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Histological and histomorphometric investigation of SiO_2 nanoparticle-coated PEEK implants on the osseointegration in rabbit

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Abstract

INTRODUCTION. Metal, polymer, and ceramic implants are examples of biocompatible implants that are frequently suggested to enhance osseointegration and promote bone formation. The goal of this study was to improve osseointegration by developing a new interface. This contact exists between bone tissue and the polymeric component. Silicon dioxide material is applied to PEEK (polyether ether ketone) plastic in this work coat.

MATERIALS AND METHODS. Thirty New Zealand rabbits received sixty implants. Each rabbit has two implants in its tibia. Ten rabbits were scarified at two, four and six, weeks following implantation. The left tibia implant was experimental (coated with silicon dioxide), while the right tibia implant was control (uncoated) for every animal. Following hematoxylin and eosin staining, each section was histologically inspected and evaluated for histomorphometric analysis to count bone cells (osteoblast, osteocyte, and osteoclast) and the area of new bone is formed.

RESULTS. In comparison to control groups, histological results for silicon dioxide-coated PEEK implants showed a more rapid of bone growth, mineralization, and maturation. Significant difference of histomorphometric examination of every bone parameter was very high between the control and experimental groups during the course of all healing intervals.

CONCLUSIONS. According to the current study, the nano-silicon dioxide coating promotes faster osseointegration than an uncoated implant, improving bone formation and increasing the healing rates.

Keywords: bone formation, silicon dioxide, implant, osseointegration, PEEK

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Гистологическое и гистоморфометрическое исследование имплантатов PEEK с наночастицами SiO_2 и их влияние на остеоинтеграцию у кроликов

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Резюме

ВВЕДЕНИЕ. Металлические, полимерные и керамические имплантаты являются примерами биосовместимых имплантатов, которые часто рекомендуются для улучшения остеоинтеграции и стимуляции формирования костной ткани.

ЦЕЛЬ. Повышение остеоинтеграции за счет разработки нового интерфейса, который формируется между костной тканью и полимерным компонентом. В этой работе на пластик PEEK (полиифенилсульфонэтилкетон) наносится покрытие из диоксида кремния.

МАТЕРИАЛЫ И МЕТОДЫ. Тридцати кроликам породы Новая Зеландия имплантировали в общей сложности шестьдесят имплантатов. У каждого кролика были по два имплантата в большеберцовой кости. Десять кроликов забивали на сроках 2, 4 и 6 недель после имплантации. Имплантат в левой большеберцовой кости был экспериментальным (покрыт диоксидом кремния), а в правой – контрольным (без покрытия). После окрашивания гематоксилином и эозином каждый срез подвергался гистологическому исследованию и гистоморфометрическому анализу для подсчета костных клеток (остеобластов, остеоцитов и остеокластов) и оценки площади вновь образованной кости.

РЕЗУЛЬТАТЫ. В сравнении с контрольными группами, гистологические данные показали, что имплантаты PEEK с покрытием из диоксида кремния способствовали более быстрому росту кости, минерали-

зации и ее созреванию. Различия по всем гистоморфометрическим параметрам костной ткани между контрольной и экспериментальной группами были высокими на всех этапах заживления.
ВЫВОДЫ. Согласно результатам исследования, нанопокрытие из диоксида кремния ускоряет остеоинтеграцию по сравнению с непокрытыми имплантатами, улучшает формирование костной ткани и повышает скорость заживления.

Ключевые слова: формирование кости, диоксид кремния, имплантат, остеоинтеграция, PEEK

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INTRODUCTION

The goal of biomedical implants is to get the patient's biomechanical activity back to normal. Using a range of technical materials, the implant device is matched with the surrounding tissue to produce effective results. which, given the complexity of biological systems, is a difficult undertaking [1; 2]. In order to support a dental prosthesis, a dental implantation surgical process is utilized to connect the implanted tooth or teeth to the jaw bone. Osseointegration, the biological process that allows a close link to the materials with bone, serves as the foundation for modern implants [3–5].

On the other hand, osseointegrated dental implants are those with pores that osteoblast cells and the connective supportive tissue can migrate into. In the context of oral implantology, this describes bone that grows directly up to the surface of dental implants without a layer of soft tissue in between [6]. It can also be defined as a process that is immune-driven which leads to the formation of the new bone surrounding the surface of the implant rather than a pure response of the bone [7]. Due to earlier differentiation and proliferation of bone-forming cells, osseointegration can be augmented by the new bone formation and mineralization that accelerate the treatment of surface of the implant site [8].

The topography and chemical characteristics of the implant surface are crucial for osseointegration. In order to promote cell growth and proliferation, and improve wettability. There are also chemical, physical and mechanical techniques used for treating implant surface that alter implant surface energy and topography [9]. Implant surface roughness has a direct impact on the quality of the bone-implant contact [10]. The primary techniques that are known to produce roughness in implants are titanium plasma spraying, sandblasting, acid etching, and coating with bioactive material. To enhance biological properties of dental implants modern innovation like microtopography and nano-topography are being used [11]. In general, the implant rough surface is preferable for osteoblasts for the adhesion, multiplication, and collagen making. While implant smooth surface is preferable for the secure adhesion of epithelial and fibroblast cells. Additionally, implants with moderate surface roughness demonstrated improved stability and bone ingrowth, enabling early mechanical loading [12; 13].

The thermoplastic (PEEK) polyether ketone ketone is suitable for a variety of sterilizing processes, including as autoclaving, ethylene oxide, and gamma irradiation, because to its inert chemical structure and high temperature tolerance. Furthermore, PEEK's modulus of elasticity is half that of cortical and trabecular bone. Because PEEK has fewer mechanical incompatibilities with bone tissue than metal implants, which could lessen stress shielding, it is favored in a range of therapeutic applications [14]. Consequently, the majority of implant applications began utilizing polymers in addition to metals since they offer superior peri-implant tissue recovery detection [15]. Some employ polymers as basic components in the design, while others use them as a material to coat the metal component [16]. To raise the likelihood that the implanted portion will be accepted and to enhance the body's capacity to integrate with it. Reduce the inflammatory reaction as well [17; 18].

Inorganic nanomaterials made of silicon dioxide, and silica nanoparticles are classified as either nonporous or mesoporous. They have high biocompatibility, easily modifiable surfaces because of the presence of silanol groups (Si-OH), and customizable size and pore volume [19]. Because Si-OH groups can react with biological fluids, it has been demonstrated that they contribute to bioactivity. This study sought to assess the impact of covering PEEK implants with SiO_2 nanoparticles on the osseointegration of rabbits both histologically and histomorphometrically.

MATERIALS AND METHODS

A circular target with a diameter of 50 mm and thickness of 3 mm was made to accomplish the RF plasma sputtering of SiO_2 powder. To create a uniform target and prevent target fracture, 30 grams of SiO_2 powder were mixed, then loaded and pressed in a cylindrical stainless-steel mold that measured with a diameter of 50 mm and height of 3 mm. Pressing is carried out using an electrical press for two minutes at 8 tons of pressure. To prevent cracks, the samples were left to cure gradually at room temperature for a whole day.

In the stainless-steel system chamber, radio frequency magnetron sputtering was carried out. The substrates were connected to the rotating cathode (negative charge) disc, while the target was connected to the system's anode (positive charge).

After inserting the PEEK substrates and target into the sputtering chamber, the chamber window was securely shut. A set distance of 10 cm separated the substrates and the target. To improve distribution consistency, the deposition was carried out at 2 rpm. There were three runs or sputtering intervals [20].

Thirty male New Zealand white rabbits, all weighed from 1.5 to 2 kg, aged from 10 to 12 months, had sixty machined surface implants from PEEK placed in them. Each rabbit had two implants put in its tibia: one in the experimental left tibia and one in the control right tibia.

Ten rabbits were sacrificed at two, four- and six-weeks following implantation. The implants were divided into two groups: the experimental group (30 PEEK coated implants) for three healing periods, 10 implants for each period, for the control group there has also been (30 uncoated implants).

The sterilizing implants were inserted into the 5 mm prepared hole in the tibiae of both rabbits. The uncoated implants were fixed straight into the right tibial bone of the rabbits, while those implants with coated implant was inserted into the left tibia following the rabbits' sacrifice in the conclusion of the suggested times. To create implant-contained bone blocks, the soft tissue was removed from the right and left tibias after the rabbit model had been sacrificed, to reveal the whole bone, which was then sliced 5 mm from both implant sides.

The samples underwent a 48-hour fixation process in 10% formalin, followed by decalcification using a formic acid solution, alcohol-dehydrated bone tissue, and paraffin embedding. Hematoxylin and eosin were used to stain 5 µm sections that were produced as normal. A light microscope was used for the histological evaluation.

A new bone production ratio was calculated by histomorphometrically evaluating the bone cells (osteoblast, osteocyte, and osteoclast).

RESULTS

The histology and histomorphometry performance of PEEK covered with SiO_2 were examined in this work. All animals generally recovered well from surgery and experienced no notable negative effects. They moved normally during the study period and showed no symptoms of infection, inflammation, or allergic reaction at the surgery site. The animal's general health, temperament, or diet were unaffected by the interventional procedures. There was no way to manually move the screws in order to assess osseointegration. Two groups were examined: the control group, which had no coated material, and the second group, which had SiO_2-coated PEEK specimens.

The degree of osseointegration of coated and uncoated samples with the bone, however, is revealed by the histological analysis. Following the completion of the implantation phase, several processing procedures are carried out to get ready for histological analysis. Various magnifications are used to capture the images. In terms of bone development, the first and second groups are compared.

Specimens of new bone of the experimental group were deposited more than the control group specimens at intervals of 2, 4, and 6 weeks. At increased magnification, it was discovered that the osteoblastic osteoid of the experimental group lining the immature bone surface was more recognizable.

Control 2-week

In certain areas, the histological findings revealed the deposition of osteoid tissues with the fibrous connective tissue, which was populated by progenitor cells, fibroblasts, inflammatory cells, and osteoblasts. (Fig. 1).

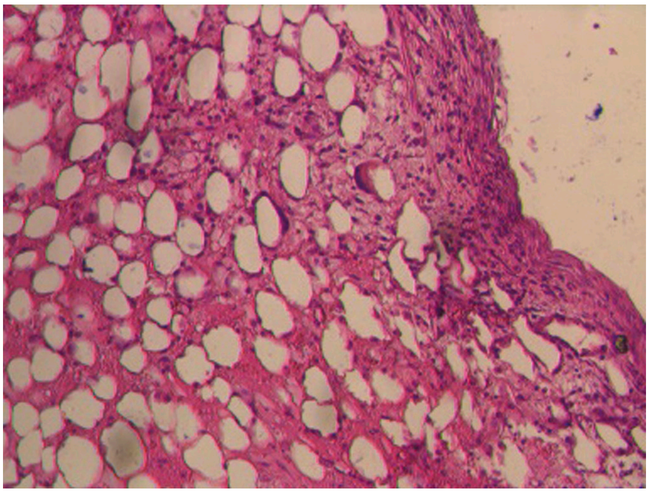

Fig. 1. Histological view of 2-week duration control group shows area filled with fibrous C.T. and osteoid tissue) H&E ×10

Рис. 1. Гистологическая картина контрольной группы на 2-й неделе: область заполнена фиброзной соединительной тканью и остеоидной тканью; окраска гематоксилином и эозином (H&E) ×10

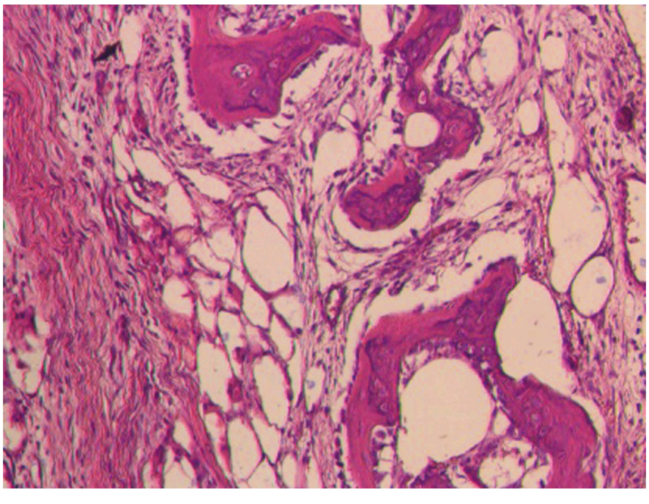

Fig. 2. View of two-week experimental group reveals woven bone a and blood vessels H&E ×4

Рис. 2. В препаратах экспериментальной группы на 2-й неделе выявляются грубоволокнистая костная ткань и кровеносные сосуды; окраска гематоксилином и эозином (H&E) ×4

Experimental 2-week

The histology results showed that the implant shape was followed by woven bone in the thread area. Higher magnification revealed that the thread area was packed with thin bone trabeculae, with osteocytes occupying their vast lacunae and osteoblasts grouped in a single mass at their margins. (Fig. 2).

Control 4-week

A few thin bone trabeculae filled with osteocytes and reosteocytes were seen in the histological image of the woven bone (Fig. 3).

Experimental 4-week

The histological view of experimental group in a 4-week period shows new bone trabeculae which demarcated from basal bone by reversal line. These bone trabeculae filled with osteocytes and surrounded by osteoblasts and osteoclasts (Fig. 4).

Control 6 week

The histological view shows formation of dense bone trabeculae filled with large-size osteocytes and surrounded by active osteoblasts. Also, the presence of osteoclasts and reversal line in higher magnification this indicates continuous bone remodelling (Fig. 5).

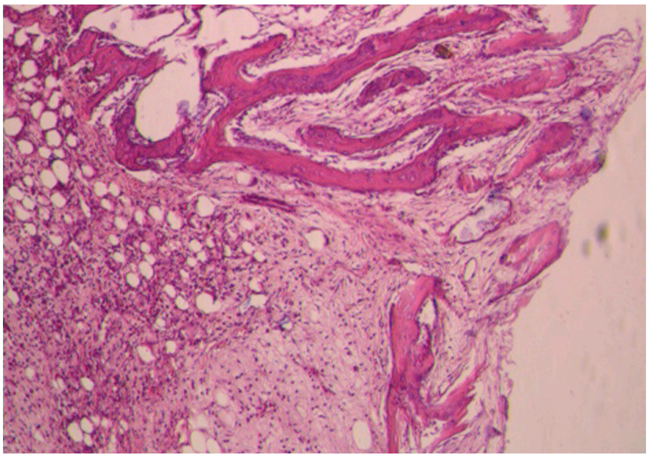

Fig. 3. Histological view of 2-week control group shows woven bone and thin bone trabeculae filled thread area, H&E ×10

Рис. 3. Гистологическая картина контрольной группы на 2-й неделе: выявляются грубоволокнистая кость и тонкие костные трабекулы, заполняющие область дефекта; окраска гематоксилином и эозином (H&E) ×10

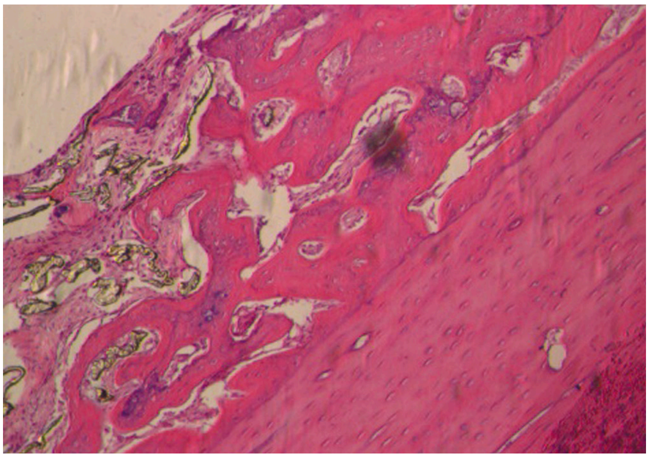

Fig. 5. View of 6-week control group shows osteoblasts, osteoclasts (OCL), and osteocytes (OC) H&E ×20

Рис. 5. Гистологическая картина контрольной группы на 6-й неделе: выявляются остеобласты, остеокласты (OCL) и остеоциты (OC); окраска гематоксилином и эозином (H&E) ×20

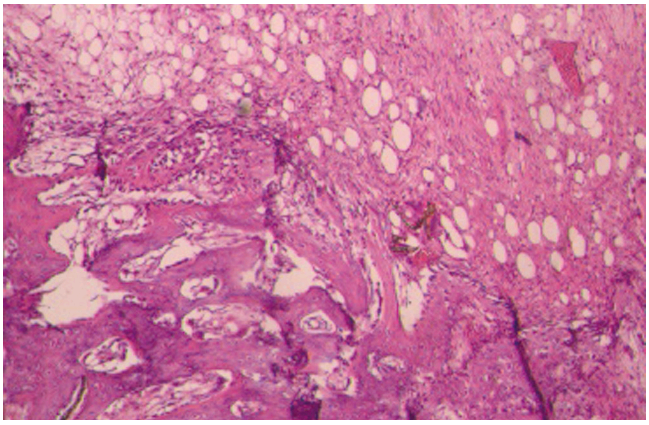

Fig. 4. View of 4-weeks experimental group shows woven bone (WB), new bone trabeculae (BT) filled thread area with osteoblasts (OB) and osteocytes (OC) H&E ×10

Рис. 4. В препаратах экспериментальной группы на 4-й неделе определяется грубоволокнистая кость (WB) и новообразованные костные трабекулы (BT), заполняющие область дефекта, с наличием остеобластов (OB) и остеоцитов (OC); окраска гематоксилином и эозином (H&E) ×10

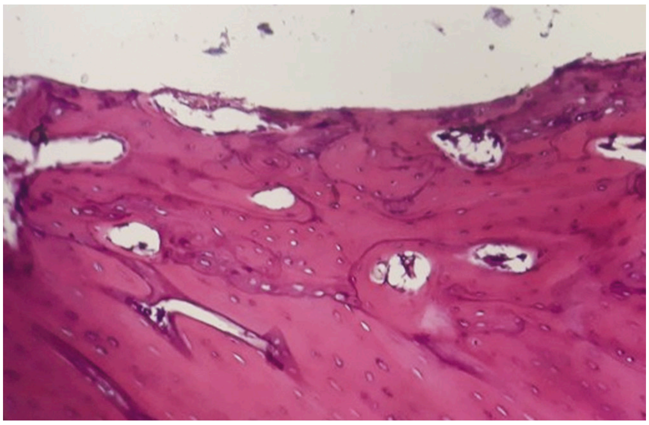

Fig. 6. Histological view of 6 weeks experimental group shows mature bone rimmed by osteoblasts, Haversian lamellae with osteocytes (OC) that are arranged in a circle around Haversian canal (HC) and reversal line (RL) H&E ×20

Рис. 6. Гистологическая картина экспериментальной группы на 6-й неделе: зрелая кость, окаймленная остеобластами, с гаверсовыми ламеллами, содержащими остеоциты (OC), расположенные вокруг гаверсового канала (HC), и линией реверсии (RL); окраска гематоксилином и эозином (H&E) ×20

Table 1. Statistical analysis of the bone architecture parameters for the experimental and control groups

Таблица 1. Статистический анализ параметров структуры кости для экспериментальной и контрольной групп

Variable	Duration	N	Control				Coating			
			Mean	SD	*F*-test	*p*-value	Mean	SD	*F*-test	*p*-value
Bone formation area	2-week	10	1.32	0.08	9.283	0.004	1.45	0.07	22.134	0.000
	4-week	10	1.53	0.04			1.67	0.04		
	6-week	10	1.92	0.06			2.81	0.07		
Osteoblasts N.	2-week	10	6	0.9	5.292	0.000	6.3	1.4	25.603	0.000
	4-week	10	12.1	1.2			16.7	2.6		
	6-week	10	15.4	2.3			14.4	2.7		
Osteocytes N.	2-week	10	0	0	6.358	0.000	3	0.4	18.407	0.000
	4-week	10	6.2	0.95			8.7	0.3		
	6-week	10	10.5	1.7			11.3	1.8		
Osteoclasts N.	2-week	10	0.5	0.46	4.547	0.000	0.47	0.2	22.896	0.000
	4-week	10	2.14	1.63			1	0.3		
	6-week	10	0.9	0.51			0.9	0.12		

Experimental 6-week

The histological view reveals well established mature bone rimmed by osteoblast and filled with osteocytes occupied their small lacunae in thread area. In higher magnification the mature bone characterized by presence of Haversion lamellae (Fig. 6).

Histomorphometry analysis of study groups for bone architecture parameters

The mean values of the descriptive statistics of the bone architecture parameters for the experimental and control groups rise with over time in all healing intervals. In every recovery period, the SiO_2-coated group's mean values are greater than the control groups. Both the experimental and control groups' mean numbers of osteoblasts and osteocytes rose over time, with the SiO_2-coated group's mean values rising faster than the control group's at intervals of two, four, and six weeks. The osteoclasts number was lower in the SiO_2-treated group than in the control group at the same interval, according to the mean values for both groups in the week.

The assessment region's quantitative histomorphometry analysis showed that while both groups' new bone area (%) increased, the experimental group's specimens showed substantially greater growth than the control group's at two-, four-, and six-week intervals. Normality test showed normal distribution of data for all groups (for Shapiro test, p value greater than 0.05).

DISCUSSION

Employing SiO_2 to analyze and compare the bone response to PEEK implants coated and uncoated. A histomorphometric analysis of the implant-bone contact in rabbits at various healing times was used to conduct the evaluation. The findings of the current study showed that because coated implants osseointegrate more quickly than uncoated implants, they had a better bone response. In dental implantology, altering implant surfaces to encourage osseointegration with bone is a relatively new strategy. Even though histomorphometry analysis is a destructive test, it is nonetheless regarded as a representative test for assessing implant stability and researching the quality of implant-to-tissue contact. It can be used whenever the implant has been surgically inserted. Many researchers have evaluated bone-to-plant contact using this analytical method [21; 22].

Two weeks following implantation, the histological analysis of the coated groups revealed higher signs of new bone formation encircled by osteoblast cells and an abundance of osteocytes with active blood vessels at the thread-interface region than the uncoated group. Histological characteristics of the uncoated group specimens at the six-week mark of the healing interval showed that the implant-bone interface region was filled with new bone, which was plentiful, and that the Harversian canal was lined with osteoblasts and osteocyte cells. These results suggest that processing was ongoing in the bone deposition area. However, six weeks after implantation, the coated group specimens had almost mature, new, and thick bone with osteocytes distributed regularly throughout the Harversian system. The current study demonstrated that superior osseointegrated implants were produced when implants were placed into living bone in an environment that was more favorable for bone regeneration due to the addition of encouraging elements. This outcome is consistent with Ibrahim et al. [23].

The high osteoconductivity of the coated surface was demonstrated by the notable changes in the overall amount of bone tissue growth surrounding the implant in the cortical region and bone marrow. At the nanoscale, a highly ordered nanoporous silica structure formed, and the silica-covering particles were homogeneous and free of microdefects. These findings show that adding silica covering with a nanoporous structure, which was shown in a prior work to promote cell adhesion and differentiation processes [24; 25].

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