,C)+12 vol% Mg composite. Because of the ultrafine-grained Ti structure (grain size of 0.82 μm), the as-extruded Ti(PA)+17 vol% Mg composite exhibited an attractive combination of reproducible UTS = 450 ± 1 MPa, $YS_{0.2}$ = 331 ± 5 MPa, and ε = 8.9 ± 1% accompanied with decreased E = 88 ± 0.1 GPa (**Table 2** and **Fig. 6**).

Table 2 *Ultimate tensile strength (UTS), 0.2% strain offset yield stress ($YS_{0.2}$), elongation (ε), and Young`s modulus (E) of the as-extruded Ti(PA) and Ti(HDH,C) Mg free materials, and Ti(PA)+Mg and Ti(HDH,C)+Mg composites. The minimum requirements for the type 4 biomedical material by the ISO 22674 standard.*

Material	UTS (MPa)	$YS_{0.2}$ (MPa)	ε (%)	E (GPa)

Ti(PA)	508 ± 1	328 ± 2	23 ± 0.5	100 ± 0.1
Ti(PA)+12 vol% Mg	469 ± 9	333 ± 10	11.2 ± 0.3	-
Ti(PA)+17 vol% Mg	450 ± 1	331 ± 5	8.9 ± 1	88 ± 0.1
Ti(PA)+24 vol% Mg	392	335	1	-
Ti(HDH,C)	598 ± 3	411 ± 1	31.3 ± 1.8	99.7 ± 0.2
Ti(HDH,C)+12 vol% Mg	498 ± 26	400 ± 19	2.7 ± 0.6	92.6 ± 0.2
Ti(HDH,C)+17 vol% Mg	443 ± 1	367 ± 5	1.5 ± 0.1	88.6 ± 0.6
Ti(HDH,C)+24 vol% Mg	409	340	1.1	81 ± 1
ISO 22674 type 4 minimum	-	360	2	-

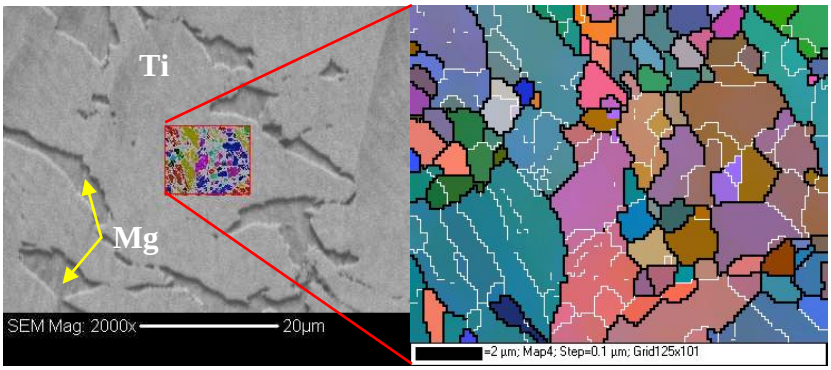


Fig. 6. *Grain orientation map of the Ti phase in the transversal direction of the as-extruded Ti(PA)+17 vol% Mg composite.*

The EDS analysis did not confirm evidence of a chemical interlayer between the virtually immiscible Ti and Mg elements or the presence of voids and pores (**Fig. 7a, b, c**). The EBSD analysis of the Ti(PA)+17 vol% Mg composite revealed a micrometric Mg grain structure, whereas typically a few grains with the size of a few µm existed in the cross-section of a separated Mg filament (**Fig. 7d**). Furthermore, a detailed microstructural study confirmed no enrichment of the Ti–Mg interfaces by O and TiO_2 (**Fig. 8**).

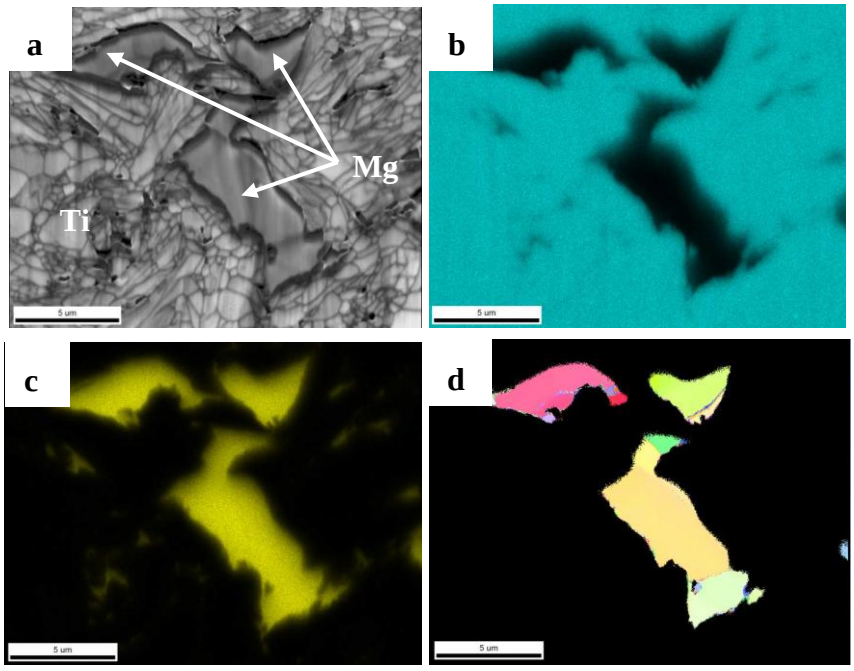


Fig. 7. *(a) SEM micrograph with the corresponding EDS map of the (b) Ti and (c) Mg elements, and (d) a grain orientation map of the Mg phase in the transversal direction of the as-extruded Ti(PA)+17 vol% Mg*

composite.

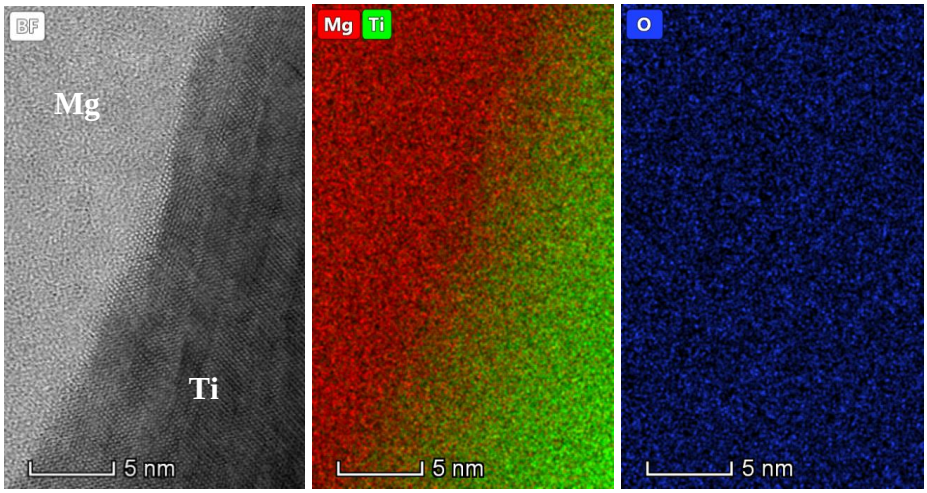


Fig. 8. *STEM micrograph with the corresponding combined EDS map of the Ti, Mg, and O elements of Ti–Mg interface in the Ti(PA)+17 vol% Mg composite in the transversal direction.*

Owing to a high damage tolerance of the Ti matrix and the interconnected skeleton of the fine Mg filaments, a fatigue stress of 260 MPa at $1.5 \cdot 10^7$ cycles was attained, which was higher than that published for the wrought Ti Grade 1 and 2 reference materials (~ 250 MPa at 10^7 cycles) (**Fig. 9**). Moreover, the fatigue stress of the Ti(PA)+17 vol% Mg dental implants, after corrosion in the HBSS for 6 days, was 180 N at $5 \cdot 10^6$ cycles and still remained at a reasonable level compared to the one obtained for the as-processed Ti Grade 4 dental implants of identical design (i.e. 300 N) (**Fig. 10**).

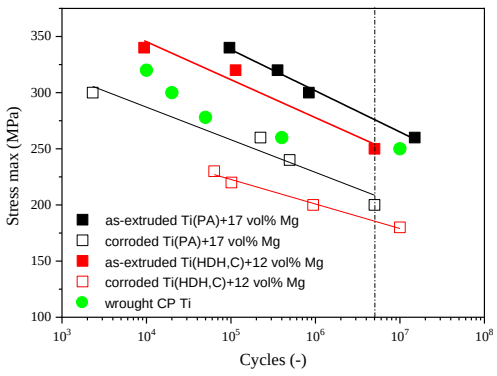


Fig. 9. *S-N curves of the Ti(PA)+17 vol% Mg and Ti(HDH)+12 vol% Mg composites tested in the as-extruded condition and after corrosion in the HBSS for 6 days. Data for the wrought CP Ti Grade 1 are included for comparison.*

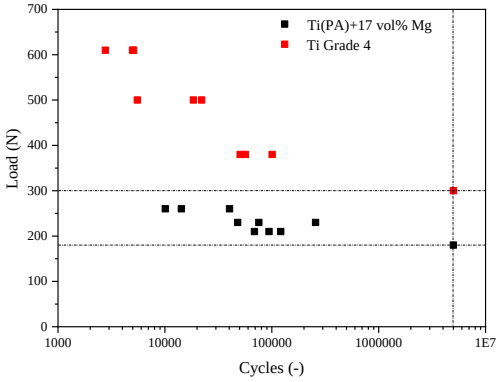


Fig. 10. *Fatigue life of MV4.5-10 dental implants fabricated from the Ti(PA)+17 vol% Mg after corrosion in the HBSS for 6 days. Ti Grade 4 dental implant of an identical design was tested in the as-extruded condition for comparison.*

Exposure of the Ti(PA)+17 vol% Mg composite to the HBSS resulted in the degradation of the biodegradable Mg component and formation of pores at the former Mg sites. The DR was extremely pronounced at the early stage of the exposure, and it started to stabilize after 5 days (**Fig. 11**). The DR determined after 21 days exposure was reduced

down to ~ 0.06 mg.cm^{-2}.day^{-1} because of the refined Mg grain structure. The degradation of Mg allowed for a further decrease in the E of the composite down to 84.3 ± 0.3 GPa (**Table 3**). The tensile strengths (UTS = 456 ± 2 MPa and $YS_{0.2}$ = 346 ± 3 MPa) remained mostly unaffected by the Mg degradation, while the corroded composite exhibited a decreased fatigue stress of 200 MPa at 5·10^6 cycles and ε = 5 ± 0.7% (**Table 4** and **Fig. 9**).

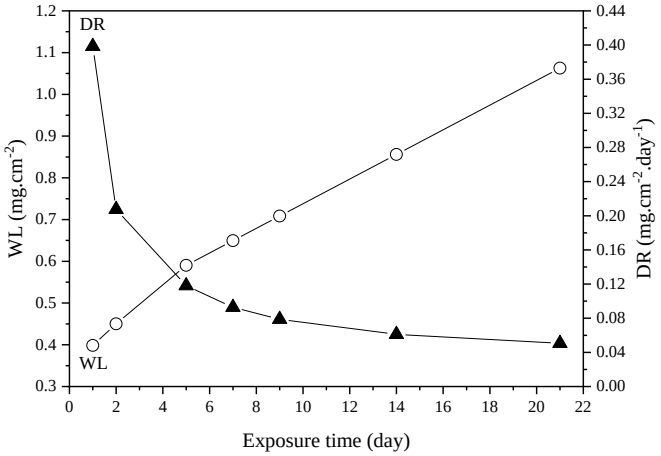


Fig. 11. *Weight loss (WL) and degradation rate (DR) of the Ti(PA)+17 vol% Mg composite after exposure to the HBSS for different time intervals.*

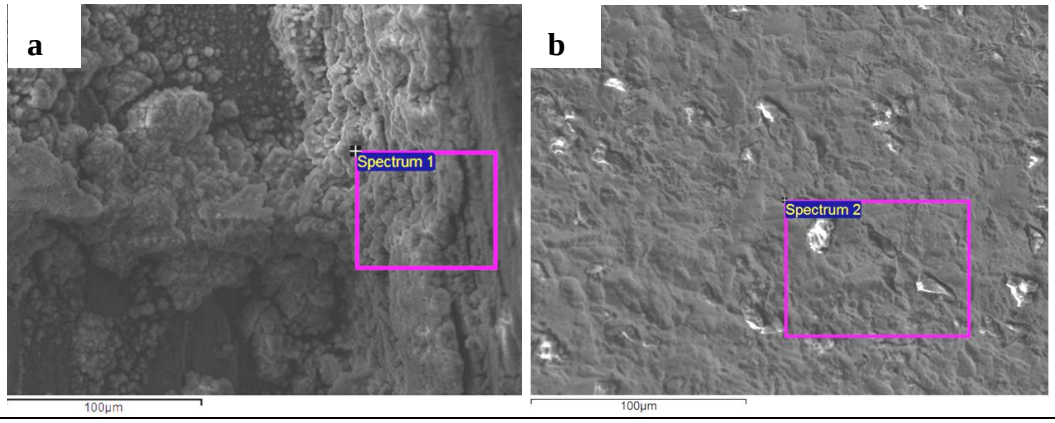
Table 3 *Evolution of Young`s modulus (E) of the as-extruded Ti(PA)+17 vol% Mg samples immersed in the HBSS for 1, 7, 14, and 21 days.*

Condition	**as-extruded**	**corroded for 1 day**	**7 days**	**14 days**	**21 days**
E (GPa)	88 ± 0.1	85.6 ± 0.3	84.8 ± 0.2	84.5 ± 0.1	84.3 ± 0.3

Table 4 *Ultimate tensile strength (UTS), 0.2% strain offset yield stress (YS0.2), and elongation (ε) of the Ti(PA)+17 vol% Mg samples exposed to the HBSS for 21 days.*

Condition	**UTS (MPa)**	**$YS_{0.2}$ (MPa)**	**ε (%)**
as-extruded	450 ± 1	331 ± 5	8.9 ± 1
corroded for 21 days	456 ± 2	346 ± 3	5 ± 0.7

The mechanical properties and fatigue life of the corroded composite were at the level of the ISO 22674 specification of Type 4 material for its application in dental implants. To make progress with a potential use of the Ti(PA)+17 vol% Mg (referred as Ti17Mg) composite for the fabrication of dental implants, it is necessary to evaluate how host living tissues that are in contact with the implant materials respond to them. The in-vitro response, including cytotoxicity, genotoxicity, and oxidative stress, of the pre-washed Ti17Mg composite and dental implant (DI) was evaluated. The results showed the surface pretreatment by 20 h pre-washing in the HBSS at a ratio of 20 ml.cm^{-2} minimized the hypermagnesemia effect. Compared with the Ti Grade 4 (TiGR4) reference, EDS analysis confirmed the formation of a Ca/P-rich biomimetic layer on the surface of the Ti17Mg DI after extraction in the DMEM (**Fig. 12**).



Element (wt%)	Oxygen (O)	Magnesium (Mg)	Carbon (C)	Phosphorus (P)	Titanium (Ti)	Calcium (Ca)	Aluminum (Al)
Spectrum 1	59.42	15.94	14.46	4.22	3.86	2.10	-
Spectrum 2	33.96	-	3.90	0.81	54.59	1.24	5.50

Fig. 12. *SEM micrograph of surface of the (a) Ti17Mg DI and (b) TiGR4 DI after extraction in DMEM at 37 °C for 72 h with the corresponding EDS results of the chemical composition of the formed by-products from the purple rectangle area.*

The indirect MTT assay revealed that the Ti17Mg extracts exerted a mild level of cytotoxicity without any induced morphological alteration of the incubated cells (**Fig. 13**). The direct assays showed that the initial cell adhesion and proliferation were improved (**Fig. 14**). No DNA damage was induced, and No detection for smearing or loss of plasmid DNA was found (**Fig. 15** and **Fig. 16**). The oxidative stress confirmed no increase in the ROS levels (**Fig. 17**). In-vitro evaluation study confirmed a desirable in-vitro response of the pre-washed Ti17Mg that was comparable to that of the TiGR4 reference.

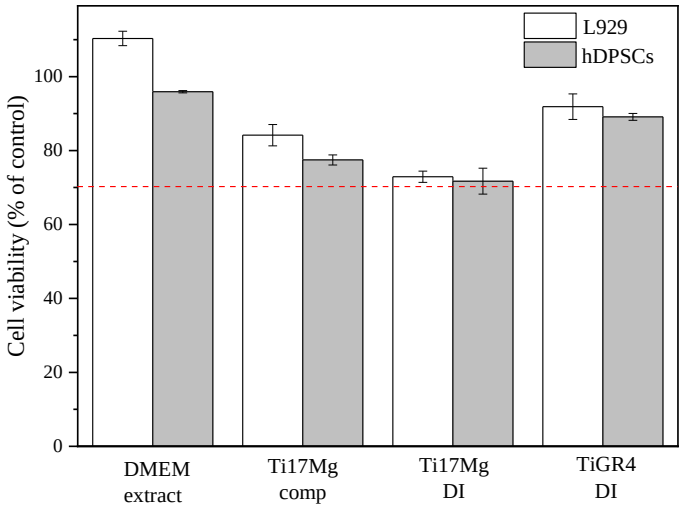


Fig. 13. *The cell viability of L929 and hDPSCs treated with the extracts of the investigated specimens and controls for 24 h evaluated by the indirect MTT assay. The dashed line represents the cytotoxicity level.*

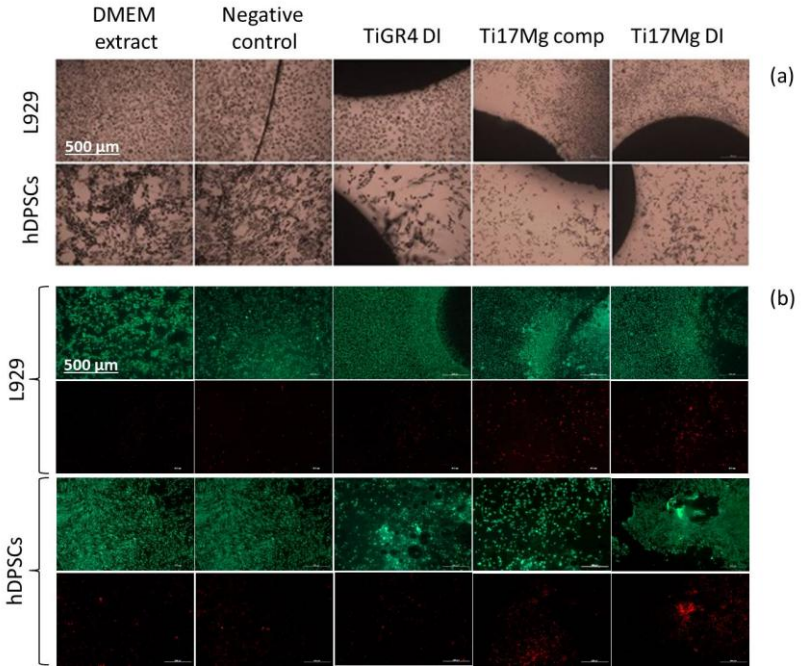


Fig. 14. *Direct cytotoxicity of the TiGR4 DI reference, Ti17Mg comp, and Ti17Mg DI discs using the L929 and hDPSCs by means of the (a) MTT and (b) Live/dead assays. All images were taken at 40x magnification; the scale bars are identical for all images.*

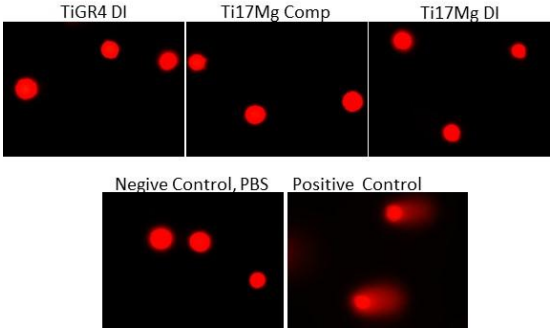


Fig. 15. *Representative microscopic images of the comet assay using single-cell gel electrophoresis for the TiGR4 DI reference, Ti17Mg comp, and Ti17Mg DI sets. Negative control: PBS treated cells, Positive control: Ethyl methanesulfonate (EMS) treated cells, under 40x magnification.*

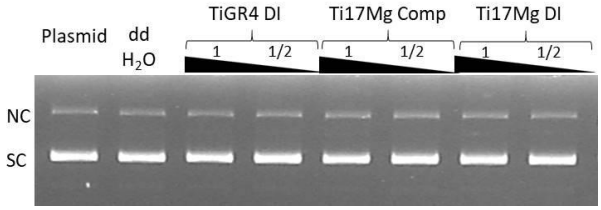


Fig. 16. *Migration pattern of plasmid DNA incubated with extracts of the TiGR4 DI reference, Ti17Mg comp, and Ti17Mg DI. The bands are labeled as NC: Nicked circular, SC: Supercoiled. ddH_2O was served as a negative control.*

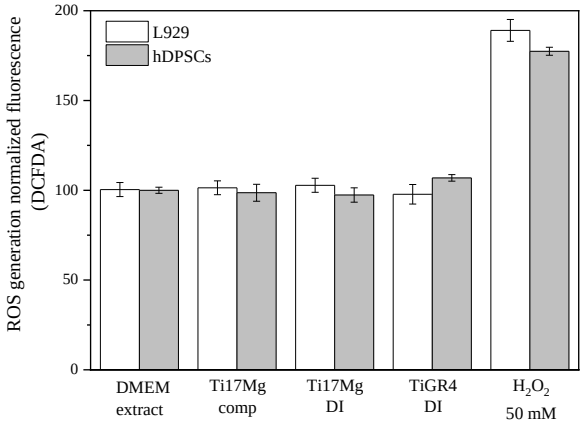
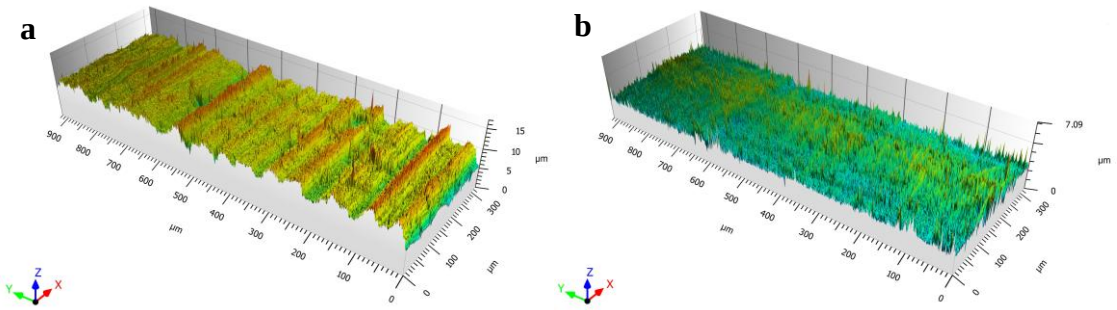


Fig. 17. *ROS generation of the L929 and hDPSCs upon 24 h exposure to the extracts of the TiGR4 DI reference, Ti17Mg comp, and Ti17Mg DI. The results for the H_2O_2 postive control are also shown..*

Although the chemical pre-washing is effective, it formed pores on the surface, which can act as deep pockets for bacterial infections after implantation. Moreover, this approach is not scalable for the massive production of dental implants. Consequently, the mechanical treatment by grinding and polishing was introduced as an alternative way. This approach can boost implant surface characteristics and is more feasible for the intended application. The representative parameters of surface roughness for the Ti17Mg-P specimen had profoundly smaller values, decreased by ~ 3 times, compared with those of the Ti17Mg-T specimen (**Fig. 18**). The existence of the fine-grained substructure formed by low angle grain boundaries along with an increased surface area make the Mg component present at the surface vicinity of the Ti17Mg-T more prone to corrosion than Mg under the surface. Therefore, the Ti17Mg-T specimens had a DR higher than that of the Ti17Mg-P specimens (**Fig. 19**). Whatever the solution the Ti17Mg-P was exposed to, the DR reached very comparable values.



	Parameter (μm)		
Sample	**Sa**	**Sq**	**Sz**
Ti17Mg-T	0.934	1.20	17.10
Ti17Mg-P	0.376	0.49	7.09

Fig. 18. *3D maps of surface of the (a) Ti17Mg-T and (b) Ti17Mg-P specimens and the corresponding surface roughness parameters Sa, Sq, and Sz.*

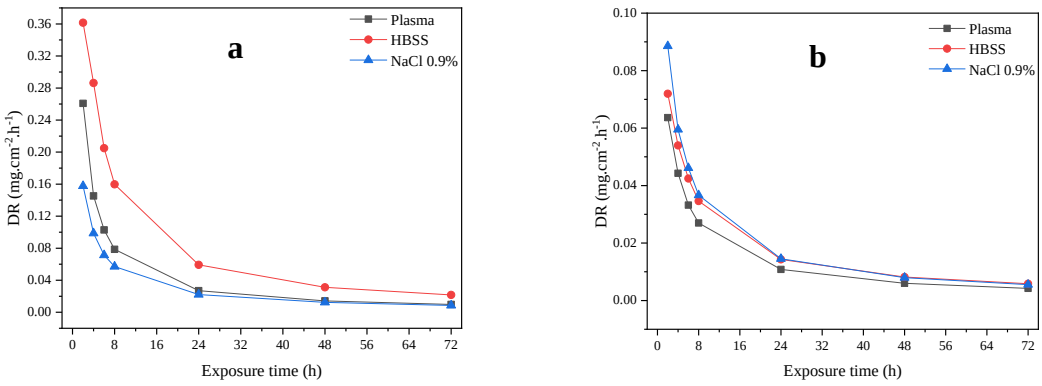


Fig. 19. *Degradation rates (DR) of the (a) Ti17Mg-T and (b) Ti17Mg-P specimens as functions of exposure time in the artificial Plasma, HBSS, and NaCl 0.9% solutions using a V/SA ratio of 20 ml.cm^{-2} at 37 °C.*

The Ti17Mg-T extracts exhibited a higher cytotoxic behavior compared with that

of the Ti17Mg-P extracts but also did not exceed the limit of 30% defined by the ISO 10993-5 standard (**Fig. 20**). This behavior of the Ti17Mg-T was mainly attributed to the accumulative concentration of Mg in the extraction medium, the corrosion by-products, and a subsequent rise of the pH value due to the extreme DR at the early stage of exposure (up to 24 h).

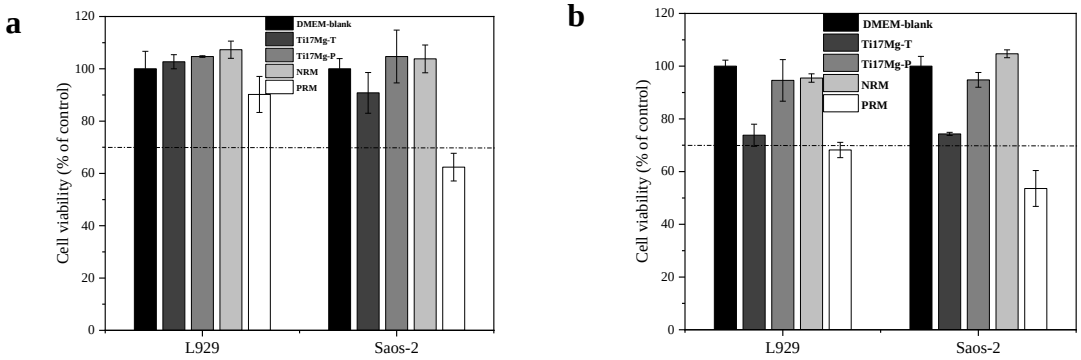
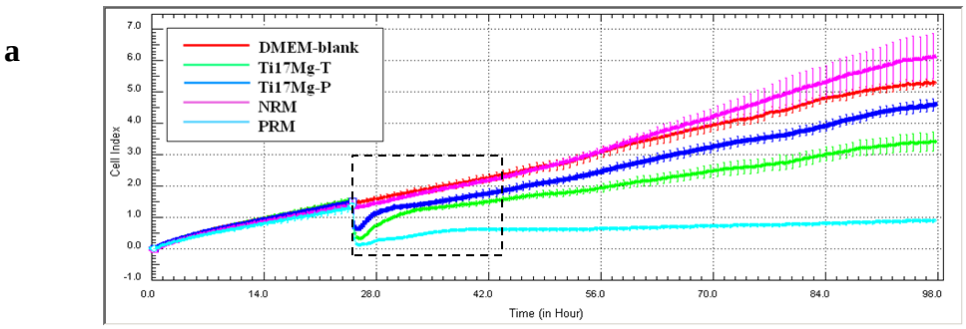
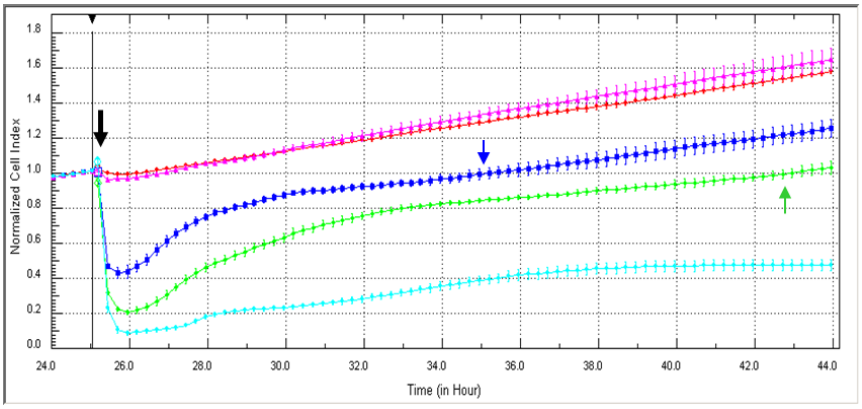


Fig. 20. *Cell viability of L929 and Saos-2 incubated with the extracts of the investigated specimens and controls for (a) 24 h and (b) 72 h evaluated by the MTT assay. The dashed line represents the cytotoxicity level (70%). The controls are labeled as NRM: Negative Reference Material, and PRM: Positive Reference Material.*

xCELLigence showed to be a highly accurate platform to monitor cell adherence and proliferation. Variation in the CI correlated with cell adhesion, spreading, and proliferation. The immediate and transient reduction in adhesion of the incubated Saos-2 cells induced by the addition of the Ti17Mg-T, Ti17Mg-P, and PRM (plain Mg) extracts was mainly related to the concentration of accumulative Mg in the extracts (**Fig. 21**). As the concentration of liberated Mg increased, the response was acute and the percentage of adhesion reduction raised. This also explains why the Ti17Mg-P had a proliferation or growth rate (difference in CI) higher than that of the Ti17Mg-T. This behavior was consistent with the results of the MTT cytotoxicity assay.



b



c

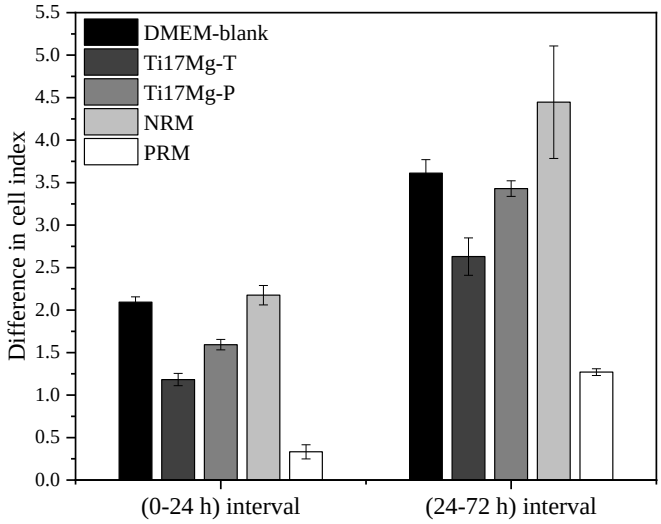


Fig. 21. *(a) Representative graph from the xCELLigence system that compares the proliferation rate of Saos-2 cells when incubated with the extracts of the Ti17Mg-T and Ti17Mg-P samples and the NRM, PRM, and DMEM-blank controls for 72 h. (b) The magnification of the dashed rectangle highlighted on the xCELLigence profile illustrates the Normalized Cell Index (CI) at the moment of the extracts addition. (c) Comparison of the difference in the mean CI of the incubated Saos-2 cells with the extracts of all studied samples for two intervals, (0–24 h) and (24–72 h). The controls are labeled as NRM: Negative Reference Material, PRM: Positive Reference Material.*

Pronounced differences in the number and area of FA can be detected for the cells that were treated with the Ti17Mg-T and Ti17Mg-P for 72 h (**Fig. 22**). The cells exposed to the Ti17Mg-P extract for 72 h had an almost similar number of FA and corresponding total area compared to those of the DMEM-blank control. This was rationalized by the superior metabolic activity, and cell growth and spreading. The preliminary in-vivo study of the Ti+Mg implant-like samples after 12 weeks revealed complete bone healing in the peri-implant region in the vicinity of threads, and no signs of gaseous enclosures or any undesired effects were determined (**Fig. 23** and **Table 5**).

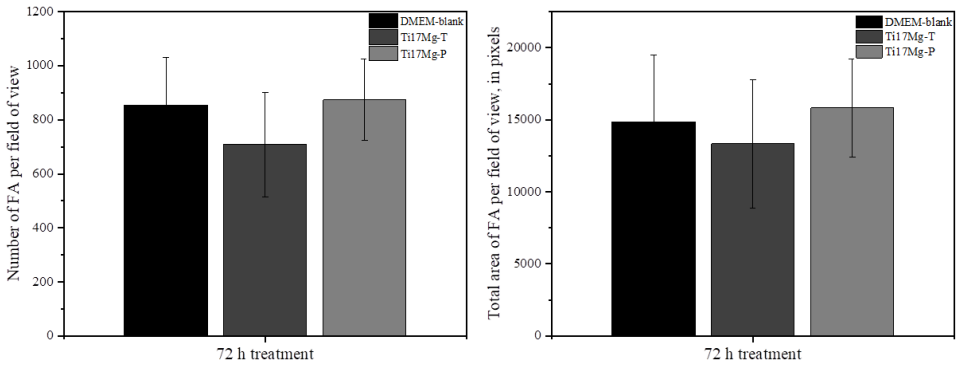


Fig. 22. *Number and corresponding total area of the focal adhesions (FA), monitored by the paxillin staining, for the Saos-2 cells incubated for 72 h with the extracts of the Ti17Mg-T, Ti17Mg-P, and DMEM-blank.*

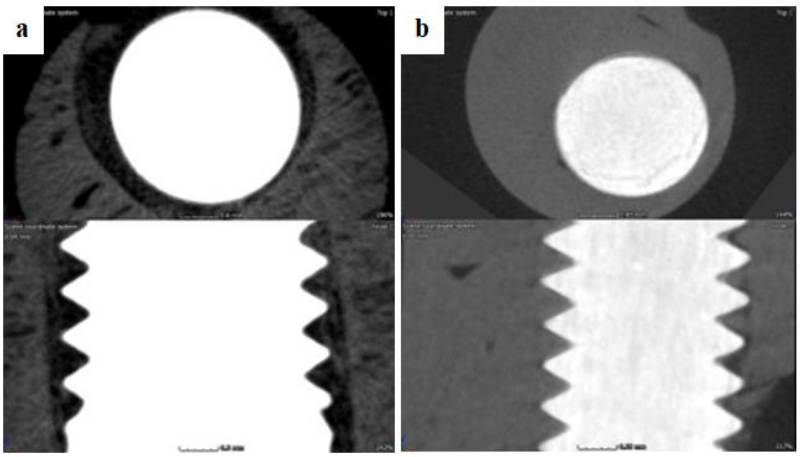


Fig. 22. *Micro-CT scans of the extracted Ti(HDH,C)+12 vol% Mg specimens after (a) 4 and (b) 12 weeks of implantation.*

Table 5 *Average values of the bone-implant contact (BIC) for the implanted Ti(HDH,C)+12 vol% Mg specimens at different time intervals.*

	BIC (%)		
Location	**4 weeks**	**8 weeks**	**12 weeks**
Cortical bone	59	89	98
Bone marrow	52	81	91

4. CONCLUSION

The Ti(PA)+17 vol% Mg composite reduces the drawbacks of the established Ti-based materials, and concurrently maintains excellent mechanical performance and fatigue endurance when compared to those of a pure Ti reference material. Its Ti matrix assures adequate mechanical characteristics, and the Mg component leads to a reduction in E and improves the surface bioactivity and thus enables a long-term fixation. The smooth and less strained surface of the Ti17Mg-P composite eliminates the need for pre-washing or further surface treatments and concurrently maintains the bioactive surface nature when compared with the pre-washed Ti17Mg composite or conventional Ti-based materials. The study confirmed that the Ti17Mg-P composite is an immensely promising material for applications in endosseous dental implants subjected to intense and cyclic loading.

PUBLICATIONS AND SCHOLARLY WORK

ARTICLES

1. M. Balog, **A.M.H. Ibrahim**, P. Krizik, O. Bajana, A. Klimova, A. Catic, Z. Schauperl, Bioactive Ti + Mg composites fabricated by powder metallurgy: the relation between the microstructure and mechanical properties, *Journal of Mechanical Behaviour of Biomedical Materials*, **90**(2019), pp. 45–53.
2. **Ahmed Mohamed Hassan Ibrahim**, Martin Balog, Peter Krizik, Frantisek Novy, Yuksel Cetin, Peter Svec Jr., Oto Bajana, Marian Drienovsky, Partially biodegradable Ti-based composites for biomedical applications subjected to intense and cyclic loading, *Journal of Alloys and Compounds*, **839**(2020), pp. 1–13.
3. Yuksel Cetin, **Ahmed Mohamed Hassan Ibrahim**, Aysen Gungor, Yasemin Yildizhan, Peter Krizik, Martin Balog, In-vitro evaluation of a partially biodegradable TiMg dental implant: The cytotoxicity, genotoxicity, and oxidative stress, *Materialia*, **14**(2020), pp.100899
4. **Ahmed Mohamed Hassan Ibrahim**, Martina Takacova, Lenka Jelenska, Lucia Csaderova, Martin Balog, Juraj Kopacek, Eliska Svastova, Peter Krizik, The effect of surface modification of TiMg composite on the in-vitro degradation response, cell survival, adhesion, and proliferation, *Materials Science and Engineering C*, submitted a revised version.

CONFERENCES

5. Martin Balog, Peter Krizik, Lubomir Orovcik, **Ahmed Mohamed Hassan Ibrahim**, Amir Catic, Zdravko Schauperl, Microstructural characterization and testing of BIOACTIVE Titanium-Magnesium Metal Composite For dental implants (BIACOM®), *International Conference DF PM 2017*, Oct. 22–25 in Stara Lesna, Slovak Republic. **Poster** presentation.
6. **Ibrahim Ahmed Mohamed Hassan**, Balog Martin, Krizik Peter, Catic Amir, Schauperl Zdravko, Optimization of Mg Content in Novel Bioactive Ti-Mg Composite Produced by Warm Powder Consolidation Process, *World Powder Metallurgy Congress*, Beijing, China, pp.1825–1831, 2018. **Full-length paper**.
7. Zdravko Schauperl, Amir Ćatić, Martin Balog, Peter Križik, **Ahmed Mohamed Hassan Ibrahim**, Innovative Ti-Mg composite for dental implants, *26th International Conference on Materials and Technology*, 3–5 October 2018, Portorož, Slovenia. **Oral** presentation.
8. Martin Balog, **Ahmed Mohamed Hassan Ibrahim**, Peter Krizik, Oto Bajana, Alena Klimova, Amir Catic, Zdravko Schauper, On the development of PM fabricated bioactive Ti + Mg composite (BIACOM®) with low Young`s modulus, *PMTi2019 Conference*, 24–27 September 2019, University of Utah, Salt Lake City, Utah USA. **Oral** presentation.
9. **Ahmed Mohamed Hassan Ibrahim**, Martina Takacova, Lenka Jelenska, Lucia Csaderova, Martin Balog, Juraj Kopacek, Eliska Svastova, Peter Krizik, TiMg composite with a mechanically modified surface: the in-vitro degradation response and cytocompatibility, *21th International Conference on Materials and Tribology MATRIB*, 30th June–2nd July 2021, Vela lake, Croatia. **Full-length paper**. (proceedings in progress).
10. Martin Balog, **Ahmed Mohamed Hassan Ibrahim**, Peter Krizik, Amir Catic, Zdravko Schauperl, Yuksel Cetin, HajrudinBesirovic, From the powder to implantation–the development of novel biomedicalTiMg composite(BIACOM©), *21th International Conference on Materials and Tribology MATRIB*, 30th June–2nd July 2021, Vela lake, Croatia. **Full-length paper**. (proceedings in progress).