ABSTRACT
of the Thesis for the Philosophy Doctor (PhD) degree
Specialty "6D074000-Nanomaterials and Nanotechnology"
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Bioactive composite material based on bacterial cellulose, hydroxyapatite and nano-antiseptics: structure and properties

General characteristics of the dissertation work. This work is devoted to the experimental development of a method for producing composite materials based on a nanofibrillar hydrogel film of bacterial cellulose (BC) with embedded $Ti_3C_2T_x$MXene and hydroxyapatite (HAP) nanoparticles. The structural and phase state of the resulting nanocomposites, their morphological characteristics, antibacterial activity, and *in vitro* biocompatibility are studied.

Relevance of the topic. The current stage of development in nanomaterials and biomedical engineering is characterized by the active development of antimicrobial structures that combine functional effectiveness and biocompatibility. This is particularly true for materials designed to combat wound infections associated with antibiotic-resistant pathogens that form biofilms. An innovative approach to the development of such nanocomposite materials involves the incorporation of novel inorganic antiseptic components or the modification of biomaterials with bioactive ions to ensure not only antimicrobial activity but also the initiation of a controlled tissue reaction that promotes wound healing. Wound dressings based on BC hydrogel, a biopolymer with an ultrafine three-dimensional nanomesh structure, have been the subject of many developments in this area. However, BC primarily functions as a physical barrier against wound infection, so an alternative strategy is needed to develop a bifunctional platform that inhibits the formation and/or breakdown of biofilms at the wound site while stimulating skin regeneration. One component of such a platform could be MXenes – two-dimensional (2D) nanomaterials belonging to the family of transition metal carbides, nitrides, and carbonitrides. There is encouraging evidence that MXenes or materials containing them inhibit biofilm formation and even promote their destruction.

On the other hand, there is evidence that MXenes can exhibit cytotoxicity at high concentrations, limiting their use in certain medical applications. The challenge of balancing efficacy and biocompatibility can be addressed by adding HAP to the composite, which can reduce cytotoxicity and accelerate wound healing through calcium-mediated coagulation, which is essential for tissue regeneration. It is believed that integrating MXene and HAP into the BC scaffold will combine the antibacterial action of MXene with the bioactivity of HAP, creating a multifunctional platform.

Despite active research in this area, information on composites consisting of BC with incorporated HAP and MXene nanoparticles is lacking. Previously, only two-component systems of BC/MXene, BC/HAP, and HAP/MXene have been reported.

Therefore, it is necessary to develop a method for synthesizing a hybrid BC/MXene/HAP nanomaterial, which requires careful optimization of all process stages. Particular attention should be paid to the selection of formation conditions for each component, the sequence of their introduction, and ensuring stable interactions between the organic matrix and inorganic nanophases. Furthermore, the composite production method must preserve the gel form of BC with its nanofibrillar structure, as it is in this state that the material can exhibit its characteristic physicochemical properties.

Aim of the study: To develop and characterize the structural and antimicrobial properties of a three-component nanocomposite material made of bacterial cellulose, MXene and hydroxyapatite.

Objectives of the work:

- analyze the structural and mechanical properties of BC gel films obtained in a molasses-containing nutrient medium;
- obtain BC-based composite materials with reinforcing components: MXene and hydroxyapatite;
- study the elemental, phase, and functional compositions and morphology of the BC nanogel film after intercalation of MXene and hydroxyapatite nanocomplexes;
- select a sterilization method for the composite hydrogel that preserves its structural and functional properties;
- study the antimicrobial and antibiofilm properties of the three-component composite material in a time-kill test and on a chronic wound biofilm (LCWB) model;
- determine the cytotoxicity and biocompatibility of the three-component composite material *in vitro*.

Research objects: BC gel films, as well as experimental composite samples containing hydroxyapatite nanoparticles and $Ti_3C_2T_x$MXene incorporated into their structure.

Research methods: BC production – surface cultivation; $Ti_3C_2T_x$ – chemical etching; HAp – chemical precipitation; BC/HAp – *in situ* and *ex situ* modifications; HAp/MXene – physicochemical hybridization; BC/HAp/MXene and BC/MXene composites – immersion method (*ex situ*). Film and composite morphology – scanning electron microscopy (JSM-7800F); Structural-phase state – transmission electron microscopy, X-ray structural analysis; Functional composition – IR spectroscopy (Bruker Alpha II FTIR); Elemental composition and spatial distribution – energy-dispersive X-ray spectroscopy (JEOL JED-2300T CENTURIO SDD); Crystalline structure of composites – X-ray diffraction (XRD) (PANalytical Empyrean, Malvern Panalytical); Chemical composition – high-resolution X-ray photoelectron spectroscopy (HR-XPS); Film strength – tensile testing machine “Instron”; Antibacterial activity – Time-kill test; Antibiofilm activity – microplate method, *in vitro* model of chronic wound biofilm (LCWB); Biocompatibility – viability, adhesive properties, metabolic activity, morphology, and proliferation of NIH3T3 mouse fibroblasts.

Scientific novelty of the research results.

A new nanocomposite material, BC/HAP/MXene, has been developed for the creation of a multifunctional wound dressing with antiseptic, antibiofilm, and antioxidant properties.

For the first time, it has been demonstrated that *ex situ* incorporation of the HAP/MXene complex into the BC matrix minimizes oxidation of two-dimensional titanium carbide and preserves the nanofibrous hydrogel structure of BC.

For the first time, the composition of an economical molasses-based nutrient medium has been proposed and optimized, ensuring the preservation of the nanofibrillar structure and mechanical strength of BC with an increased level of its biosynthesis.

Theoretical significance of the research.

The choice of nanocomposite material composition, in which the antimicrobial and antibiofilm effects are determined by the interaction of functional nanophases, is theoretically and experimentally substantiated.

The pathways for an economically efficient method of obtaining BC and its functionalization with inorganic bioactive components—MXene and HAp—to obtain a biocompatible composite material with high antimicrobial activity, low cytotoxicity, and pro-regenerative properties were defined.

Possible mechanisms of the antimicrobial action of the composite's functional components are presented, including MXene-mediated generation of reactive oxygen species and HAp-mediated inhibition of fibrin polymerization, leading to the disruption of biofilm structure.

Practical value of the study.

The synergistic interaction of two-dimensional MXene nanostructures and HAP nanoparticles integrated into a three-dimensional nanofibrillar BC scaffold mimicking the extracellular matrix represents a promising next-generation material for the treatment of chronic wounds infected with multidrug-resistant microorganisms.

An optimized nutrient medium based on sugar production waste, as well as selected process parameters for culturing the producer strain, ensure the production of BC with a reproducible nanofibrillar structure and stable physical and mechanical properties. This material can be used in the scalable industrial synthesis of BC as a nanostructured matrix for the creation of functional nanocomposite materials.

Basic statements for the defense:

- BC synthesized in a modified nutrient medium supplemented with 2% molasses is characterized by a hierarchically organized nanofibrillar architecture, represented by a three-dimensional mesh matrix of tightly interwoven cellulose fibrils of nanoscale diameter, which determines the material's porous structure and its high mechanical strength (37.12 ± 0.2 MPa).

- The formation of a nanostructured composite material with a uniform distribution of reinforcing nanoparticles is due to the *ex situ* incorporation of a HAP/MXene nanocomplex preformed by physicochemical hybridization into the BC matrix.

- BC functionalized with MXene, which generates reactive oxygen species, exhibits pronounced antimicrobial properties, and HAP-mediated inhibition of fibrin polymerization and Ca^{2+}-dependent inactivation of the Bap protein accelerates the wound healing process.

The main research results and conclusions:

1. Mechanically strong (37.12 ± 0.2 MPa) and elastic (3.28 ± 0.2% elongation) nanostructured BC, consisting of nanofibers 15–35 nm in diameter and fibrillar bundles 50–150 nm in size, is formed on a modified medium containing molasses (2%). Cultivation of the *Komagataeibacter xylinus* C-3 strain on this medium for 7 days increases film formation productivity to 12.8 g/L and reduces the cost of the synthesized biopolymer fivefold.

2. A nanostructured composite material BC/HAP/MXene was obtained by integrating a pre-formed HAP/MXene complex into the BC matrix using the *ex situ* method, ensuring a uniform distribution of HAP nanoparticles and MXene sheets in the BC structure.

3. Comprehensive structural analysis of the BC/HAP/MXene composite confirmed the successful integration of all components at the nanoscale. SEM and EDS revealed individual HAP microagglomerates (1–5 μm) embedded in the BC/MXene matrix, with elemental mapping confirming the colocalization of Ca and P from HAP and Ti from MXene. Cross-sectional imaging with TEM and FIB provided direct evidence of HAP nanoparticles (50–200 nm) anchored at the interface between the BC and MXene layers, confirming the hierarchical phase-separated architecture. XPS analysis demonstrated a significant shift in the Ti 2p binding energy ($\Delta \approx 2.5$ eV) in the BC/HAP/MXene composite compared to BC/MXene, indicating strong interfacial electronic interactions between MXene and HAP. The addition of MXene/HAP increased the tensile strength to 43 MPa and increased the tensile elongation by 8%.

4. Methods of sterilization of BC/HAP/MXene nanocomposite with cold plasma or ethylene oxide are preferable, since they ensure minimal changes in the structure and functional properties of the material while maintaining electrical conductivity and photothermal response at a level of 94–98% of the original.

5. The incorporation of the HAP/MXene nanocomplex into BC imparts high antibacterial and antibiofilm activity to the BC/HAP/MXene composite against clinically significant multidrug-resistant pathogens. Bacteriostatic growth inhibition reached 95% (*P. aeruginosa*) and 99% (*S. aureus*). In a physiologically relevant chronic wound biofilm (LCWB) model, a reduction in MRSA viability of up to 98% and FQRPA viability of up to 86% was observed.

6. The nanostructured architecture of the BC/HAP/MXene composite promotes high adhesion of NIH-3T3 fibroblasts to the material surface and their active proliferation. Cells cultured in the medium containing the composite demonstrated high metabolic activity in the MTT assay (>81%), indicating the absence of significant cytotoxicity and acceptable biocompatibility of the composite.

Connection with the plan of basic scientific works.

The dissertation was carried out within the framework of the following projects: 1) 2679/GF4 “Development of biocomposite materials based on bacterial cellulose for the creation of transdermal therapeutic systems”; 2) AP23488697 “Strategy for developing a hydrogel photothermal platform with enhanced antibacterial and regenerative properties for the treatment of infected wounds.”

Publications. The main content of the dissertation is reflected in 11 published works, including 4 articles in journals indexed in the Web of Science and Scopus databases, 3 articles in republican scientific journals included in the list of CQAFSHE MSHE RK, 4 abstracts in materials of international conferences.

The author's personal contribution lies in carrying out the main volume of theoretical and experimental research, analysis, interpretation, presentation of the results obtained, and preparation of manuscripts for publications.

The volume and structure of the dissertation. The dissertation is presented on 148 pages of computer-typed text and consists of the following sections: designations and abbreviations, introduction, literature review, materials and research methods, results and discussion, conclusion, and a list of 285 references. The work includes 11 tables, 41 figures, and 1 appendix.