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Microplasma-Sprayed Titanium and Hydroxyapatite Coatings on Ti6Al4V Alloy: *in vitro* Biocompatibility and Corrosion Resistance: Part II

Coatings enhance cell proliferation, corrosion resistance and implant integration

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Part II presents the results which show that HA coatings significantly enhance MSC proliferation by 13% compared to the titanium alloy base, while titanium coatings also exhibit an 11% increase. Porosity inversely affects CP-Ti's elasticity. Coatings with lower porosity demonstrate better corrosion resistance. HA coatings promote osteogenic activity and angiogenesis, which is crucial for implant integration.

Keywords

endoprosthesis implants; biocompatible coatings; porosity; surface roughness; *in vitro* test; elastic modulus

This work follows from Part I (1).

1. Results and Discussion

The surface roughness of the substrate and coatings, porosity and adhesive tensile strength of the coatings are presented in **Table I**.

3D laser scanning images of the surface of a titanium alloy substrate subjected to gas abrasive treatment and all three types of microplasma-sprayed coatings are presented for comparison in **Figure 1**.

In this study, the mean roughness (Sa) and root mean square roughness (RMS) (Sq) were assessed. RMS roughness offers a more thorough evaluation of surface roughness in contrast to Sa roughness. Substrate sample revealed the lowest roughness of 4.6 ± 0.1 µm (Sa) and 5.84 ± 0.14 µm (Sq), respectively, whereas sprayed coatings revealed a significant increase in roughness (2–7 times) **(Figure 1(a)–1(d))**. Sa and Sq revealed a similar trend in roughness. Titanium spraying led to an increase in roughness and the highest value for CP-Ti coating (Group 2), was achieved (27.6 ± 2.6 µm (Sa) and 34.4 ± 3.5 µm (Sq)). HA coating revealed roughness higher than reported usually in the literature (4–8 µm) (2–4). At the same time, Gross and Babovic reported roughness of HA coatings up to 24 µm (5). Borsari *et al.* explored the impact of roughness on cell proliferation, affirming that surface morphology significantly influences cell behaviour, with excessively high roughness (over 40 µm) resulting in decreased cell proliferation (6).

SEM images of the surface of the substrate and coatings and cross sections of coatings on the substrate are shown in **Figure 2** and **Figure 3**.

As can be seen in **Figure 2** and **Table I**, CP-Ti coatings have different surface roughness and porosity and at the same time, satisfactory adhesion to the substrate, which was achieved by correct selection of microplasma spraying parameters. Thus, as planned, it was possible to obtain two groups of CP-Ti coatings with significantly (3.85 times) different porosity, while, as expected, the more porous coating (Group 2) has a 2.5 times rougher surface. Since a porous coating with high surface roughness can be formed due to large solid heated particles moving towards the substrate at low speed and, accordingly, not being greatly deformed when hitting the substrate, while the size of the sprayed particles and the degree of their heating in the plasma jet can be varied by the microplasma spraying parameters, therefore the coating morphology was controlled by microplasma spraying parameters such as electric current,

Table I Characteristics of Composition of Titanium and Hydroxyapatite Coatings and Titanium Alloy Substrate

Characteristics	Materials			
	CP-Ti coating, Group 1	**CP-Ti coating, Group 2**	**HA coating**	**Ti6Al4V alloy after gas abrasive treatment**
Porosity	Porosity up to 6.5% and pore sizes up to 20 μm	Porosity up to 25.0% and pore sizes up to 300 μm	Porosity up to 10% and pore sizes up to 50 μm	N/A
Mean surface roughness (Sa), μm	11.5 ± 1.0	27.6 ± 2.6	9.8 ± 0.5	4.6 ± 0.1
Root mean square roughness (Sq), μm	14.8 ± 1.2	34.4 ± 3.5	12.7 ± 0.4	5.8 ± 0.1
Mean static tensile strength of the coating, MPa	35.1 ± 2.9	27.6 ± 0.9	16.6 ± 1.4	N/A
Modulus of elasticity of the coating in the tensile zone (E_T), GPa	20.2 ± 1.8	12.8 ± 1.0	Not measured	N/A
Modulus of elasticity of the coating in the compression zone (E_c), GPa	47.9 ± 0.5	19.5 ± 1.5	Not measured	N/A

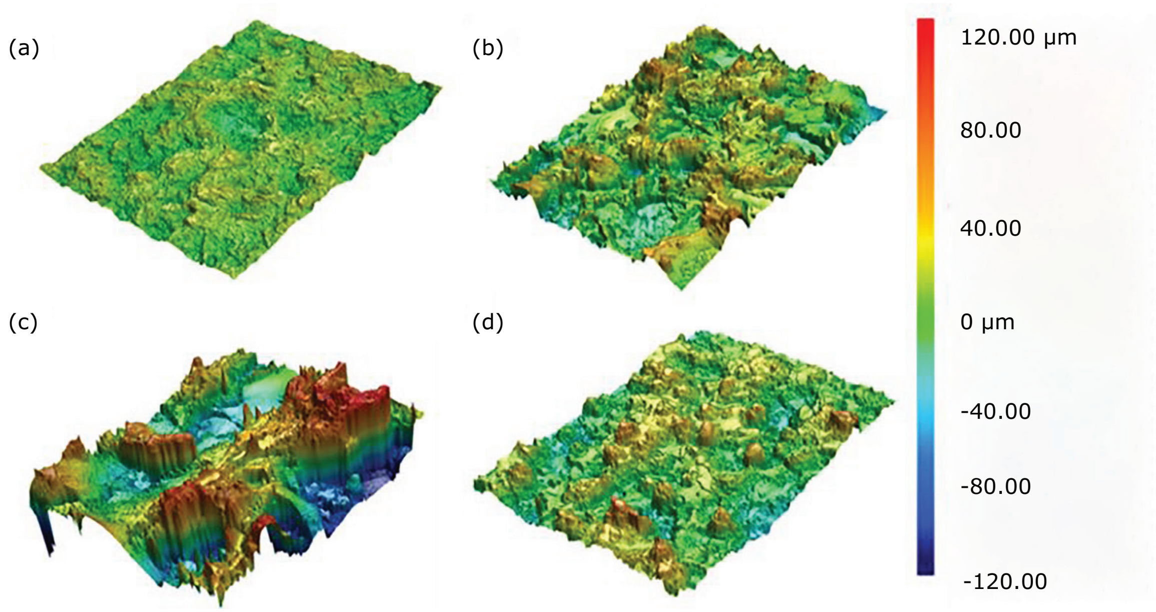

Fig. 1. 3D laser scanning of: (a) the surface of titanium alloy substrate and microplasma-sprayed coatings: (b) CP-Ti of Group 1; (c) CP-Ti of Group 2; (d) HA

plasma gas flow rate, spraying distance and wire or powder feed rate. As was shown in previous articles, the maximum surface roughness of the coatings (more than 50 μm) corresponded to a combination of the variable microplasma spraying parameters minimum values, at which the formation of thick (about 100 μm) slightly deformed splats occurs, which makes it possible to obtain porous coatings with large pores. In the articles, high-speed small and completely molten particles can form a dense coating with relatively low surface roughness (7–10).

The adhesion to the substrate is higher for dense CP-Ti coatings (35.1 ± 2.9 MPa) compared to

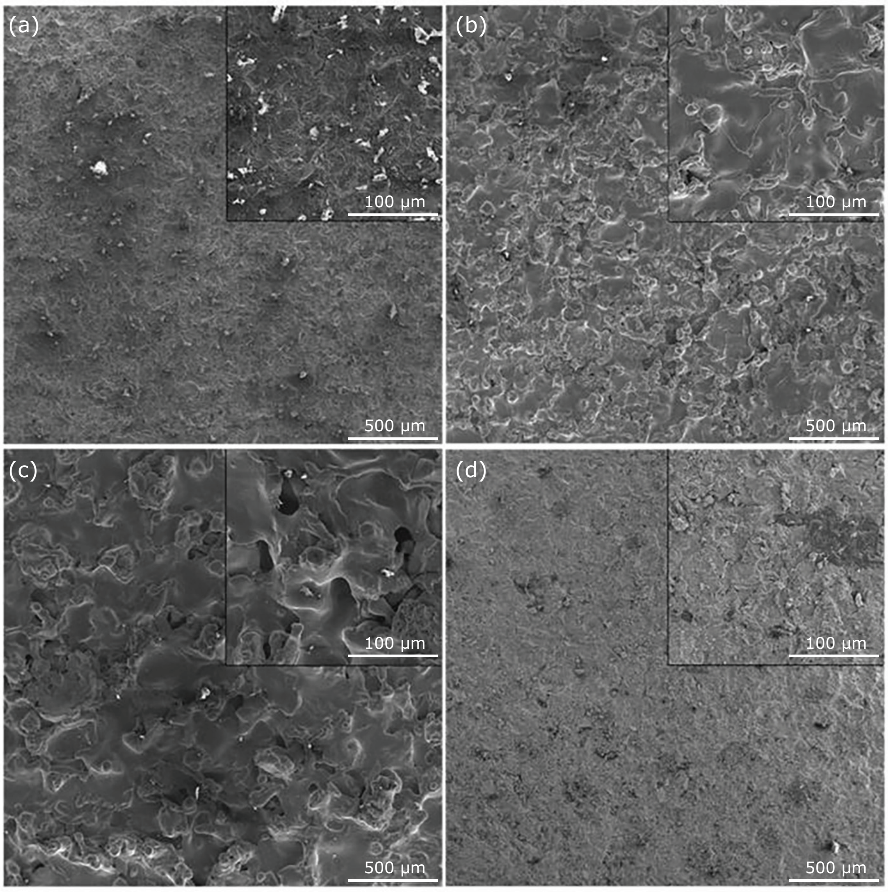

Fig. 2. SEM images of: (a) the surface of titanium alloy substrate after gas abrasive treatment and microplasma-sprayed coatings: (b) CP-Ti of Group 1; (c) CP-Ti of Group 2 ; (d) HA

porous ones (27.6 ± 0.88 MPa). However, in both groups of CP-Ti coatings, the mean static tensile strength meets the ISO 13179-1 requirements for plasma-sprayed coatings derived from titanium (not worse than 22 MPa) (11). Notably, the mean static tensile strength of HA coatings is 16.6 ± 1.39 (**Table I**), which meets the recommended minimum of 15 MPa, under the ISO 13779-2 standard (12). Meanwhile, the porosity of the microplasma-sprayed HA-coating is up to 10%, with pore sizes up to 50 µm (**Table I**). Consequently, the possibility of microplasma spraying porous HA coatings with satisfactory adhesion to a titanium alloy substrate has been confirmed, as previously shown (7–10).

It should be noted that all microplasma-sprayed coatings, as well as the substrate after gas abrasive treatment, have surface roughness (Sa) ranging from 4.6 µm to 26.6 µm (**Table I**), which are noted as promoting good cell proliferation and viability. In particular, for orthopaedic titanium implants, the average surface roughness (Ra) recommended by researchers is in a wide range from 0.07 µm to 100 µm However, there have been no systematic studies on the effect of surface roughness on biocompatibility (13, 14).

The surface roughness is to some extent related to the choice of the coating thickness (or its layers thickness), because the coating must cover the highest protrusions on the surface of the substrate (or on the underlying layer surface for the multilayer coating), ensuring the coating consistency. Therefore, a dense CP-Ti coating on the surface of a titanium alloy after gas abrasive treatment can have a thickness of about 100–120 µm (**Figure 3(a)**) and the roughness of a dense CP-Ti coating is significantly less than that of a porous CP-Ti coating (**Figure 1**). The main criteria for choosing the biocompatible coating thickness are its desired porosity and the subsequent possibility to increase the surface area available for bone attachment with increasing coating thickness. The thermal plasma sprayed porous titanium coating with pore sizes up to 300 µm, which corresponds to the optimal pore size range for bone ingrowth (**Table I**) in Part I of this review (1), should be sufficiently thick, with a thickness of about 250 µm (**Figure 3(b)**). For example, Canabarro *et al.* investigated thermally sintered powder titanium discs with porous titanium coating of various thicknesses (0.5 mm, 1.0 mm and 1.5 mm) and found that the largest thickness of 1.5 mm provided the best *in vitro* osteoblastic

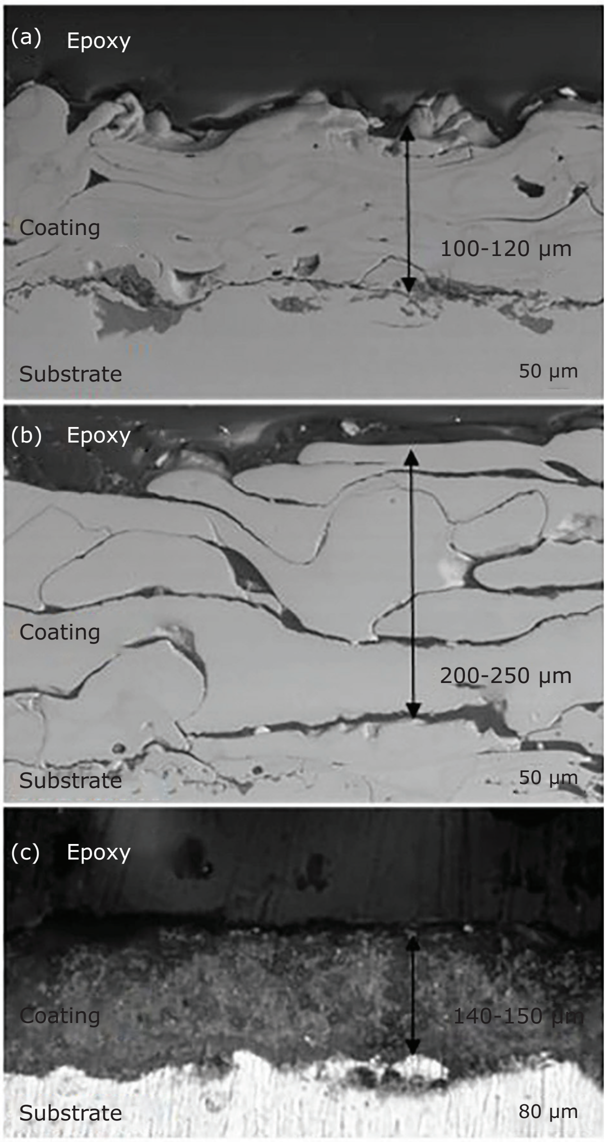

Fig. 3. SEM images of the cross-sections microplasma-sprayed coatings: (a) CP-Ti of Group 1; (b) CP-Ti of Group 2; and (c) HA

cell response in terms of proliferation, osteoblast phenotype expression and extracellular matrix mineralisation (15).

Recently, the issue of the influence of coating thickness on the biological response of the coated implant has been very relevant (16, 17) and studies of coating bioactivity demonstrate that thicker coatings might have more pronounced bioactive properties (15, 16). However, Nuswantoro *et al.* studied the effect of electrophoretic-deposited HA coating thickness on the inflammation and osseointegration properties of titanium orthopaedic implants. They concluded that, compared to the thin (50–70 μm) and thick (90–110 μm) HA coatings, the medium (70–90 μm) thickness coating provided the lowest inflammation levels, relatively high osseointegration rates and optimal new bone tissue growth (17). However, for a microplasma-sprayed HA coating on a titanium alloy after gas abrasive treatment, it was necessary to choose a thickness higher than the optimal range (70–90 μm) recommended in the paper (11) to ensure the consistency of the coating. Therefore, in this study, the average thickness of HA coatings was chosen to be about 140 μm (**Figure 3(c)**).

It is important to note that varying the porosity of the coatings by selecting the microplasma spraying parameters made it possible to change the elastic modulus of the CP-Ti coatings accordingly. According to literature data (18, 19), the elastic modulus of titanium and its alloys is in the range of 100–110 GPa, which is significantly higher than the elastic modulus of cortical bone tissue (10–30 GPa), but substantially less than the elastic modulus of stainless steel (210 GPa). Measurement of the elastic modulus of CP-Ti coatings of Group 2 with a volume porosity of 25.0% showed that in the compression zone its value decreases to 19.5 GPa and in the tensile zone it decreases to 12.8 GPa, which provides greater similarity with the elastic modulus of bone, which can vary in the range from 5 GPa to 30 GPa (20) than that of CP-Ti coatings of Group 1 with a porosity of 6.5%, which has an elastic modulus of 47.9 GPa (**Table I**). Similar elastic modulus values for porous titanium were established from the results of acoustic measurements in the paper (21). It should be noted here that the nanoindentation method cannot be used to measure the elastic modulus of porous CP-Ti coating with large pores (up to 300 μm). It is more likely that the elastic modulus of CP-Ti decreases so sharply due to the high porosity (up to 25%) of the CP-Ti coating rather than due to any other characteristics.

Thus, it can be assumed that it is possible to reduce the likelihood of the occurrence of the stress shielding effect in the implant coating bone system not only by changing the material from which the implant itself is made but also by forming the necessary volumetric porosity in the coating layer on its surface. Moreover, this study indicates specific microplasma spraying parameters (Table IV) in Part I (1), which ensure the formation of a coating with increased porosity and reduced elastic modulus. Based on the data analysis in **Table I**, it is clear that the elastic modulus of CP-Ti coatings in the tension zone (E_T) is significantly less than the corresponding value in the compression zone (E_c) at the same volumetric porosity content. It should also be noted that with an increase in the volumetric porosity, the difference between the elastic modulus in the tension and compression zones decreases. Thus, with a volume porosity of 25%, the elastic modulus of CP-Ti coatings of Group 2 in the tensile zone is 65.4% of the corresponding characteristic of the same coating in the compression zone. The minimum E_c of 12.8 GPa for CP-Ti coatings is quite close to the values given in the literature for plasma coatings made of titanium powder with volumetric porosity (15.4 ± 0.8)%, for which the modulus of elasticity in the tensile zone was 11.6 GPa (22). This indicates that microplasma spraying CP-Ti coatings with porous structures make it possible to reduce the elastic modulus of the main implant material on the surface by almost ten times.

The corrosion potential is the potential of a corroding surface in an electrolyte, which is related to a reference electrode. It is determined either from the plateau in the potential transient when the working electrode is not polarised or from Tafel extrapolation of the anodic and cathodic curves in the potentiodynamic polarisation curves. The current density at the corrosion potential is likewise calculated from potentiodynamic diagrams and is directly related to the corrosion rate. The higher the E_{corr} and the lower the i_{corr}, the better the corrosion performance of the material (23). Polarisation curves of the samples are shown in **Figure 4**.

As can be observed in **Figure 4**, the lowest E_{corr} and highest i_{corr} values were measured in the Ti6Al4V sample. This also means that Ti6Al4V has the lowest corrosion resistance. On the other hand, the i_{cor} value decreased and the E_{corr} value increased in porous CP-Ti (Group 2) and HA coated specimens compared to Ti6Al4V. However, the highest corrosion resistance was obtained in the dense CP-Ti coating (Group 1). The passivation of unalloyed titanium based materials is given by a two layer oxide layer consisting of titanium dioxide. In this oxide film structure comprising an inner and outer layer, corrosion resistance is mainly provided by the inner layer. However, the oxide structure of Ti6Al4V consists of alumina and vanadia in addition to titania. Under the aggressive body fluid, alumina and vanadia dissolve and disrupt the passivity of Ti6Al4V (24, 25). Therefore, the corrosion performance of Ti6Al4V is lower than that of unalloyed CP-Ti. On the other hand, HA with a ceramic structure is expected to improve the corrosion resistance of Ti6Al4V. This is because ceramic materials are more resistant to corrosion than metallic materials.

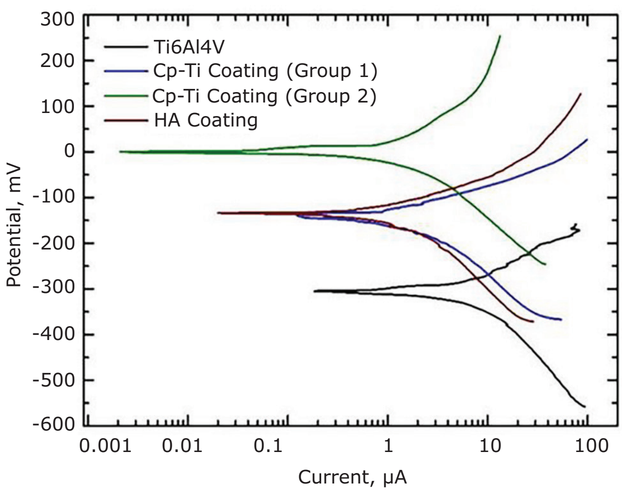

Fig. 4. Tafel extrapolation graph of the specimens of coatings and substrate

For example, Niespodziana *et al.* (26) produced Ti–HA biocomposites by powder metallurgy and investigated the effect of HA on the corrosion behaviour of unalloyed titanium. In this study, an increase in the corrosion performance of the material was observed with increasing HA content. In a similar study, Gnanavel *et al.* (27) deposited HA on Ti6Al4V using a pulsed laser deposition technique. Corrosion examinations were performed on coated and uncoated samples using potentiodynamic polarisation experiments in simulated bodily fluid (Hanks solution). Similarly, it was observed that the HA coating increased the corrosion resistance of the Ti6Al4V substrate.

As mentioned above, the highest corrosion resistance was attained with a dense CP-Ti (Group 1) coating on a Ti6Al4V substrate. The corrosion performance of the material increased with decreasing porosity in the coating. Pores are natural defects that weaken the material mechanically and electrochemically, causing a decrease in corrosion resistance due to the larger surface area of the area exposed to the electrolyte in porous materials compared with non-porous materials (28). In this sense, it is feasible to claim that the corrosion results are consistent with the literature.

The cytotoxicity of various implant groups was evaluated using the LDH assay, a widely recognised method for detecting cellular damage and assessing cell viability. The LDH assay measures the release of LDH enzyme into the cell culture medium, which occurs when cell membranes are compromised, typically indicating cytotoxicity. The results of this assay were particularly noteworthy.

All groups of implants, which presumably included different materials or treatments, demonstrated no cytotoxic effects on rat MSCs (**Figure 5**).

MSCs are a type of multipotent stem cell capable of differentiating into a variety of cell types, making them a crucial element in tissue regeneration and repair studies. Therefore, their survival and health in the presence of the implants are vital indicators of the biocompatibility of the implant materials. Moreover, when the results from these various implant groups were statistically compared to those obtained from the commercially available titanium alloy, a standard in the field of implant materials, no significant difference was observed. This lack of statistical difference in cytotoxicity levels suggests that the new implant materials or treatments are as biocompatible as the conventional Ti6Al4V alloy after gas abrasive treatment.

The study employed the CCK-8 assay to evaluate cell proliferation, a critical factor in assessing the biocompatibility and effectiveness of various coatings applied to implants. This assay is a sensitive and reliable method for quantifying cell viability and proliferation, based on the detection of cellular metabolic activity. The coatings under investigation included HA-coating, CP-Ti coating (Group 1) and CP-Ti coating (Group 2). Each of these coatings showed a remarkable performance in the assay (**Figure 6**).

The results indicated a statistically significant increase in the proliferation rate of approximately 32% for cells exposed to these coatings compared to the control group, which did not have any implant extracts. This finding is substantial as it suggests that the HA and both CP-Ti (Group 1 and Group 2) coatings create an environment conducive to cell growth, which is a crucial aspect of successful implant integration. In contrast, when the proliferation rate was assessed for cells exposed to Ti6Al4V alloy after gas abrasive treatment (**Figure 6**), a widely used material in implants, the increase in proliferation was somewhat lower, at 23%, but still statistically significant compared to the control group. This indicates that while the Ti6Al4V alloy is effective in supporting cell proliferation, the new coatings from CP-Ti and HA, are potentially more effective. Furthermore, a direct comparison between the microplasma spraying coatings and their substrate from Ti6Al4V alloy after gas abrasive treatment revealed insightful differences. Both HA and CP-Ti (Group 1 and Group 2) coatings showed a statistically significant increase in cell proliferation by 13% and 11% respectively, compared to the Ti6Al4V alloy after gas abrasive treatment.

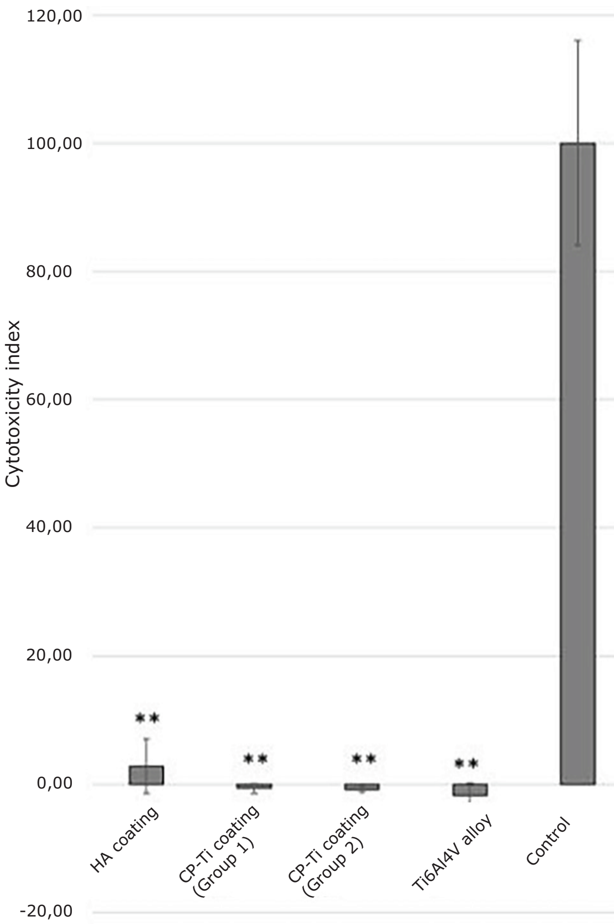

Fig. 5. LDH cytotoxicity assay. MSCs were cultured in implant-enriched media (3-day extract) for 24 h. CyQUANT™ LDH Cytotoxicity Assay (C20300, Thermo Fisher) was used to evaluate the cytotoxicity. ** - p value ≤ 0.005 compared to the positive control

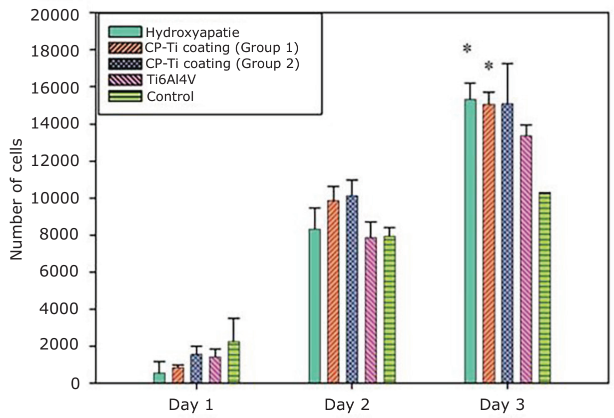

Fig. 6. Proliferation assay with CCK-8. MSCs were cultured in implant-enriched media (3-day extract) for 72 h. CCK-8 (96992, Sigma Aldrich) was used to evaluate the number of proliferated cells. ** - p value≤ 0.005, * - p value≤ 0.05

This comparison is critical as it benchmarks the new coatings against a well-established standard in the field, providing a clear indication of their superior performance in supporting cell growth. These results are significant in the context of implantology and regenerative medicine. The ability of a material to support cell proliferation is a key factor in its suitability for use in implants, as it directly impacts the healing process and the integration of the implant with the host tissue. The findings suggest that the HA and both groups of CP-Ti coatings not only meet but exceed the performance of Ti6Al4V alloy after gas abrasive treatment in this regard. This implies a potential for these microplasma-sprayed coatings to improve the outcomes of implant surgeries, offering better integration with the host tissue and promoting faster healing and recovery.

Early osteogenesis, which is a critical phase in bone formation and healing, was evaluated using the alkaline phosphatase (ALP) assay. The ALP assay is a widely accepted method for measuring the activity of ALP, an enzyme that is indicative of osteoblastic activity and bone formation. Higher levels of ALP activity are generally associated with increased osteogenesis. Among the various groups tested, the one that received HA coatings exhibited notable results (**Figure 7**).

This group showed a statistically significant increase in ALP activity by approximately 5% compared to the control group, which received no treatment (**Figure 7**). This increment, albeit modest, is significant in the context of bone healing and regeneration. It indicates that the HA coating has a positive impact on early osteogenesis, enhancing the activity of osteoblasts, which are bone forming cells. The importance of this finding lies in the potential of HA as a biomaterial for improving bone healing and regeneration.

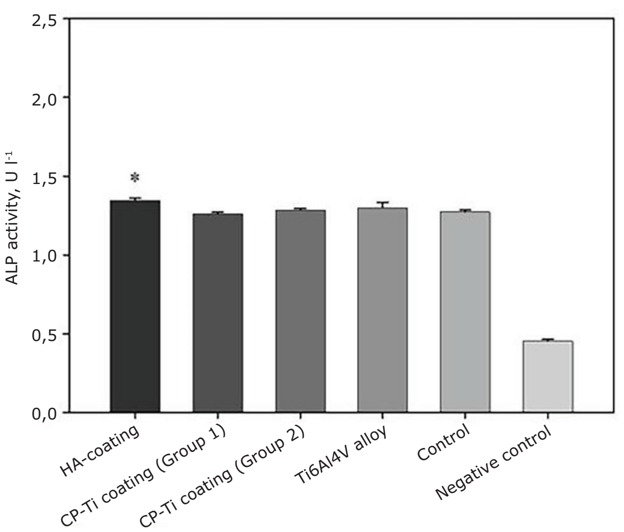

Fig. 7. ALP assay. MSCs were cultured in implant-enriched osteogenic media (3-day extract) for two weeks. ALP Assay Kit (ab83369, Abcam) was used to assess the osteogenic differentiation. * - p value ≤ 0.05 compared to the control group

HA is known for its biocompatibility and similarity to natural bone minerals, making it a popular choice for orthopaedic and dental applications. The observed increase in ALP activity in the HA group suggests that this material could provide an environment that is more conducive to bone formation at the early stages of osteogenesis compared to untreated controls. This increase in ALP activity is particularly relevant in the field of implantology and bone tissue engineering, where early and effective osteogenesis is crucial for the success of implants and bone grafts. The results imply that HA coatings might enhance the integration of implants with the surrounding bone tissue, potentially leading to improved outcomes in bone repair and regeneration procedures. However, the increase in ALP activity was specific to the HA group and was relatively modest. This underscores the need for further research to optimise the properties of HA and explore its full potential in promoting osteogenesis. Future studies might focus on comparing HA with other biomaterials, testing different formulations or modifications of HA and investigating the long-term effects of HA on bone healing and integration.

The assessment of prolonged osteogenic differentiation was conducted using the Alizarin Red S Assay across various groups. This assay is a well-established method for detecting calcium deposits, which are indicative of osteogenesis. The results of this assay were quite revealing (**Figure 8**). Each group of coating showed a statistically significant increase in osteogenic differentiation, estimated at around 30%, when compared to the control group that received no treatment, referred to as 'Osteo' (**Figure 8**). This finding is significant as it highlights the effectiveness of the coatings being tested in promoting bone growth and development. The control group, 'Osteo', with no implant extract, served as a baseline to measure the efficacy of the treatments. The approximately 30% increase observed in the other groups suggests a robust enhancement in osteogenic activity due to the treatments.

The angiogenesis assay was aimed at determining the effect of different implant coatings on the

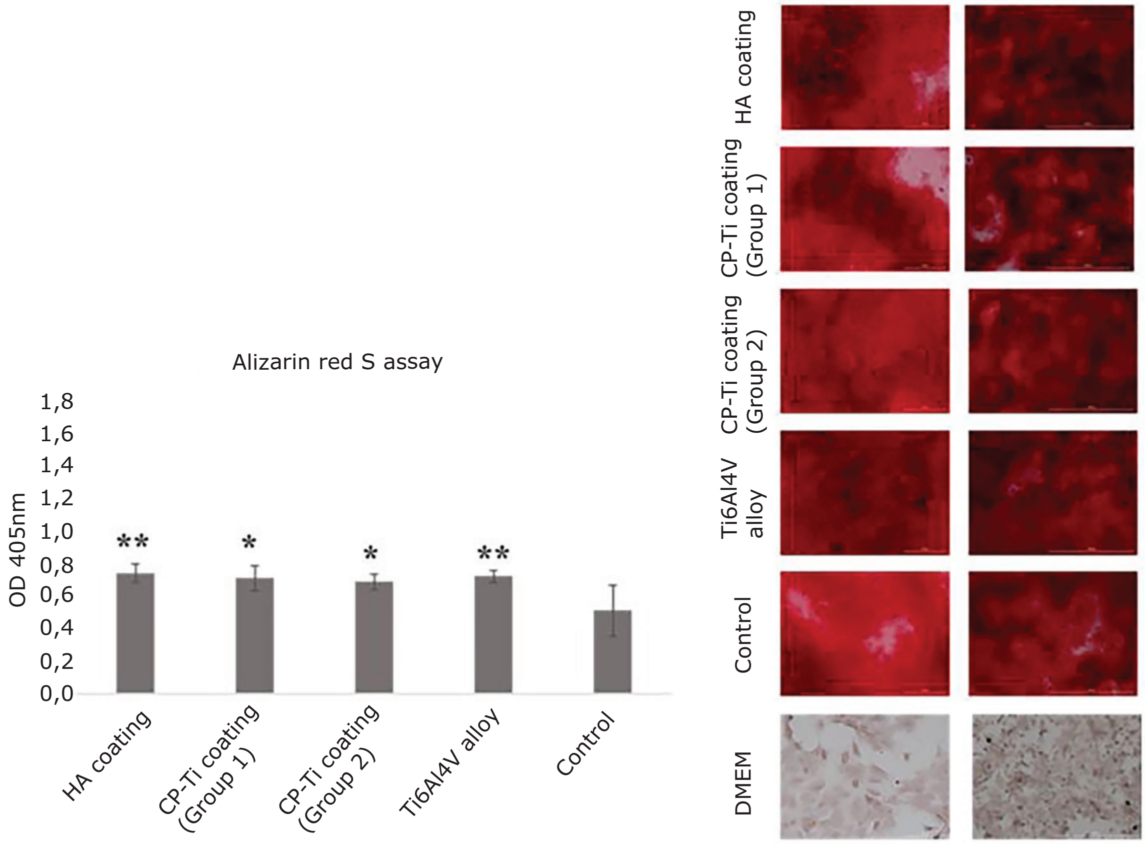

Fig. 8. Alizarin Red S Assay. MSCs were cultured in implant-enriched osteogenic media (3-day extract) for 5 weeks. Alizarin Red S (A5533, Sigma Aldrich) was used to assess the osteogenic differentiation. ** - p value ≤ 0.005, * - p value ≤ 0.05 compared to the control group (Osteo)

behaviour of HUVEC and their ability to form vessels. This ability was assessed by comparing commonly tested parameters such as the number and lengths of branches, segments, junctions, master junctions, master segments and the branching interval of the tubular structures formed by the HUVECs cultured in different implant enriched media and a standard non-treated medium. The results of the angiogenesis assay are presented in **Figure 9**. The findings revealed that some branches had risen for both HA coating and CP-Ti coating (Group 1), with 1.9 and 2-fold increases, respectively, although the former result was not as significant. CP-Ti coating (Group 1) showed a greater enhancement, which might suggest that this coating offers a more conducive surface (**Figure 9**). Similarly, media enriched with HA coating and CP-Ti coating (Group 1) increased the number of segments (3.3 and 3.8-fold increases, respectively), number of master segments (3.7 and 4.1-fold increases) and the total master segment length (3.6 and 2.6-fold increase, respectively) when compared to the Ti6Al4V alloy group. The number of segments is the number of individual sections delimited by two junctions, master segments are primary segments or major vessels in the network from which smaller branches or secondary segments emerge. The increase in the number of master segments indicates a more complex and hierarchical vascular network, important for the stability and functionality of newly formed vessels. It suggests that the HA coating and CP-Ti coating (Group 1) coatings not only promote the quantity but also the quality of the vascular structures. Hence providing a strong framework for the overall vessel network. The total master segment length suggests the formation of longer and potentially more durable vessel like structures.

The HA coating and CP-Ti coating (Group 1) exhibited notable elevation in other parameters. Specifically, the number of meshes (4.5 and 5.3-fold increases for HA and titanium fine, respectively), the number of junctions (2.7 and 3-fold increases) and the number of master junctions (3.4-fold and 3.5-fold increases) were significantly higher when compared to the Ti6Al4V alloy group (**Figure 9**). The increase in the number of meshes implies that the network formed is not only abundant but also intricately connected. Another factor that

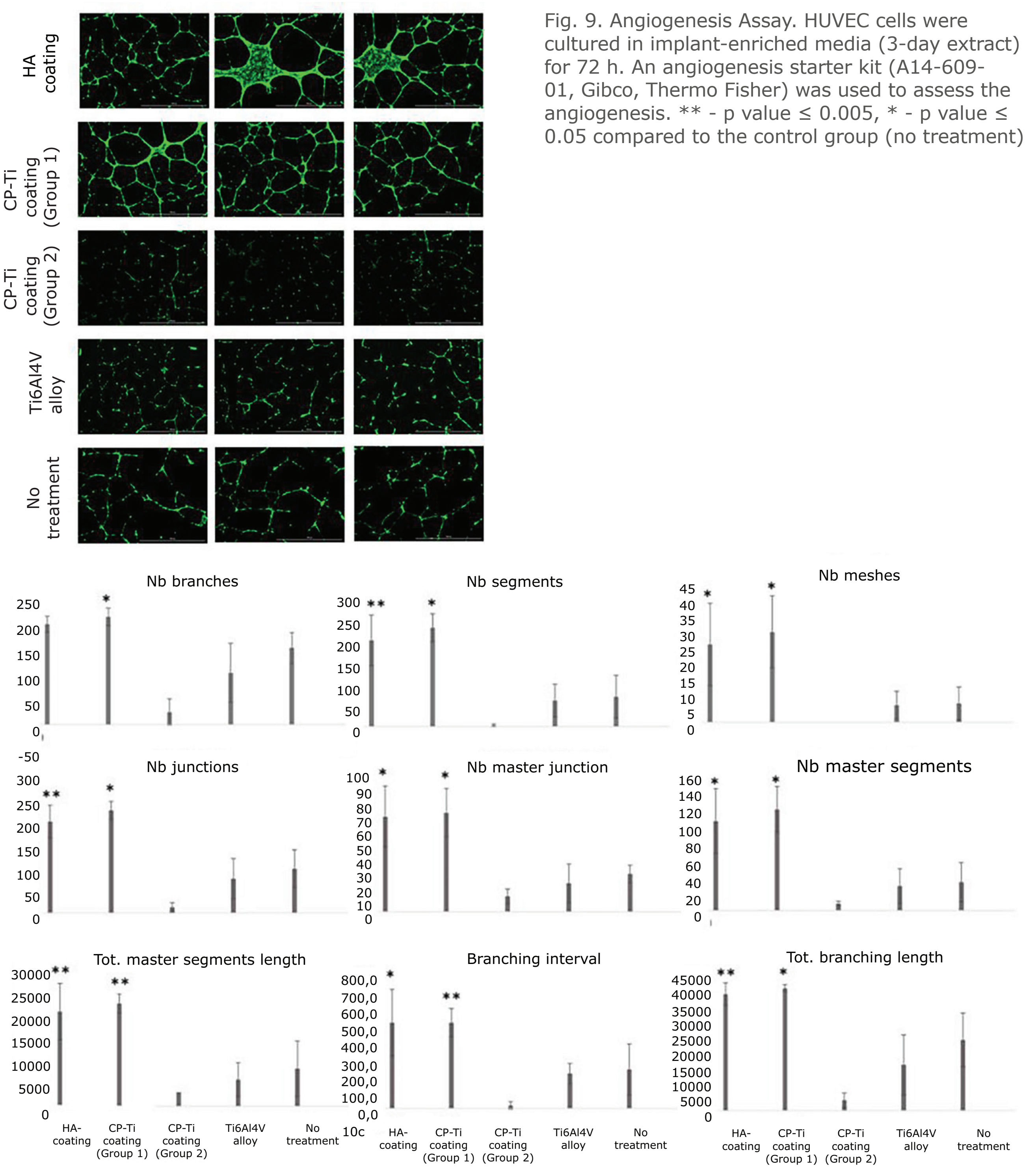

Fig. 9. Angiogenesis Assay. HUVEC cells were cultured in implant-enriched media (3-day extract) for 72 h. An angiogenesis starter kit (A14-609-01, Gibco, Thermo Fisher) was used to assess the angiogenesis. ** - p value ≤ 0.005, * - p value ≤ 0.05 compared to the control group (no treatment)

illustrates the complexity of the formed networks is the number of junctions, which indicates where the vessels branch or merge. The rise in the number of master junctions demonstrates more stable and functional vessel networks. Cells in HA coating and CP-Ti coating (Group 1) had shown a 2.6-fold and 2.7-fold increase, respectively, in the total branching length, while branching intervals for both HA-coating and CP-Ti coating (Group 1) were almost identical, with both groups showing a 2.5-fold increase compared to Ti6Al4V alloy. The branching interval represents the distance between two branches, while the total branching lengths in HA coating and CP-Ti coating (Group 1) underscore the ability of the vessels to develop extensive and complex networks.

Thus, the most prominent and statistically significant difference was observed in groups of HA and CP-Ti (Group 1) compared to both the no-treatment group and Ti6Al4V alloy after gas abrasive treatment.

During three days of *in vitro* testing, significant increases in cell proliferation rates were observed for the HA coatings (13%) and for both CP-Ti coatings (11%) compared to the Ti6Al4V alloy substrate (**Figure 6**). These differences were statistically significant. Thus, all coatings increased the biocompatibility of the titanium substrate, with HA coatings appearing to have the most significant potential for improving the biocompatibility of the titanium implant (see **Figure 9**). Thus, the *in vitro* test in this experiment did not allow us to establish the patterns of the influence of porosity and roughness of CP-Ti coatings on the proliferation and viability of the MSC however, an increase in the porosity of a CP-Ti coating significantly affects the decrease in its elasticity modulus, as shown earlier (29).

The interest in how cells attach to bone grafts remains high, with SEM emerging as the premier technique for visualising cell adhesion on various materials. Studies have shown how bone-like cells interact with titanium surfaces, recognising and adapting to their 3D shape due to a versatile cytoskeleton. Changes influence this adaptation in surface texture, which affects cell adhesion strength and leads to variations in critical cellular processes, including proliferation, migration and differentiation. Through SEM analysis, it was observed that after two days in culture, MSCs exhibited adherence within the pores of the discs, displaying a spindle-shaped fibroblast-like morphology characteristic of MSCs, as indicated by arrows in **Figure 10** for CP-Ti coating (Group 1) and in **Figure 11** CP-Ti (Group 2).

Therefore, for the formation of biocompatible coatings from CP-Ti, those microplasma spraying parameters that provide maximum porosity of the coating are preferable. Considering the results from measuring the adhesive strength (Table V)

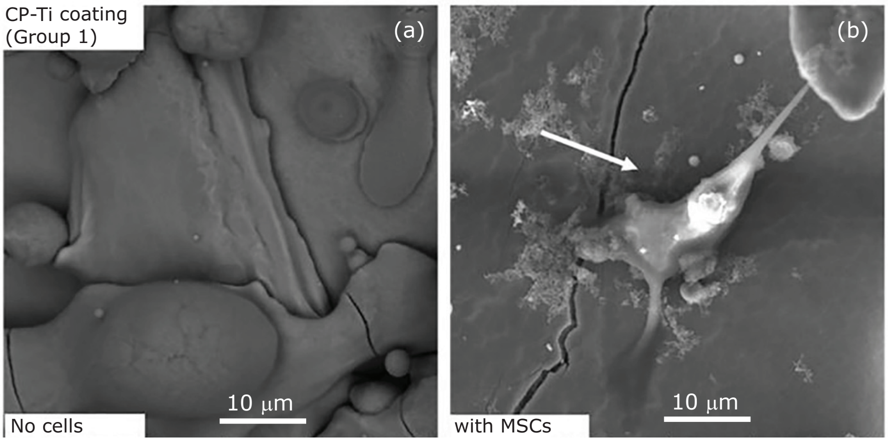

Fig. 10. SEM image of: (a) CP-Ti coating (Group 1) without cells; (b) with MSCs cultured for 48 h

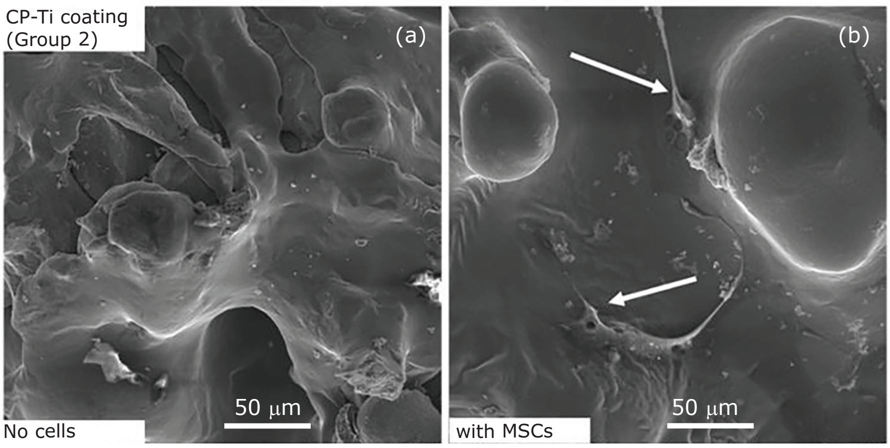

Fig. 11. SEM image of: (a) CP-Ti coating (Group 2) without cells; (b) with MSCs cultured for 48 h

and the corrosion characteristics of the coatings, it is feasible to recommend the microplasma spraying parameters listed in Table IV for applying a three-layer coating to titanium alloy implants. This proposed coating structure includes a lower layer of dense CP-Ti (Group 1), an intermediate layer of porous CP-Ti (Group 2) and a top layer of HA.

In this case, a dense lower titanium layer could provide reliable corrosion protection and good adhesion to the substrate. An intermediate porous titanium layer could decrease the elastic modulus and a top HA layer could increase the biocompatibility of the surface of such a three-layer coating. In addition, the HA coating would not close the large pores on the surface of the intermediate titanium coating due to the smaller size of the HA particles forming the top layer compared to the pore size of the intermediate coating. Currently, thanks to the use of robotic microplasma spraying, specifically an intelligent robotic system for microplasma surface processing, presented in detail in the conference paper (30), it has become possible to microplasma spray multilayer coatings on surfaces of very complex shapes with precise adherence to such spraying parameters as the spraying distance, the perpendicularity of the incidence of the plasma jet onto the substrate and the constant linear speed of the microplasmatron movement along the substrate's surface. The above advantages were used in the development of a method for robotic microplasma spraying of three-layer coatings (dense CP-Ti bottom layer porous CP-Ti middle layer and HA top layer) on titanium implants, protected by a patent for utility model of the Republic of Kazakhstan (31). However, the biocompatibility and corrosion resistance of such three-layer coatings have not been tested and no choice was made of the optimal average thickness of each layer.

Based on the analysis of the characteristics of all three types of coatings, it is proposed to form a three-layer coating on a titanium alloy substrate by microplasma spraying of a dense CP-Ti layer 100 μm thick, an intermediate porous CP-Ti layer 250 μm thick, and an upper HA layer 140 μm thick with the microplasma spraying parameters indicated in Table IV in Part I (1) to ensure good adhesion of the coating to the substrate and between layers, good corrosion resistance to human body fluids, reduced modulus of elasticity of the coating compared to the substrate alloy and increased biocompatibility of the surface for rapid implant ingrowth.

In general, the parameters of microplasma-sprayed coatings indicated in Table IV in Part I (1) as well as the results of measurements of a number of coating characteristics presented in **Table I** are of practical value both for specialists in the production of coated implants and for researchers in the processes of TPS of coatings. The microplasma spraying parameters indicated in Table IV in Part I (1) can be recommended for specific materials, with the confidence that these parameters will provide the porosity and roughness values indicated in **Table I**, as well as the biocompatibility and corrosion resistance of coatings, higher than that of an uncoated Ti6Al4V alloy.

2. Future Research Perspectives

In the future, it seems promising to conduct a systematic study of the influence of porosity and surface roughness on the biocompatibility and corrosion resistance of microplasma-sprayed CP-Ti coatings, as well as to obtain a three-layer coating: a lower dense CP-Ti layer on the substrate, a middle porous CP-Ti layer and an upper HA layer, as described above, and study its characteristics to test the hypothesis of increased biocompatibility and corrosion resistance properties of a three-layer coating compared to single-layer coatings of these materials. As previously shown (8), the average static tensile strength of HA coatings on a porous CP-Ti microplasma-sprayed sublayer is 24.2 ± 0.85 MPa, which is 1.6 times higher than the minimum 15 MPa recommended by ISO 13779-2 standard in implants for surgery for thermally sprayed coatings of HA (12). The logical next step after *in vitro* testing could be *in vivo* testing of this three-layer coating. It is also of interest to include in a systematic study of the influence of the roughness and porosity of microplasma-sprayed coatings on their biocompatibility and corrosion resistance for such promising coatings of medical implant metals as tantalum and zirconium, in order to select the optimal microplasma spraying parameters for obtaining biocompatible coatings of medical implants or endoprostheses.

3. Conclusion

It has been established that the greatest influence on the proliferation and viability of MCSs is exerted by the coating material, in particular, microplasma spraying of HA coatings on a titanium alloy substrate provides an increase in the rate of cell proliferation within three days by 45%, whereas CP-Ti coatings increase this rate by 40% compared to the substrate, which remains neutral, having neither a positive nor a negative effect on proliferation. The porosity and roughness of the coating in the studied ranges do not significantly affect the proliferation of MCSs; however, CP-Ti coatings with lower roughness and porosity demonstrate better resistance to corrosion in a physiological solution. It is important that with an increase in the volumetric porosity of CP-Ti coatings from 6.5% to 25.0%, the elastic modulus of the coatings in the compression zone decreases from 47.9 to 19.5 GPa and in the tensile zone it decreases from 20.2 Gpa up to 12.8 GPa, which brings the elastic modulus of porous CP-Ti coatings closer to those for human bones.

Thus, insight into tailoring the microstructure and, therefore, the properties of CP-Ti and HA coatings by adjusting the coating deposition parameters were obtained, opening up new opportunities for the introduction of TPS technologies for the manufacture of biocompatible coatings for medical endoprosthesis implants, which is significant for medicine and the development of advanced biomaterials technologies.

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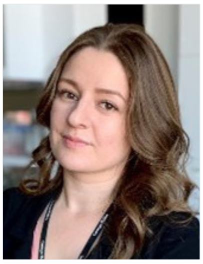

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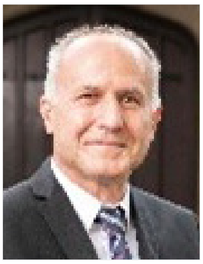

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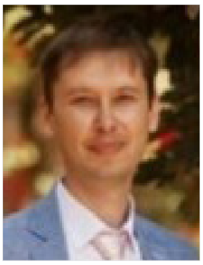

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