Kamila Narojczyk[1], Barbara Nasiłowska[2], Marta Kutwin[3], Robert Szczepaniak[4]
1. Doctoral School, Military University of Technology, gen. S. Kaliskiego 2, 00-908 Warsaw, Poland
2. Department of Nanobiotechnology, Institute of Biology, Warsaw University of Life Sciences, Ciszewskiego 8, 02-786 Warsaw, Poland
3. Institute of Optoelectronics, Military University of Technology, gen. S. Kaliskiego 2, 00-908 Warsaw, Poland
4. Faculty of Aviation, Polish Air Force University, Dywizjonu 303 No. 35, 08-521 Deblin, Poland

SELECTED PERFORMANCE CHARACTERISTICS OF GRAPHENE – OXIDE – COATED DENTAL IMPLANTS

1. Introduction

The steady increase in surgical procedures to replace missing teeth has led to a growing demand for dental implants, which are primarily made of titanium or its alloys — materials that are commonly used in dental surgery due to their excellent biocompatibility. Various types of implant are used in clinical practice, but the most common are endosseous implants, which are screw-shaped and replace missing tooth roots [1, 2]. Pure titanium (98.9–99.6% Ti) and alloyed titanium (Ti-6Al-4V) are the main materials used in dental surgery. They are characterised by high biocompatibility and resistance to various types of corrosion. The TiO_2 layer formed on their surfaces creates favourable conditions for the formation of bone-like tissue and cellular reactions [3, 4, 5]. However, this process prolongs osseointegration and slows down tissue regeneration. Although this method of treating tooth loss is generally considered safe, with an approximate success rate of 90–95%, the risk of complications increases as implant-based tooth replacement procedures become more popular [6]. Introducing such a biomaterial into the human body triggers an immune response and initiates inflammatory and reparative processes. The success of the osseointegration process is influenced by several properties of the material itself, including wettability, roughness, surface energy and corrosion resistance [7]. A method for producing hyperhydrophilic titanium surfaces is presented in publication [8]. The modified surface demonstrated a reduced number of monocytes and macrophages (inflammatory cells), along with the formation of a thick, well-developed clot and a stable, continuous layer of platelets and plasma proteins permanently bound to the strongly hydrophilic surface under study. Therefore, it can be concluded that hydrophilic surfaces strongly promote tissue integration.

Corado [9] presented the results of studies on the mechanical and structural properties of titanium materials coated with graphene oxide. The Ti + GO material demonstrated good wettability, with a contact angle (θ) of 44.35° between water and the tested surface. Electrochemical corrosion tests also showed an increase in corrosion resistance. Roughness analysis revealed a decrease in the parameters for the graphene oxide-coated material.

Various types of complications can occur, including mechanical, biological and patient non-compliance. Common bacterial infections include peri-implantitis, which affects approximately 20% of patients, and peri-implant mucositis, which is a milder form of infection affecting up to 50% of cases [10].

In the human oral cavity, an implant is constantly exposed to bacteria and biofilm formation, whereby microorganisms adhere to one another and to the surface in a multilayered structure. This structure is surrounded by an extremely durable outer layer that protects the bacteria from the penetration of antibiotics. Consequently, treating peri-implant infections poses a significant challenge to modern implantology. Efforts are constantly being made to improve the surface properties and antibacterial properties of the implants used [11].

Consequently, research is underway to develop new methods of modifying implants to give them improved biological, mechanical and physicochemical properties. Using nanomaterials, such as graphene oxide, could solve this problem. Due to its unique properties, including biocompatibility, the promotion of adhesion and proliferation of human osteoblast cells, and antibacterial activity, graphene oxide may represent an interesting alternative to current implantation methods [12]. Researchers conducted studies on the behaviour of cells and tissues in contact with graphene oxide [13]. They created a scaffold by coating a collagen sponge with a graphene oxide dispersion at concentrations of 0.1 and 1 μg/mL, which they then implanted into the alveolar socket following tooth extraction. It was found that graphene oxide improved mechanical strength, enzymatic resistance and the adsorption of calcium ions and proteins. Additionally, the proliferation of MC3T3-E1 osteoblasts increased significantly. Studies conducted on rats showed that the implantation of a prepared scaffold containing 1 μg/mL of graphene oxide stimulated cell ingrowth. The tissue ingrowth area was 2.5 times larger and the genetic material content in the tested scaffolds increased by a factor of 1.4 compared to the control group. Furthermore, the GO scaffold promoted bone formation, which was five times greater than in the control group.

The antibacterial properties of graphene oxide have also been demonstrated. A research group led by Ravinkumar et al. conducted a study analysing the viability of a Staphylococcus aureus bacterial strain cultured in the presence of graphene oxide. They observed a reduction in bacterial survival after just four hours, with survival rates at around 50%. However, after a further 20 hours of incubation, this figure had increased to around 93%. Scanning electron microscopy analysis showed that graphene oxide structures surrounded the bacteria, damaging their cell membranes. Additionally, the induction of intracellular reactive oxygen species was observed, which was associated with a decrease in microbial viability, as well as changes in the expression of cell membrane-associated proteins [14].

A preliminary literature review has demonstrated graphene oxide's great potential for modifying the surfaces of titanium dental implants. It combines desirable mechanical and biological properties, and exhibits strong antimicrobial activity — an extremely important quality in such a demanding environment as the human oral cavity. The review indicates that surfaces modified with graphene oxide stimulate cell adhesion and osteoblast differentiation, thereby favouring osseointegration processes.

This presentation outlines the preliminary findings of mechanical, biological and structural analyses of titanium materials coated with a graphene oxide layer. The following analyses were conducted: structural surface analyses using a scanning electron microscope, IR spectroscopy and LIBS; bacteriological analyses of the viability of bacteria seeded on the surface of the tested samples and the degree of growth inhibition; and preliminary mechanical tests.

2. Materials and Methods

Dental screw posts purchased from Shenzhen Felyang Medical Equipment Co., Ltd. were used in this study. The sample preparation process included disinfecting the materials in their as-delivered state with ethanol, cleaning them and activating their surfaces with low-temperature plasma using a Plasma Prep III (Garfield Ave, Westchester, PA, USA) at a power output of 100 W for 5, 10 or 15 minutes. Next, graphene oxide was applied by immersing the samples in an aqueous GO suspension with a concentration of either 500 mg/L or 5 g/L (Graphene Supermarket, NY 11933, USA) for a specified time. Excess suspension was then removed using an Eppendorf 5702 RH device. The samples were then dried at room temperature for approximately 10 minutes. In the next step, gold nanoparticles were deposited using an EM ACE600 high-vacuum sputter coater (Leica Microsystems GmbH, Germany) to form a 3–5 nm thick layer. By combining the specified parameters in this way, 25 measurement samples were obtained.

3. Research results

Structural analyses using SEM, Raman spectroscopy and LIBS were performed, along with factor analysis. Preliminary mechanical tests were also conducted, including static compression testing of implants embedded in bone tissue. Additionally, tests were performed to determine the extent of bacterial growth inhibition in Staphylococcus aureus.

Selected results of the SEM (Quanta 250 FEG) analysis of the implant surfaces are presented in Fig. 1. The arrows in this figure indicate the structure of graphene oxide flakes deposited on the surface.

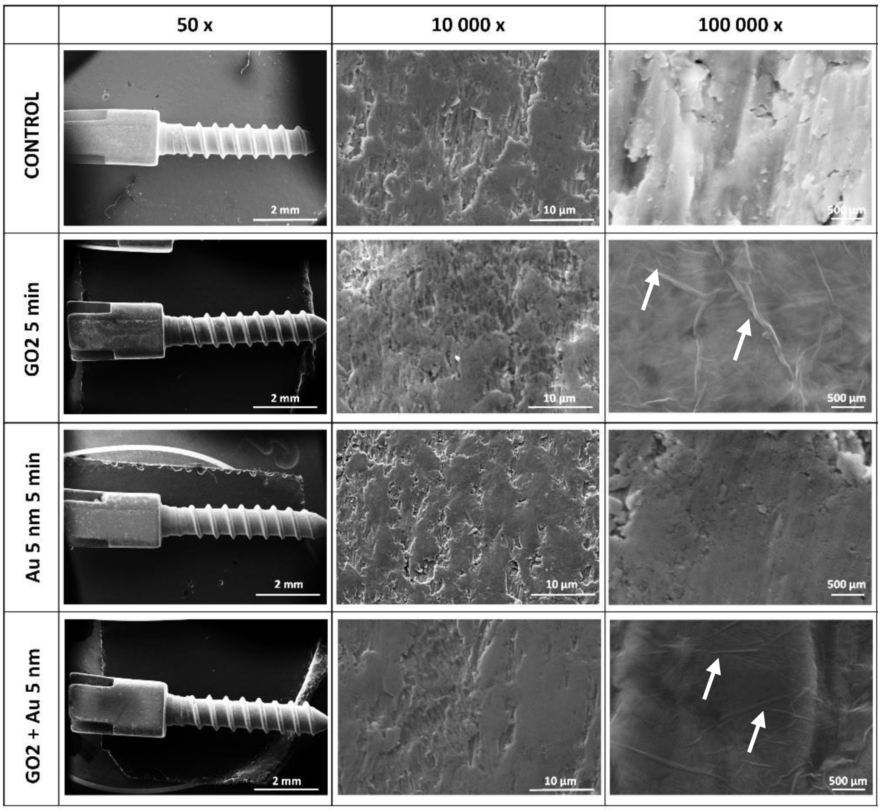

Fig. 1. The sample surfaces were analysed using SEM at magnifications of 50×, 10,000× and 100,000×.

The antibacterial properties of graphene oxide deposited on the surface of the tested samples (Fig. 2) were analysed by determining the viability of Staphylococcus aureus (ATCC 25923) after a 24-hour incubation period under standard conditions at 37 °C. The PrestoBlue assay (ThermoFisher, Waltham, MA, USA) was performed by adding the reagent to the liquid culture and incubating for 30 minutes. The absorbance was then measured at a wavelength of 570 nm with a reference wavelength of 600 nm. The test was performed in triplicate.

Cultures on pure titanium exhibited the highest viability value, reaching over 140%. In contrast, for the sample with a deposited layer of GO1 graphene (500 mg/L) and a 5-nm-thick gold layer following 5 minutes of plasma treatment, the viability of the microorganisms dropped to approximately 60%. Similar results were obtained for cultures on a sample with a 3 nm thick gold layer following 10 minutes of plasma surface activation.

3. Conclusions

Based on the studies conducted, it was found that graphene oxide nanoparticles had been successfully deposited on the surface of the samples tested. The boundaries of the graphene flakes and the characteristic carbon groups were visible, indicating the presence of GO. Tests on the viability of Staphylococcus aureus bacteria showed reduced viability in all samples tested compared to the control group.

Referencje:

[1] Gaviria, L., Salcido, J. P., Guda, T., & Ong, J. L. (2014). Current trends in dental implants. Journal of the Korean Association of Oral and Maxillofacial Surgeons, 40(2), 50–60. https://doi.org/10.5125/jkaoms.2014.40.2.50

[2] Begum, S., Peeran S. Classification of dental implants. In: 9 Int Dental Students, 7th Dental Surgeons Conf 2021 Souvenir. 65.

[3] Oldani, C., Dominguez, A. "Titanium as a Biomaterial for Implants." Recent advances in arthroplasty 218 (2012): 149-162.

[4] Hansson, H. A., Albrektsson, T., & Brånemark, P. I. (1983). Structural aspects of the interface between tissue and titanium implants. The Journal of prosthetic dentistry, 50(1), 108–113. https://doi.org/10.1016/0022-3913(83)90175-0

[5] Legeros, R. Z., & Craig, R. G. (2009). Strategies to affect bone remodeling: Osteointegration. Journal of Bone and Mineral Research, 8(S2), S583–S596. doi:10.1002/jbmr.5650081328

[6] Karoussis, I. K., Salvi, G. E., Heitz-Mayfield, L. J., Brägger, U., Hämmerle, C. H., & Lang, N. P. (2003). Long-term implant prognosis in patients with and without a history of chronic periodontitis: a 10-year prospective cohort study of the ITI Dental Implant System. Clinical oral implants research, 14(3), 329–339. https://doi.org/10.1034/j.1600-0501.000.00934.x

[7] Rupp, F., Liang, L., Geis-Gerstorfer, J., Scheideler, L., & Hüttig, F. (2018). Surface characteristics of dental implants: A review. *Dental materials : official publication of the Academy of Dental Materials*, *34*(1), 40–57. https://doi.org/10.1016/j.dental.2017.09.007

[8] Milleret, V., Tugulu, S., Schlottig, F., & Hall, H. (2011). Alkali treatment of microrough titanium surfaces affects macrophage/monocyte adhesion, platelet activation and architecture of blood clot formation. *European cells & materials*, *21*, 430–444. https://doi.org/10.22203/ecm.v021a32

[9] Corado, H. P. R., Moura de Souza Soraes, F., Barbosa, D. M., Lima, A. M., & Elias, C. N. (2022). Titanium Coated with Graphene and Niobium Pentoxide for Biomaterial Applications. *International journal of biomaterials*, *2022*, 2786101. https://doi.org/10.1155/2022/2786101

[10] Lee, C. T., Huang, Y. W., Zhu, L., & Weltman, R. (2017). Prevalences of peri-implantitis and peri-implant mucositis: systematic review and meta-analysis. *Journal of dentistry*, *62*, 1–12. https://doi.org/10.1016/j.jdent.2017.04.011

[11] Mombelli A. (2012). Antimicrobial advances in treating periodontal diseases. *Frontiers of oral biology*, *15*, 133–148. https://doi.org/10.1159/000329676

[12] Li, X., Liang, X., Wang, Y., Wang, D., Teng, M., Xu, H., et al. (2022b). Graphene-based nanomaterials for dental applications: principles, current advances, and future outlook. Front. Bioeng. Biotechnol. 10, 804201. doi:10.3389/fbioe.2022.804201

[13] Nishida, E., Miyaji, H., Kato, A., Takita, H., Iwanaga, T., Momose, T., Ogawa, K., Murakami, S., Sugaya, T., & Kawanami, M. (2016). Graphene oxide scaffold accelerates cellular proliferative response and alveolar bone healing of tooth extraction socket. International journal of nanomedicine, 11, 2265–2277. https://doi.org/10.2147/IJN.S104778

[14] Ravikumar, V., Mijakovic, I., & Pandit, S. (2022). Antimicrobial Activity of Graphene Oxide Contributes to Alteration of Key Stress-Related and Membrane Bound Proteins. *International journal of nanomedicine*, *17*, 6707–6721. https://doi.org/10.2147/IJN.S387590