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Peri-implant osteogenesis on alumina-coated titanium implants in rat femur: morphological and elemental analysis of implant surfaces

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A – концепція та дизайн дослідження; B – збір даних; C – аналіз та інтерпретація даних; D – написання статті; E – редагування статті; F – остаточне затвердження статті

Keywords: orthopedics, implants, functional-protective coatings, corundum ceramics, aluminum oxide, osteoinduction, osteoconduction, osseointegration, scanning electron microscopy, energy dispersive X-ray spectroscopy, peri-implant osteogenesis.

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Peri-implant bone tissue regeneration involves complex processes that are not yet fully understood at the cellular and molecular levels, leaving significant gaps in our knowledge that require further investigation.

Aim. The study aimed to compare peri-implant osteogenesis on titanium femoral implants with alumina composite coatings applied by different methods to conventional titanium implants in an animal model.

Materials and methods. Implants underwent sandblasting with silicon carbide, plasma torch treatment, and coating with titanium, corundum, sprayed titanium wire, or hydroxyapatite, resulting in seven different surfaces. 105 female Wistar rats received implants in their right femurs and were divided into 7 groups based on implant type and exposure duration (1, 2, or 4 weeks). Implant fragments were analyzed using scanning electron microscopy and energy dispersive X-ray spectroscopy to quantify chemical elements. Ratios of carbon to nitrogen and calcium to phosphorus were calculated. Data were analyzed using the U-Mann–Whitney test, with $p < 0.05$ as a significant value.

Results. The energy dispersive X-spectrometry results confirmed morphological analysis findings by quantitatively and qualitatively assessing implants surface chemical composition. The key elements were evaluated, relevant for identifying bone tissue components like collagen (C and N) and hydroxyapatite (Ca and P), as well as implant coatings (Ti, Al, Ca, and P). Carbon and phosphorus showed fluctuations over time, with notable differences among groups. Aluminum appeared stable in some groups but varied in others. Calcium levels remained low initially and increased steadily in hydroxyapatite coated implants. Titanium levels were high initially, decreasing slightly over time. Morphological analysis correlated with surface roughness measurements. Notably, fibrin, collagen, and bone tissue presence varied among groups over time, with some groups showing significant mineralized bone tissue accumulation. After four weeks, blood clots persisted in some groups, while others exhibited bone tissue remodeling with the presence of osteoblasts and osteoclasts. Alumina-based coatings showed signs of degradation, with alumina cement scales found among macrophages and fibers.

Conclusions. Our study found that stable bone implants outperform alumina-composite coatings in long-term osseointegration due to mechanical stability. Although ceramic composites initially enhance osteoinductive properties, better attachment to titanium substrates is needed.

Ключові слова: ортопедичні імплантати, функціонально-захисні покриття, корундова кераміка, оксид алюмінію, остеоіндукція, остеокондукція, остеоінтеграція, растрова електронна мікроскопія, енергодисперсійна рентгенівська спектроскопія, періімплантатний остеогенез.

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Періімплантатний остеогенез на титанових імплантатах із корундовим покриттям у стегновій кістці щура: морфологічний та елементний аналіз поверхонь імплантату

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Регенерація періімплантатної кісткової тканини включає складні процеси, що досі остаточно не вивчені на клітинному та молекулярному рівнях. Це робить доцільними наступні дослідження в цій галузі.

Мета роботи – порівняти періімплантатний остеогенез на титанових стегнових імплантатах з алюмооксидними композитними покриттями, що нанесені різними методами, зі звичайними титановими імплантатами на тваринній моделі.

Матеріали і методи. Імплантати піскоструминно обробили карбідом кремнію, плазмовим пальником і покрили титаном, корундом, напиленим титановим дротом або гідроксиапатитом. У результаті отримали сім різних поверхонь. Імплантати встановили в праві стегнові кістки 105 самицям щурів лінії Вістар. Тварин поділили на 7 груп залежно від типу імплантату та тривалості експозиції (1, 2 або 4 тижні). Фрагменти імплантатів проаналізували за допомогою сканувальної електронної мікроскопії та енергодисперсійної рентгенівської спектроскопії для кількісного визначення хімічних елементів. Обрахували співвідношення вуглецю до азоту та кальцію до фосфору. Дані проаналізували за допомогою критерію U-Манна–Вітні, різницю при $p < 0,05$ визначили як статистично значущу.

Результати. Результати енергодисперсійної рентгенівської спектрометрії підтверджено даними морфологічного аналізу, який здійснили шляхом кількісного та якісного оцінювання хімічного складу поверхні імплантатів. Визначили ключові елементи, важливі для ідентифікації компонентів кісткової тканини, як-от колаген (C і N) і гідроксиапатит (Ca і P), а також покриття імплантатів (Ti, Al, Ca і P). Вміст вуглецю та фосфору змінювався з часом, з істотними відмінностями за групами. Алюміній виявився стабільним в одних групах, але змінювався в інших. Рівень кальцію спочатку залишався низьким, а в імплантатах з гідроксиапатитовим покриттям неухильно зростав. Частка титану спочатку була високою, але з часом дещо знижувалася. Дані морфологічного аналізу корелювали з результатами вимірювання шорсткості поверхні. Так, вміст фібрину, колагену та кісткової тканини в різних групах з часом змінювався, і в окремих групах

визначили істотне накопичення мінералізованої кісткової тканини. Через чотири тижні в окремих групах зберігалися згустки крові, а в інших виявили ремоделювання кісткової тканини з наявністю остеобластів і остеокластів. Покриття на основі глинозему мали ознаки деградації – серед макрофагів і волокон виявлено лусочки цього покриття.

Висновки. Результати дослідження дали підстави зробити висновок, що стабільні кісткові імплантати перевершують алюмокомпозитні покриття в довгостроковій остеоінтеграції завдяки механічній стабільності. Хоча керамічні композити спочатку покращують остеоіндуктивні властивості, необхідне краще прикріплення до титанової основи.

The success of surgical procedures for bone defect replacement or joint function restoration hinges on the proper selection of implant material. This material must possess mechanical properties that allow the joint and bone to perform their locomotory functions – specifically, adequate strength and elasticity [1]. Additionally, it must exhibit biocompatibility, characterized by low toxicity and high osseointegrative properties; corrosion resistance is also essential for implant materials [1,2]. Titanium (Ti) is a widely used material in modern implantology that meets all these criteria [3]. It is biomechanically strong, has a high strength-to-weight ratio, and is resistant to corrosion. However, titanium requires enhanced mechanical properties for use in highly loaded applications [1,3,4]. To address this issue, various ceramic composites have been utilized as the coatings for Ti implants: hydroxyapatite (HAp), fluorapatite, aluminum oxide (alumina, corundum), bioglass, and calcium phosphates [1]. Within others, alumina ceramics demonstrated reliable osteoinductive properties apart from their low cost, availability and simplicity in manufacturing [5]. Ultimately, successful implantation is measured by the period of the normal functioning of the implant, therefore the longest duration of normal functioning is highly desirable [1]. Obviously, such a long duration of normal functioning of the implant directly depends on whether the implant has formed a full contact with mature bone tissue, i. e. whether osseointegration has occurred [1,2,3].

In general, osseointegrative properties are the properties of the implant that ensure the formation of a dense and stable contact (interface) between its surface and the newly formed peri-implant bone tissue, in other words, that ensure osseointegration [6]. In fact, the process of osseointegration is preceded by osteoinduction and osteoconduction – processes, respectively, of migration and proliferation of osteogenic cells in response to the introduction of implant material into a bone defect, which, depending on the properties of the implant, may not necessarily end in osseointegration, but to a large extent determine it course [6]. With that in mind, a successful bone implant must have a combination of physicochemical properties that would stimulate the above processes, ultimately culminating in maximally complete osseointegration [7]. This issue prompts the search for the best options for modifications of bone implants for arthroplasty and bone plastic.

Extraction of implants with consequent morphological and elemental assessment of changes on its surface using the scanning electron microscopy and energy dispersed X-ray spectroscopy appeared as a reliable study design for the evaluation of peri-implant biological performance, which allows examining the relationship between inorganic components of the implant surface and the attached biological objects [8,9,10,11].

Aim

The aim of our study was to investigate the occurrence and extent of peri-implant osteogenesis on the surfaces of extracted titanium femoral implants with alumina composite functional protective coatings applied by different methods comparing with the same properties of conventional coatings of titanium implants in animal model.

Material and methods

The procedure for implant manufacturing and their physical and chemical characteristics are described in our previous work [12]. After the sandblasting with silicon carbide, the surface of each implant was treated with a plasma torch and simultaneous coating with different substances: A – titanium powder; B – corundum, type A25 (alumina content, Al_2O_3 ~99 %), grain size 5; C – sprayed titanium wire; D – HAp, grain size 5. All these treatment methods were consequently combined with each other, in such a way that 7 different types of implant surfaces were modified for the experiment *(Table 1)*.

After the corresponding treatment of the titanium surface, the coated wire was cut into 15 mm cylindrical pins and sterilized at 135 °C for 20 min immediately prior to experiment.

The cylindrical pins were implanted intramedullary into the right femurs of 105 female Wistar rats with average age 17 weeks and weight 250 g. Animals were divided into 7 groups according to implant type (Ti, TS, TSP, TSPC, TSPT, TSPTC and TSPH) and duration of implant exposure (1, 2 and 4 weeks). 15 animals with untreated and uncoated Ti implants served as a control group, another 15 rats from group TSPH served as a comparison group with the conventional HAp-coated implants. All surgical and pharmacological interventions, including anesthesia and euthanasia, were described in our previous paper [12]. Rats were maintained on a 12 h light and dark cycle with *ad libitum* access to water and normal chow diet composed of 10% fat. All animal experiments and procedures mentioned above were performed in compliance with ethical regulations (Law of Ukraine N 3447-IV and European Directive 2010/63/EU) and the approval of Biomedical Ethics Committee of Dnipro State Medical University (meeting minutes No. 2 dated 26.10.2019).

After euthanasia, 1.5 cm fragments of the distal femurs with implants were collected and cut into three fragments of equal length of 0.5 cm: proximal (diaphysis), distal (epiphysis), and intermediate. An electric saw (Dremel-2050, Germany) was used for this purpose, using a diamond-coated disk. Proximal 0.5 cm fragments were used for the current study where bone tissue was carefully separated from the implant according to R. Depprich et al. [11]. Then detached bone samples were used for histological analysis reported in our previous report [12].

Table 1. Implant material characteristics, and their abbreviations

Implant material, coating	Abbreviation	Roughness (R_a, μm)
Untreated titanium wire	Ti	1.89
Ti + sandblasting	TS	4.87
TS + plasma with "A"	TSP	1.91
TS + plasma with "B"	TSPC	11.8
TS + plasma with "C"	TSPT	23.7
TS + plasma with "C" and "B"	TSPTC	11.5
TS + plasma with "D"	TSPH	9.4

"A": titanium powder; **"B":** corundum, type A25 (alumina content, Al_2O_3 ~99 %), grain size 5; **"C":** sprayed titanium wire; **"D":** hydroxyapatite (HAp), grain size 5.

Table 2. Peri-implant C/N and Ca/P weight% ratios

Group	C/N ratio			Ca/P ratio		
	1 week	2 weeks	4 weeks	1 week	2 weeks	4 weeks
Ti	6.59	3.88	2.97	–	1.7	0.66
TS	7.85	4.07	4.33	–	2.12	1.67
TSP	9.44	2.83	5.73	–	–	1.72
TSPT	3.39	3	3.63	–	2.23	0.88
TSPC	2.21	3.61	4.44	–	1.8	1.05
TSPTC	4.02	2.86	2.92	–	2.16	1.07
TSPH	4.86	2.78	3.23	1.07	1.12	1.2

Empty cells appeared due to missing values of one or two ratio indicators (numerator and/or denominator).

The 0.5 cm long implant fragments separated from the bone were immediately placed in a 5 % glutaraldehyde solution at +4 °C. After fixation for at least 12 hours, the samples were dried in a series of ethanol changes followed by critical point CO_2 drying (Samdri-780A, Tousimis, USA). The samples were fixed on a research table with SPI 05081-AB carbon tape and covered with a thin (20 nm) layer of conductive material (Au/Pd mixture) using a PECS Gatan 682 (Gatan, USA) device. To study the morphology, scanning electron microscope (SEM) images were captured using a MIRA3 (TESCAN, Czech Republic) microscope equipped with a field emission cathode (high-brightness Schottky cathode) and an energy dispersive X-ray spectrometer (EDS) with an X-max 80 mm² detector (Oxford Instruments, UK), operating at an accelerating voltage of 10 kV. SEM and EDS methods were carried out in Electron Microscopy Laboratory of G. V. Kurdyumov Institute for Metal Physics of the National Academy of Sciences of Ukraine (Kyiv).

The harvested implants and attached tissues were examined thoroughly at the magnification ×500. On each implant 5 representative areas of interest (AOI) were chosen for further EDS analysis under a magnification of ×500. From the perspective of relevance for the evaluation of living tissue formation, native implant surface coverage, and mineralized bone matrix formation, 6 chemical elements were quantified in mass percentages (weight%) for each AOI: titanium (Ti), nitrogen (N), carbon (C), calcium (Ca), aluminum (Al), and phosphorus (P) according to I. A. Iancu et al. [8]. Oxygen (O) was evaluated on native implants only. To determine the content of proteins (e. g., collagen), the C/N ratio was calculated [13]. Similarly for the mineral bone matrix (HAp) identifying, Ca/P ratio was computed [14].

EDS measurements data of element weight% were expressed as a median, first (25th percentile) and third (75th percentile) quartile (Q_1, Me, Q_3). Statistical analysis was performed using the U-Mann–Whitney test. P-values of <0.05 indicated an evidence, <0.01 – strong evidence, <0.001 – very strong evidence against the null hypothesis. All data analyses and drawings were performed in GraphPad Prism version 8.0.2 (263) for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com).

Results

EDS analysis of extracted implants. The results of energy dispersive X-spectrometry, which allowed analyzing the chemical composition of the implant surface, served as quantitative and qualitative confirmation of the data obtained as a result of the morphological analysis. After scanning at least five selected areas of each implant surface, seven chemical elements were evaluated, which were:

1) most relevant for identification of such components of the bone tissue as collagen (C and N) and HAp (Ca and P);

2) components of implant coatings (Ti, Al, O, Ca and P).

Besides the elemental signatures of organic and mineral bone compounds in weight percentages, the C/N and Ca/P ratios were calculated *(Table 2)*.

Quantitative elemental analysis demonstrated dynamics of changes of the weight and atomic percentages of the selected elements on implant surfaces over time and revealed several trends across the investigated groups. It is important to note that the weight% of N on the surfaces of all experimental groups and controls (Ti and TSPH) tended to increase from the first to the fourth week of implantation. However, nitrogen was absent in native implants, and did not show any statistically significant difference between the samples both within groups and in the intergroup comparison.

Carbon levels were initially present in all groups with high variability. In general, the general trend across all investigated groups was the continuous rise of C level from 1 to 4 weeks of implantation period. Although in TS group C levels occurred high at 1 week, they surprisingly decreased at week 2, then, again, increased by week 4. Nonetheless, only TSPC group demonstrated significant growth of carbon mass on the implant surfaces after 4 weeks in comparison with 1 week after implantation ($p = 0.0002$, *Fig. 1*). Additionally, comparison of C levels across the groups of the same implantation periods revealed significant difference between TSPC and all other groups without alumina-contained coatings (Ti, TS, TSP and TSPH) at 1 week of implant exposure with 0.048, 0.005, 0.01 and 0.0006 p-values respectively.

Aluminum appeared initially and remained stable over time in TSPT group. On the surface of TSPC samples Al showed up at week 1 and remains variable but present through the weeks. In the cases of TSPTC group it was surprisingly emerged at week 4, despite its absence through the previous extracting points. However, significant difference was detected neither between the samples of different implantation periods within the same group (except native implants) nor between different groups' samples of the same implantation periods.

Generally in all investigated groups phosphorus showed minimal presence or absence initially but slightly increased over time with peaks at week 2 in control, TS, TSPT, and TSPTC samples. TSPC and TSPH demonstrated continuous growth of P concentration on the implant surface. Indeed, a statistically significant difference was indicated between 1 and 4 weeks of implantation for the TSPC group ($p = 0.218$). Notably, initially present in HAp coating of TSPH samples phosphorus significantly increased its concentration at 1, 2 and 4 weeks of implantation compared with P weight% on the native samples ($p = 0.002$, $p = 0.006$ and $p = 0.005$ respectively). In case of TSP, P appeared only at week 4: 1 and 2 weeks of implantation revealed absence of this element on the TSP implants *(Fig. 1)*. Nevertheless, in case of intergroup comparison, all the studied samples demonstrated significantly lower indicators of the weight percentage of phosphorus on the implant surface compared to HAp-group at almost all implantation periods: TSPH vs. all others, 1 week, $p = 0.002$; TSPH vs. TSP at 2 and 4 weeks – $p = 0.006$ and $p = 0.048$ respectively.

Calcium showed minimal presence and remained low over time in the control group samples. In case of HAp-coated implants it demonstrated steady growth of Ca from week 1 to week 4, though weight percentages were less than in native implants and did not demonstrate significant difference between each other. In all studied groups only TSP samples revealed the peaking of Ca at week 4 ($p = 0.018$), the rest demonstrated drop of Ca concentrations at week 4, though without significance *(Fig. 1)*. Intergroup comparison revealed significantly lower Ca weight percentages on the implant surfaces in all the studied samples compared to HAp-group at 1 week of implantation periods ($p < 0.05$).

In all studied groups detected titanium levels were high initially and remain significant over time, with a slight decrease by week 4. Noteworthy, significant increase of titanium concentration was observed on the surface of the implants with corundum ceramics (TSPC and TSPTC) of one-week implantation period in comparison with native surfaces ($p = 0.003$ and 0.004 respectively). At the same time, a decrease in the level of aluminum in the elemental composition of the TSPC and TSPTC surfaces was also noted ($p < 0.05$). Subsequently, in these groups, as well as in the others, the proportion of titanium on the surface gradually decreased from 1 to 4 weeks of implantation *(Fig. 1)*. Intergroup evaluation demonstrated significant decrease in titanium leaching on the implant surface compared to the control group after 4 weeks of implantation ($p = 0.014$). On the other hand, in TSPTC group the levels of titanium on the surface after 1 week were significantly higher than in TSPH ($p = 0.006$).

SEM analysis. The morphological analysis of the surfaces of the native implants completely corresponded to the data of roughness measurements *(Table 1)*: the smoothest surface was demonstrated by implants from the Ti and TSP groups, the roughest were the implants with TSPT, TSPC and TSPTC coating, respectively. However, it should be noted that on the surface of TSPTC coated implants we observed the presence of cracks up to 1 μm width. Normally, we identified individual biological objects with the assistance of the EDS, since the elemental content and ratios allowed us to identify collagen fibers, fibrin, mineralised bone, etc.

Control group (Ti) and TISH demonstrated after 1 week of implantation was characterized by such general changes as the presence of fibrin fibers, erythrocytes, and adhesion of leukocytes. Besides that, TISH group revealed an early appearance of collagen fibers and fibroblasts adhesion, which were sparsely distributed over the implant surface. Two-week implantation period revealed gradual decrease of the blood clotting together with the much wider distribution of the collagen fibers on the surface. Moreover, TISH implants accumulated a significant amount of mineralized woven osseous tissue on about a half of the entire implant surfaces. In contrast, miniscule quantity of immature bone tissue was attached to the surface of the implants of the control group. Later, after 4 weeks of implant exposure to intramedullary tissues, the woven bone became more widespread on the surface of the control group implants, although we still found only diffusely scattered islands of immature mineralized bone, the vast majority of the surface area being covered with fibrous tissue and fibrin. In sharp contrast, the comparison group (TSPH) demonstrated a significantly higher amount of mineralized bone tissue attached almost along the entire implant surface *(Fig. 2)*.

The surface of the implants after 1 week of implantation was characterized by the same events as in the control group. Nevertheless, the extent of those events varied between different groups. For example, the largest area of the implant surface covered by fibrin clots was demonstrated in the TS group (more than two-third), while the groups with complex surface modification (TSPC, TSPT and TSPTC) showed a smaller surface area covered by fibrin: approximately one-third of the implant surface, mainly on raised areas. However, the appearance of collagen fibers and fibroblasts was observed mainly in recessed areas of the surface relief (*Fig. 3*, TSPC, 1 week).

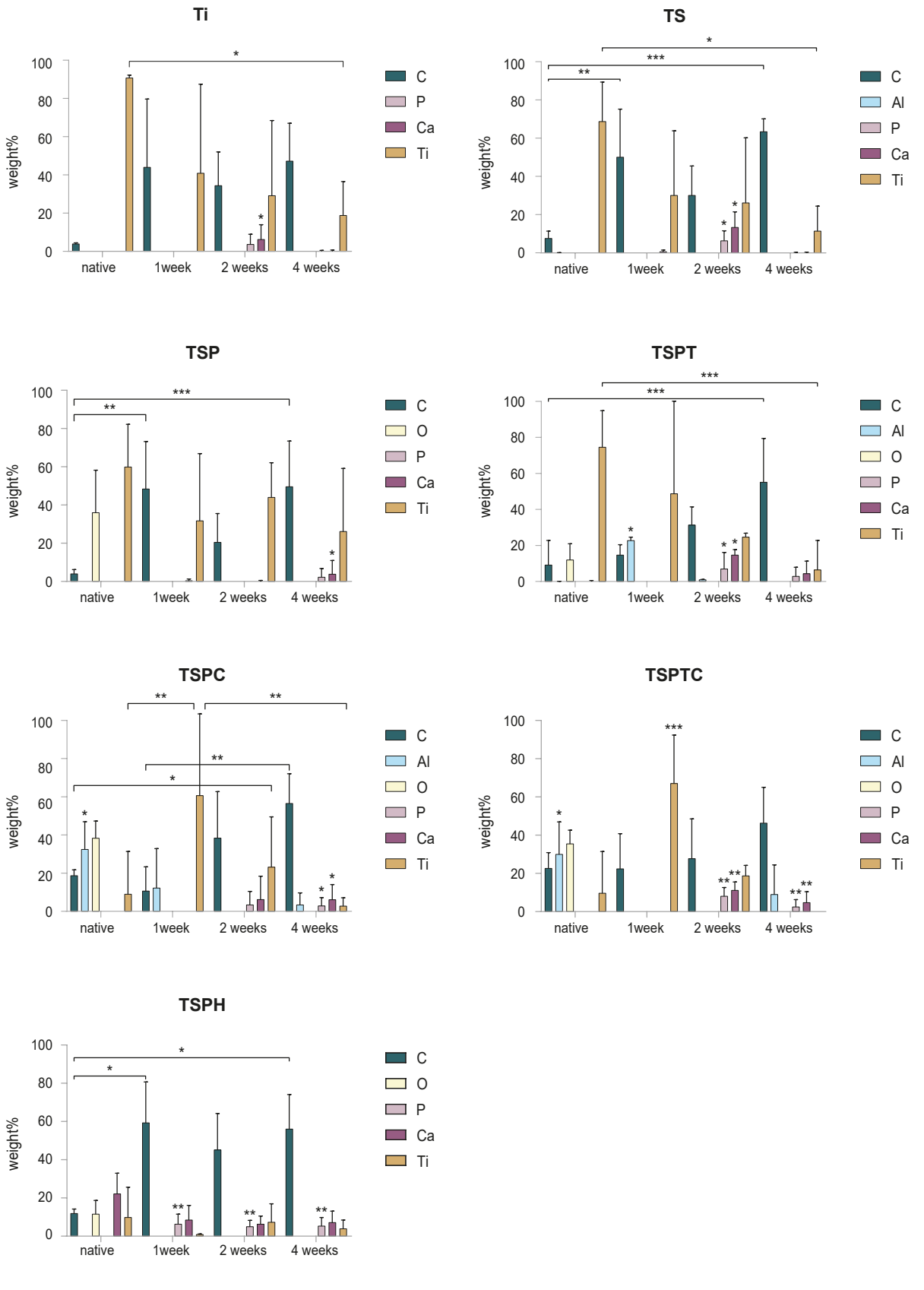

Fig. 1. The dynamics of the weight percentage (weight%) of the most relevant chemical elements that made up the surfaces of the studied implants.
:** $p < 0.05$; *:** $p < 0.01$; *****:** $p < 0.001$.

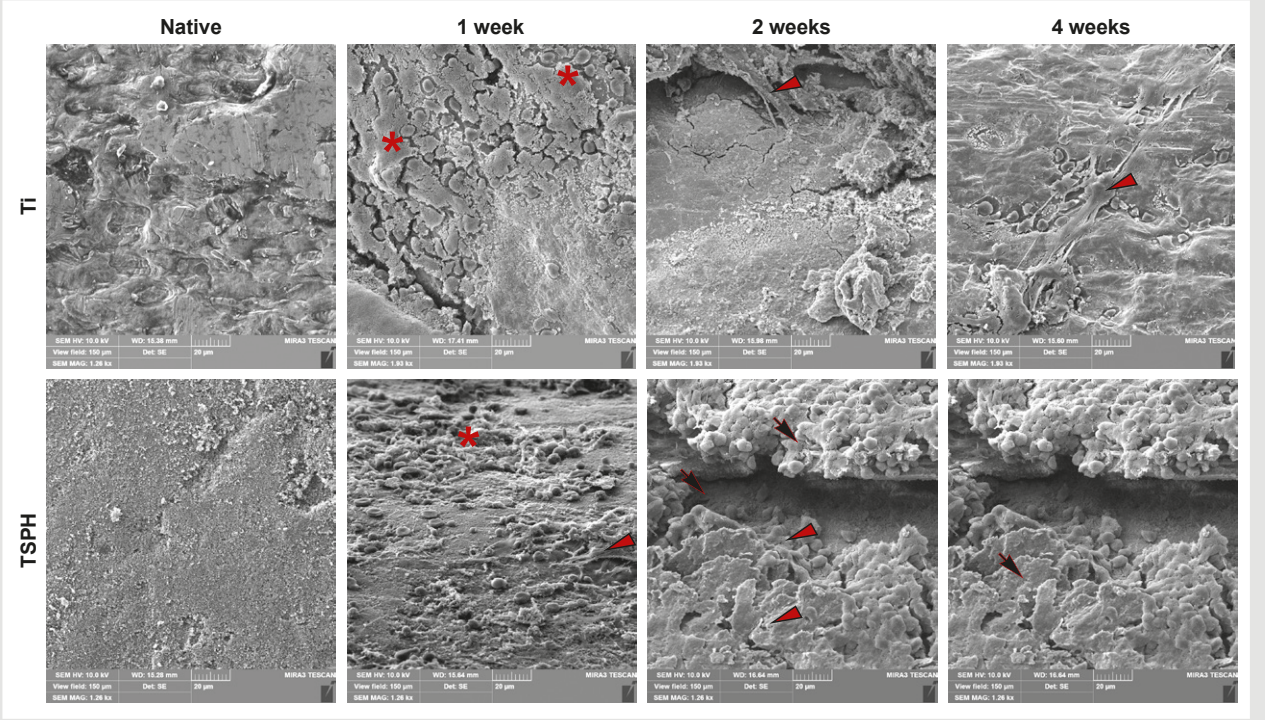

Fig. 2. Morphological characteristics of implant surfaces in control (untreated Ti) group and group of comparison with conventional hydroxyapatite coating (TSPH). ***:** asterisk indicates areas with adhered fibrin mesh; **red arrowheads** point on fibroblast clusters with collagen aggregates; **black arrows** – mineralized bone adjacent to implant surface.

Two-week changes on the modified surfaces were characterized by a decrease in the amount of fibrin, continuous accumulation of collagen, and the appearance of woven osseous tissue with the presence of osteoblasts. Inflammatory cells were preserved: macrophages and lymphocytes, but without a significant difference between the groups. The most formed woven bone tissue was observed around the implants with the TSPTC surface, not much less of it was found in the TSPC and TSPT groups. Almost similar amount of bone tissue on the surface was demonstrated by implants with sandblasting (TS). In general, these findings were similar to the events described on the surface of TSPH implants. However, a very small amount of provisional bone tissue was found on the surface of TSP implants, even less than in the control group *(Fig. 3)*.

A highly variable pattern of findings was demonstrated after 4 weeks of implant exposure. For example, blood clots continued to be observed in the control group, TS and TSPT groups, while fibrin was absent in the rest of the groups. The bone tissue was remodeled: the appearance of lamellar and trabecular bone with the presence of osteoblasts (lining cells, *Fig. 3*, TSPC, 4 weeks) and single osteoclasts was observed, although the general trend indicated a decrease in the thickness of the bone tissue around the implant. The exception included some zones on TS implants where the accumulation and adhesion of macrophages to surfaces without bone tissue was observed. Interestingly, the TSP group revealed no difference in formation of adherent bone tissue compared with other investigated groups despite it being hindered at 2 weeks of observation. Notably, TSPC and TSPTC demonstrated the presence of destruction of the coating with the finding of scales of alumina cement among macrophages and fibers *(Fig. 3)*.

Discussion

The reported results of the element composition on implant surfaces over time, assessed using energy-dispersive X-ray spectroscopy (EDS), provide comprehensive insights into the bioactivity and surface modification efficacy of different implant groups with functional protective coatings including alumina-based ceramics. It indicates how different surface coatings influence the bioactivity and osseointegration potential.

Carbon levels were high initially in most groups, with variations over time. High initial levels of carbon indicate the presence of residues from the manufacturing process: calcium carbide particles remained after sandblasting. Fluctuations over time suggest ongoing changes on the implant surface, possibly due to interactions with the surrounding biological environment. Carbon presence could also be influenced by the adsorption of organic molecules from the biological fluids [15]. For example, carbon decrease in TS group at week 2 followed by a significant increase at week 4, which might suggest initial biofilm formation followed by organic material accumulation. In contrast TSPC group revealed significant increase at week 2, continuing to rise by week 4, and indicating continuous organic deposition. Aluminum was eventually observed in composite-coated groups. Stable presence in TSPTC and TSPC groups indicates successful incorporation and retention of aluminum-based materials on the implant surfaces. Presence at week 4 in TSPTC suggests some degree of coating destabilization or/and surface

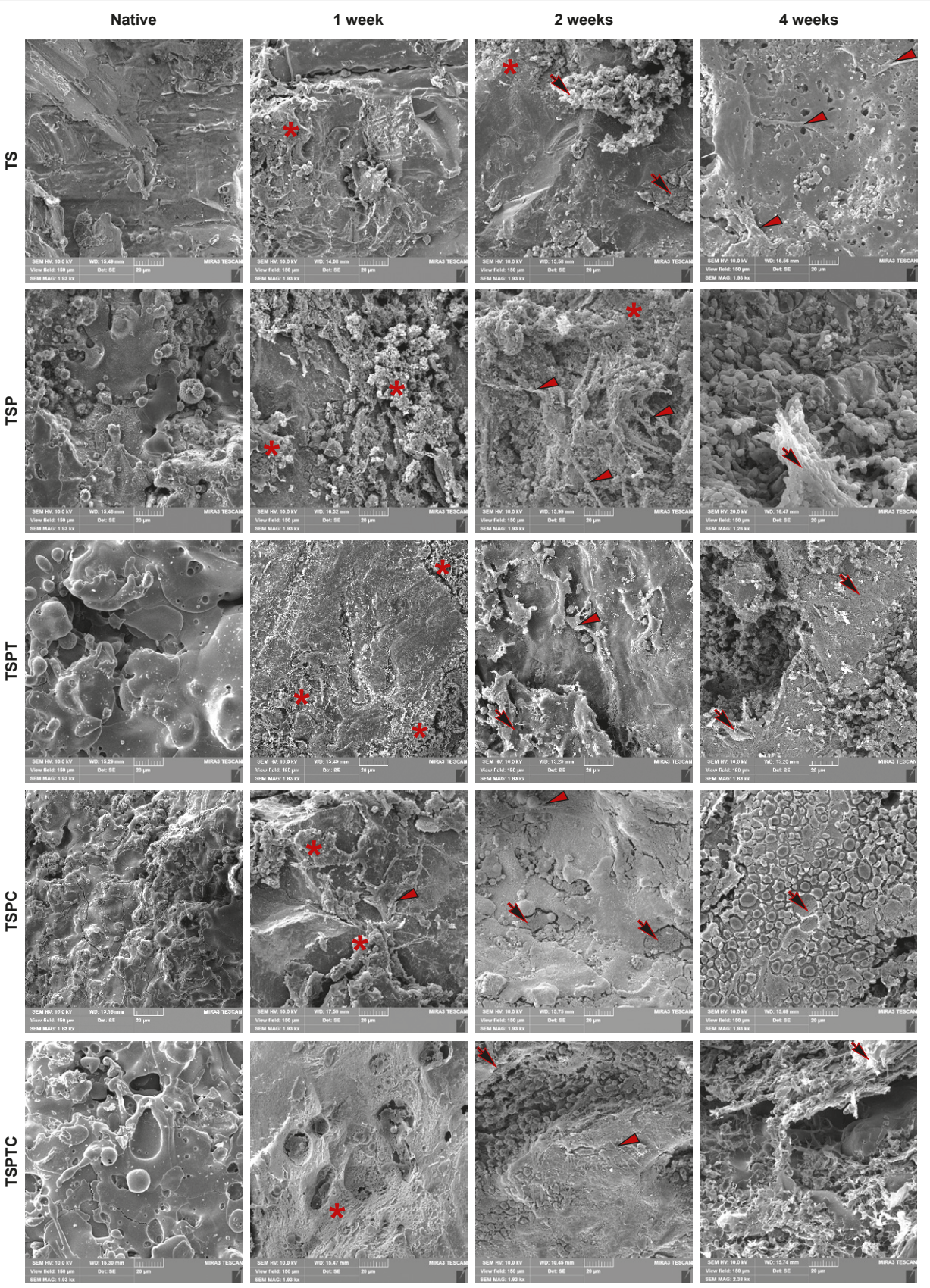

Fig. 3. Morphological characteristics of implant surfaces in studied groups. *: areas with fibrin adhesion; **red arrowheads:** collagen fibers; **black arrows:** mineralized bone. **TSPC, 4 weeks:** osteoblasts (lining cells) arranged above the bone tissue (arrow); **TSPTC, 4 weeks:** white crystalline scale (alumina coating debris) in the right lower corner.

modification that exposes areas without bone but coated with aluminum oxide. This might enhance the surface properties, potentially improving the overall bioactivity and biocompatibility of the implants. Phosphorus presence indicates bioactivity and potential bone integration [8,16].

Appearance of P over time in most groups highlights the bioactive nature of the surfaces, promoting bone cell attachment and growth [16]. Peaks in groups like TS and TSPT at week 2 followed by a reduction could suggest initial rapid bioactivity and subsequent stabilization as the bone integration process progresses. Calcium levels were consistently present across all groups. For instance, presence from week 1 in several groups (like TS and TSP) suggests early-stage mineralization, crucial for bone integration. Increased Ca-levels over time indicate ongoing bone formation and mineralization on the implant surfaces, reflecting successful osseointegration [17]. Titanium levels remained significant across all groups, confirming it as the base material. Consistent presence across all groups validates the durability and stability of titanium as a core implant material. Minor fluctuations in percentages could reflect surface modifications or the integration of other materials, such as aluminum or phosphorus, which may alter the exposed surface composition [4].

The dynamics of these elements suggest that the different implant groups exhibit varying degrees of bioactivity and surface modification efficacy. All in all, the presence and variations of phosphorus and calcium across different groups highlight the bioactivity and potential for osseointegration of the implants [8,9,10,11]. Groups with consistent phosphorus and calcium levels, like TSPT and TSPH, suggest good bone formation and integration. The detection of aluminum in specific groups confirms the successful incorporation of composite materials designed to enhance the mechanical properties of the implants. Carbon dynamics indicate significant interactions with organic tissues, which can be both beneficial (e. g., promoting bone growth) and challenging (e. g., potential for biofilm formation).

It is well known that carbon/nitrogen ratio (C/N ratio) has specific value in each protein. For instance, in collagen it comprises 3.243 [13]. Ratios significantly higher or lower than this may indicate changes in the organic matrix composition near the implant. In the control group, TS, TSPC, and TSPTC C/N ratio fluctuates over time at 1 and 2 weeks of implantation with subsequent stabilization of the matrix close to the collagen standard. TSPT shows the most stable and close-to-normal C/N ratios over time, indicating a stable organic matrix composition. However, TSP shows significant variability, indicating inconsistent organic matrix composition.

Similarly bone mineral matrix density was evaluated with Ca/P ratio, normally it varies in female Wistar rats between 1.42–1.63 [14]. According to obtained results, the TS group was characterized by normalization of Ca/P ratios over time, indicating balanced mineralization. In contrast, surprisingly, TSPH showed consistently low ratios, indicating persistent poor bone quality.

Implants showing consistent or increasing levels of elements such as phosphorus and calcium over time suggest enhanced bioactivity and potential for bone integration. Among the groups studied, TSPT, TSPC, and TSPTC exhibit sustained or increasing levels of phosphorus and calcium at 2 weeks, indicating promising osteoinductive properties which is consistent with our previous findings [2,15]. However, at 4 week all these groups revealed loss of peri-implant mineralized bone, coinciding with the appearance of a large number of macrophages, as a reaction to the detachment of the functional protective coating (the appearance of aluminum oxide scales among biological tissues, *Fig. 3*, TSPTC, 4 weeks) under the influence of mechanical stress. Indeed, for a period of 4 weeks after adequate wound healing, antibiotic therapy, anti-inflammatory therapy and pain relief, rats began active locomotion, which could cause such changes. Therefore, while surface modifications are important, the stability of the base material, typically titanium, is crucial for long-term implant success [1,2,3,6,15,16,17]. Groups with stable titanium levels, such as Ti, TS, and TSP, suggest minimal degradation or leaching of the base material, which is favorable for implant longevity. Implants showing dynamic changes in elemental composition may indicate ongoing surface interactions, which could be either beneficial (e. g., enhanced bioactivity) or detrimental (e. g., corrosion) [18,19]. Careful analysis of these dynamics is required to assess their impact on implant performance. Considering these factors, the TSPT and TSPTC groups appear promising in terms of bioactivity and potential for bone integration though required more stable base material characteristics. However, additional studies incorporating biological assays, mechanical testing, and long-term in vivo evaluations are necessary to comprehensively evaluate implant effectiveness.

Conclusions

1. Obtained results provide comprehensive insights into the performance of various implant materials and their interactions with biological tissues.

2. Bone implants with a mechanically stable surface, appropriate roughness and porosity (TS, TSP) have an advantage in the long-term development of osseointegration over investigated alumina-composite coatings (TSPT, TSPC and TSPTC) despite their pronounced osteoinductive and osteoconductive properties, due to mechanical instability.

3. Although the presence of aluminum oxide in ceramic composites confirms improved osteoinductive properties at 2 week of experimental implantation in rat femur (TSPT, TSPC and TSPTC, $p < 0.05$), the models of corundum coatings tested in our study should be improved, primarily through more reliable attachment to the titanium substrate.

4. Carbon fluctuations indicate dynamic organic interactions, crucial for understanding long-term biocompatibility and functionality of the implants. The dynamic behavior of C/N ratio revealed that TSPT coating is the most appropriate for close-to-normal matrix protein formation.

5. The observed bioactivity and mineralization processes are promising for improving osseointegration and the overall success of implants via development of more effective and biocompatible materials for orthopedic applications.

Perspectives of subsequent scientific research. In the next publication we are going to report the findings obtained after 8 weeks. It will include evaluation of bone-implant interface using grinding techniques on resin-embedded non-demineralized bone samples. Future studies should focus on optimizing these surface modifications to enhance long-term stability and bioactivity.

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References

1. Kumar M, Kumar R, Kumar S. Coatings on orthopedic implants to overcome present problems and challenges: A focused review. Materials Today: Proceedings. 2021;45(6):5269-76. doi: 10.1016/j.matpr.2021.01.831
2. Huynh V, Ngo NK, Golden TD. Surface Activation and Pretreatments for Biocompatible Metals and Alloys Used in Biomedical Applications. Int J Biomater. 2019;2019:3806504. doi: 10.1155/2019/3806504
3. Zhang LC, Chen LY. A review on biomedical titanium alloys: recent progress and prospect. Adv Eng Mater. 2019;21(4):1801215. doi: 10.1002/adem.201801215
4. Schüpbach P, Glauser R, Rocci A, Martignoni M, Sennerby L, Lundgren A, et al. The human bone-oxidized titanium implant interface: A light microscopic, scanning electron microscopic, back-scatter scanning electron microscopic, and energy-dispersive x-ray study of clinically retrieved dental implants. Clin Implant Dent Relat Res. 2005;7 Suppl 1:S36-S43. doi: 10.1111/j.1708-8208.2005.tb00073.x
5. Bahraminasab M, Arab S, Safari M, Talebi A, Kavakebian F, Doostmohammadi N. In vivo performance of Al_2O_3-Ti bone implants in the rat femur. J Orthop Surg Res. 2021;16(1):79. doi: 10.1186/s13018-021-02226-7
6. Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. Eur Spine J. 2001;10 Suppl 2:S96-101.doi: 10.1007/s005860100282
7. Davies JE. Bone bonding at natural and biomaterial surfaces. Biomaterials. 2007;28(34):5058-67. doi: 10.1016/j.biomaterials.2007.07.049
8. Iancu IA, Iancu SA, Epistatu D, Comaneanu M, Badarau IA. Scanning electron microscopy and energy-dispersive X-ray spectroscopy on the degree of bone mineralization at the bone-implant interface. Ro J Stomatol. 2024;70(1):21-6. doi: 10.37897/RJS.2024.1.7
9. Shah FA, Ruscsák K, Palmquist A. 50 years of scanning electron microscopy of bone-a comprehensive overview of the important discoveries made and insights gained into bone material properties in health, disease, and taphonomy. Bone Res. 2019;7:15. doi: 10.1038/s41413-019-0053-z
10. Mangano F, Raspanti M, Maghaireh H, Mangano C. Scanning Electron Microscope (SEM) Evaluation of the Interface between a Nanostructured Calcium-Incorporated Dental Implant Surface and the Human Bone. Materials (Basel). 2017;10(12):1438. doi: 10.3390/ma10121438
11. Depprich R, Zipprich H, Ommerborn M, Mahn E, Lammers L, Handschel J, et al. Osseointegration of zirconia implants: an SEM observation of the bone-implant interface. Head Face Med. 2008;4:25. Published 2008 Nov 6. doi: 10.1186/1746-160X-4-25
12. Loskutov O, Shponka I, Bondarenko O, Bondarenko N, Bozhko A. Histological and histochemical assessment of short-term events in peri-implant bone for osteoinductivity evaluation of functional-protective implant coatings. Medicni perspektivi. 2021;26(3):4-10. doi: 10.26641/2307-0404.2021.3.241875
13. Schwarcz HP, Nahal H. Theoretical and observed C/N ratios in human bone collagen. J Archeol Sci. 2021;131:105396.doi: 10.1016/j.jas.2021.105396
14. Hernandez-Becerra E, Londoño-Restrepo SM, Hernández-Urbiola MI, Jimenez-Mendoza D, Aguilera-Barreiro ML, Perez-Torrero E, et al. Determination of basal bone mineral density in the femur bones of male and female Wistar rats. Lab Anim. 2021;55(1):30-42. doi: 10.1177/0023677220922566
15. Nikolova MP, Apostolova MD. Advances in Multifunctional Bioactive Coatings for Metallic Bone Implants. Materials (Basel). 2022;16(1):183. doi: 10.3390/ma16010183
16. Büchter A, Joos U, Wiesmann HP, Seper L, Meyer U. Biological and biomechanical evaluation of interface reaction at conical screw-type implants. Head Face Med. 2006;2:5. doi: 10.1186/1746-160X-2-5
17. Pobloth AM, Mersiowsky MJ, Kliemt L, Schell H, Dienelt A, Pfitzner BM, et al. Bioactive coating of zirconia toughened alumina ceramic implants improves cancellous osseointegration. Sci Rep. 2019;9(1):16692. doi: 10.1038/s41598-019-53094-5
18. Ibrahim SW, Rafeeq AK, Ahmedhamdi MS. Histomorphometric assessment of implant coated with mixture of nano-alumina and fluorapatite in rabbits. Saudi Dent J. 2021;33(8):1142-8. doi: 10.1016/j.sdentj.2021.02.005
19. Lin S, Maekawa H, Moeinzadeh S, Lui E, Alizadeh HV, Li J, et al. An osteoinductive and biodegradable intramedullary implant accelerates bone healing and mitigates complications of bone transport in male rats. Nat Commun. 2023;14(1):4455. doi: 10.1038/s41467-023-40149-5