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Effect of Surface Treatment Method of Light-Cured Material on Its Toxic Properties

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Abstract

In recent decades, a large number of different nanocomposite materials have appeared, which have found wide application in almost all areas of life, including medicine. However, to date, the properties of these materials have not been completely studied. The evaluation of toxicity and biocompatibility is particularly relevant. In this regard, this study aimed to investigate the effect of the surface treatment method of light-cured material on its toxic properties for normal fibroblasts.

For this purpose, light-cured nanohybrid composite material Herculite XRV Ultra in the form of 10×5 mm plates (smooth and notched) were incubated with a culture of rat fibroblasts, after that, morphological changes were assessed, and "toxic exposure zones" (a distance from the test plate to a layer of intact fibroblasts) were measured. As a result, it was ascertained that although the investigated material has moderate toxicity to normal cells of the organism, the degree of the nanocomposite toxicity and, in particular, the size of the zone of toxic influence is significantly affected by the properties of its surface, depending on the mechanical action on the restorative material.**(International Journal of Biomedicine. 2023;13(4):356-359.)**

Keywords: nanocomposite • light-curing material • fibroblasts • cell culture • BioStation CT

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Introduction

The development of nanocomposite materials is one of the most popular but not fully studied areas of nanotechnology. In recent decades, a great step forward has been made in the creation of particles ranging in size from 1 to 100 nm .[(1)] Nanoparticles, which can be both organic (liposomes, polymers) and inorganic (metals, metal oxide, ceramics), as well as carbon-based ones,[(2)] are used in various fields of activity, among which one of the main places is occupied by modern medicine.[(3)] At the same time, materials based on nanoparticles are utilized not only in diagnostic and therapeutic practice, but also in scientific areas of medicine.[(4,5)] These include nanocomposites for manipulating biological systems, nanoparticles heated by magnetic fields and used for thermal ablation or other delicate effects on cells, systems for targeted drug delivery without biological and chemical signals, and nanoparticles applied as contrast agents for biological imaging.[(6)]

For more than four decades, composite restoration materials have been used in dentistry[(7)] not only as a filling material, but also for the production of reliable dental structures and devices.[(8)] The leading positions among restoration materials are occupied by universal light-cured composites.[(9)] These materials have a number of advantages compared to chemically curing materials, which include resistance to chemical and thermal effects from the aggressive environment of the oral cavity, reliable connection of a hydrophobic composite and hydrophilic dentin, high physical and biochemical compatibility with dental tissues, and others.[(10)] The polymerization reaction of such composites is known to occur under the influence of light energy with a wavelength of 400-500 nm, i.e., visible blue light, which is considered the safest for living cells and tissues, and without the formation of by-products.[(11,12)] In this regard, currently, light-cured composites have almost completely replaced chemical fillings. In addition to filling, photopolymers

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are widely used for the restoration of teeth, the manufacture of composite veneers and inlays, for splinting of teeth in the periodontitis treatment, as well as repair of ceramic chips on crowns. However, despite their undeniable advantages, the safety issues of using these materials remain relevant. In this connection, in biomedical research, special attention is paid to the study of potential composite material toxicity to normal cells of the organism.

Thus, this study aimed to investigate the effect of the surface treatment method of light-cured material on its toxic properties for normal fibroblasts.

Material and methods

The primary culture of fibroblasts was obtained from the omentum of three-month-old male Wistar rats weighing 200–250 g. The experiments were performed in accordance with the norms for the humane treatment of animals, which are regulated by the International Guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care, following the protocol approved by the Institutional Animal Care and Use Committee of the Irkutsk Scientific Center of Surgery and Traumatology (Protocol No. 9 of 12/16/2021).

To obtain a pure culture, fibroblasts were subcultured every 7 days. After passage 3, the resulting pure culture of fibroblasts was used for experimental studies.[13,14]

For research, a modern light-curing nanohybrid universal composite material, Herculite XRV Ultra Intro Kit (KERR, USA), a combination of a polymer, barium glass nanoparticles, and a silicon nanofiller, was chosen. From this material, two plates with a size of 10 mm × 5 mm were formed: the first plate had a smooth surface, and the second one had grooves (notches) mechanically inflicted on its surface.

The resulting samples were placed in culture plates containing fibroblast monolayer cultures, and the morphological changes in cells were evaluated during 6 days of incubation. The "zone of toxic effect" was determined by measuring the distance from the plate to the fibroblast layer in microphotographs on days 2, 3, and 6.

Photodocumentation of the morphological changes in cells and assessment of the influence of samples with different surface characteristics on fibroblast culture were carried out using the BioStation CT 4.1 (Nikon) ("Diagnostic images in surgery"). The measurement of the "toxic effect zone" was performed with Nis-Elements AR, 4.1 (Nikon) software product. Statistical analysis was carried out in the R programming environment. The Wilcoxon-Mann-Whitney test was used for pairwise comparison.

Results

As a result of observations, it was found that a day after the introduction of samples with and without notches into the fibroblast culture, in both cases, the cell monolayer looked intact and was in contact with the surface of the plates. However, in the case of testing the notched sample, slight toxic granularity was observed among the fibroblasts adjacent to the plate surface (Figure 1).

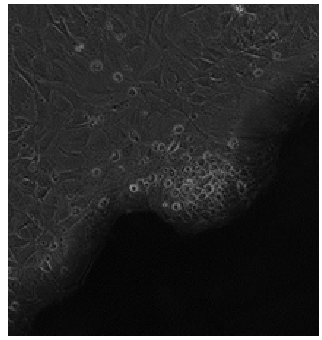
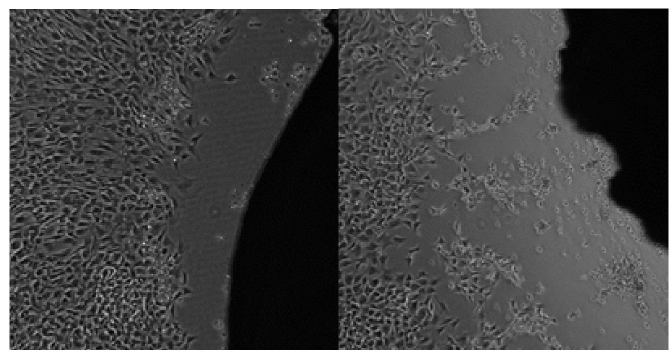


Fig. 1. *Fibroblast culture incubated with notched sample, 1 day after the start of the experiment. Phase contrast, 10x magnification.*

On the second day of the experiment, changes in cell morphology were observed, fibroblast detachment and the intact cell layer removal from the surface of the nanohybrid samples were recorded. With the sample without notches, cell detritus and altered fibroblasts were noted only near the sample surface, while the fibroblast monolayer looked intact.

When cells were incubated with notched samples, in the zone of contact with the plate, dead fibroblasts with toxic granularity were observed, followed by a layer of morphologically altered cells. During the subsequent incubation of fibroblast culture with the tested samples, the described effects continued to develop, and their progression was registered at 3 and 6 days (Figure 2A, 2B).



A B

Fig. 2. *Fibroblast culture incubated with samples without notches (A) and with notches (B), 3 days after the start of the experiment. Phase contrast, 4x magnification.*

When measuring the "zone of toxic influence," it was found that after days 2, 3, and 6, this zone was significantly wider for a sample with notches than without notches (Figure 3).

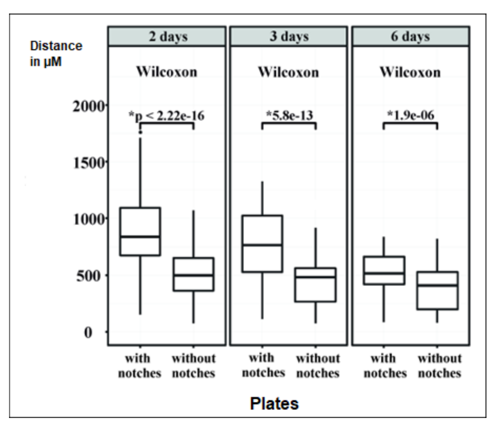


Fig. 3. *The distance between fibroblasts and plates with and without notches at various observation periods. The Wilcoxon-Mann-Whitney test.*

Discussion

The significance of nanocomposite materials with particle sizes from 1 to 100 nm is continuously increasing, as is the importance of such materials for medical practice. At the same time, it is known that the properties of synthesized materials depend mainly on the size and the characteristics of the functional surface of nanoparticles. In particular, the characteristics of nanocomposites, such as solubility, transparency, color, absorption or emission wavelength, conductivity, melting temperature, and catalytic behavior, can be controlled by changing the particle size. However, as the size decreases, the fraction of atoms found on the nanoparticle surface increases relative to their fraction inside the particle volume.[1] For example, a nanoparticle with a radius of 2.5 nm and a density of 5 g/cm^3 has an area of 240 m^2/g when assuming a spherical shape. Consequently, about 20% of the particle atoms are on its surface, which means that such nanoparticles can be more reactive.[15] In addition, due to high energy adhesive forces close to the surface, particles either agglomerate with each other or act as a filter to other small molecules, so changing the composition, size, or surface features can significantly modify the physical and chemical properties of nanoparticles whose components individually also have biological activity. Thus, the composition of light-cured materials includes, as a rule, fine-dispersed particles of silicon dioxide, pre-polymerized fillers, and organic matrix;[16] additional components are introduced to improve strength and reduce shrinkage to prevent degradation, as well as prolong the durability of the material.[17] At the same time, it is known that silicon is not only an indispensable element in the development of human bones and joints, but is also closely related to the immune system and connective tissue regulation. Silicon ions are able to induce the formation of calcium matrices, enhance bone regeneration through the ERK MAPK signaling pathway, and increase the secretion of type I collagen.[18] Some investigations have confirmed that silicon ions can activate the WNT pathway of bone marrow stromal cells, thus promoting the proliferation, differentiation, and expression of osteogenic proteins.

However, despite the unique physicochemical and biological properties of nanocomposite materials, not only their mechanical and aesthetic properties are important for their use in dental practice, but also their potential biocompatibility and non-toxicity for normal organism cells. For instance, the literature describes cases of adverse effects of light-cured material on biological tissues,[19,20] as well as a decrease in the viability of cell cultures, which is enhanced with increasing exposure time of the composite.[21] In the latter case, after 72 hours of incubation with the tested light-cured materials, the researchers observed more severe cytotoxic effects compared to the results after 24 hours of exposure. Additionally, it was found that after insufficient photopolymerization, the remaining free monomers are the main cytotoxic agents, and they can be released into the pulp tissue, which leads to inflammation and, ultimately, necrosis.[22] It should also be noted that the material strength depends not only on the proportion of inorganic components, but also on the particles' size, shape, and microstructure.[23]

In addition to the direct influence of the component composition of nanocomposite material, its toxicity and biocompatibility can be affected by such factors as the route of introduction and systemic distribution in the organism, as well as the properties of the surface in contact with living cells of the organism.[21,24] So, our study established a statistically significant dependence of the material toxicity on the roughness of its surface. It was demonstrated that the mechanical treatment of the sample, on the one hand, destroying the nanocomposite matrix integrity, and on the other hand, increasing the contact area of the material with the analyzed cells, makes a significant contribution to the toxic properties of the nanohybrid material Herculite XRV Ultra. Previously, sources have already noted that Herculite XRV Ultra has a high porosity,[25] which suggests an increase in the interaction area of the material with surrounding tissues; however, a decrease in its roughness after the polishing procedure was also demonstrated.[26] This indicates the need for careful polishing when using this composite material in dental practice. At the same time, it was found that imitation of exposure to the material identical to regular brushing of teeth during the year leads to an increase in its roughness,[27,28] which is also important to take into account, based on the changes in the toxic properties of the material established by us, depending on the surface unevenness. This study also indicates that the investigated filling material should not be under the gum and should not be in direct contact with gum tissue, since it has a cytotoxic effect on fibroblasts.

Conclusion

In this study, it was found that this light-cured nanocomposite has moderate toxicity to fibroblasts, and its toxic properties are determined not only by the chemical composition of the nanohybrid material, but also by the characteristics of its surface, depending on the mechanical influence on the restoration material. The method of toxicity assessment used in the study can be defined as universal for testing new materials, which are supposed to be applied in practice in a solid form insoluble in aqueous media.

Competing Interests

The authors declare that they have no competing interests.

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