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Bismuth apatites as the basis of biomaterials for bone tissue regeneration

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INTRODUCTION & AIM

In recent years, increasing attention has been given to the search for new antimicrobial agents capable of combating the growing threat of multidrug-resistant microorganisms to traditional antibiotics. One promising direction is the use of metals, such as **bismuth and vanadium**, as antimicrobial agents. They exhibit high toxicity to microorganisms and can be used as an alternative to traditional antibiotics.

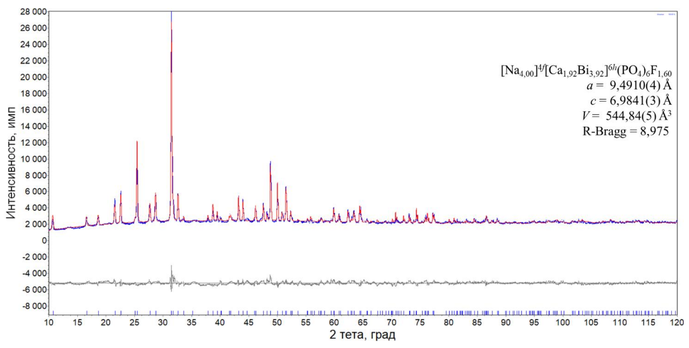
One promising material for biomedical applications is **apatite** containing one or both ions. Apatites are geomineral and biomimetic compounds that are a major component of bone tissue and have high biocompatibility with human tissues. Furthermore, this structural class is known for its high isomorphic capacity with respect to a significant portion of elements in the Periodic Table, including those possessing antimicrobial activity.

In this regard, the aim of our work is to develop new materials based on bismuth- and vanadium-substituted apatites with following compositions: $Ca_{10-2x}Bi_xNa_x(PO_4)_6F_2$ (x = 1, 2, 3, 4), $Ca_8BiNa(PO_4)_6O$ and $Ca_8BiNa(PO_4)_{5,5}(VO_4)_{0,5}O$.

METHODS

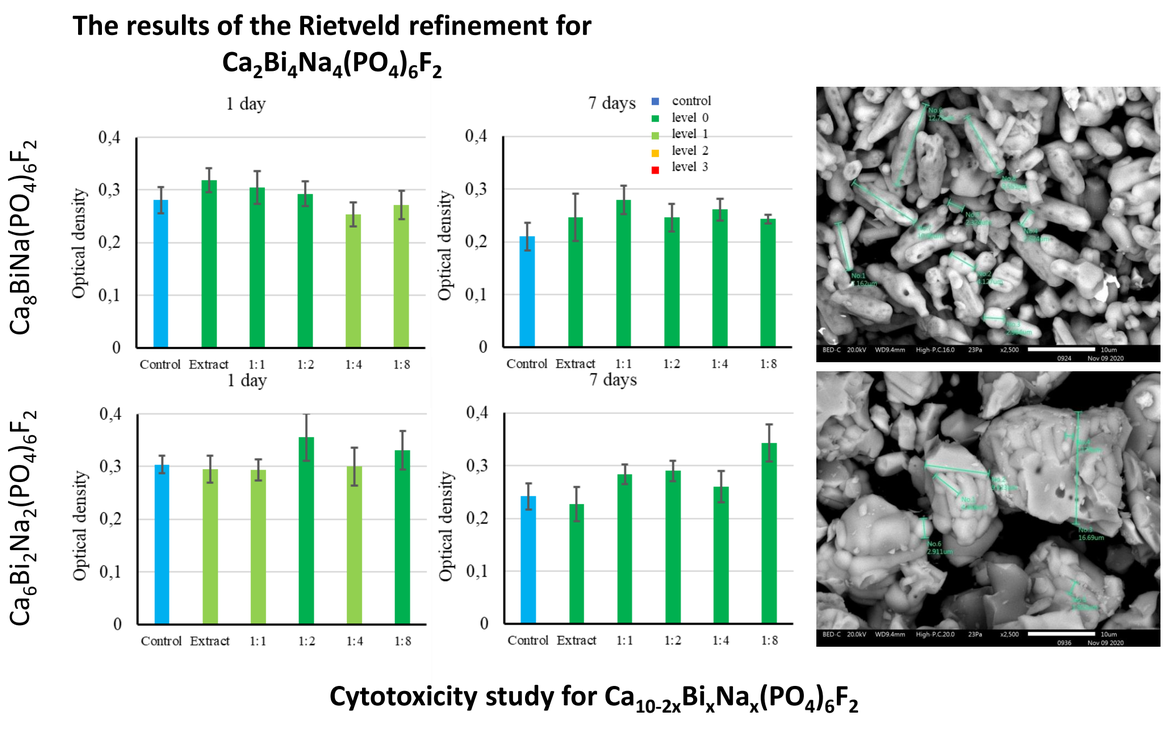
Powder X-ray diffraction method (Shimadzu XRD-6000) was used for substance **identification** based on characteristic sets of reflection peaks from crystalline planes. **Confirmation of the chemical composition** of the obtained substances was performed using energy dispersive X-ray microanalysis (EDX) with an X-MaxN 20 detector (Oxford Instruments). Scanning electron microscopy (JSM-IT300LV scanning electron microscope (JEOL) was used to study the **morphology** of the investigated substance. The study of the sample surface **topography** was performed using low-energy secondary electrons and backscattered electrons in high vacuum mode. **Refinement of the crystal structure** was performed using full-profile refinement of X-ray diffraction patterns obtained from the mentioned diffractometer using the Rietveld method. Infrared spectroscopy (Shimadzu FTIR 8400s) was used as an additional independent method for studying the **structure and group composition of the substance**. The investigation of the **phase stability** of the target compound was carried out in three media: deionized water, phosphate-salt buffer, and a 0.25% trypsin solution. The separated powders were analyzed using element analysis and X-ray phase analysis. The **cytotoxicity** of the obtained substance was determined using the standard MTT test. The **bactericidal activity** of the samples was determined against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria.

RESULTS & DISCUSSION



The results of the Rietveld refinement for $Ca_2Bi_4Na_4(PO_4)_6F_2$

x	Composition of the cationic sublattice for $Ca_{10-2x}Bi_xNa_x(PO_4)_6F_2$
1	$[Ca_{2,92}Na_{0,90}Bi_{0,19}]^{4f}[Ca_{4,88}Bi_{0,81}]^{6h}$
2	$[Ca_{1,71}Na_{2,00}Bi_{0,29}]^{4f}[Ca_{4,09}Bi_{1,65}]^{6h}$
3	$[Ca_{2,85}Na_{3,00}]^{4f}[Ca_{1,05}Bi_{3,00}]^{6h}$
4	$[Na_{4,00}]^{4f}[Ca_{1,92}Bi_{3,92}]^{6h}$
5	$[Na_{2,08}Bi_{1,92}]^{4f}[Na_{2,31}Bi_{3,69}]^{6h}$

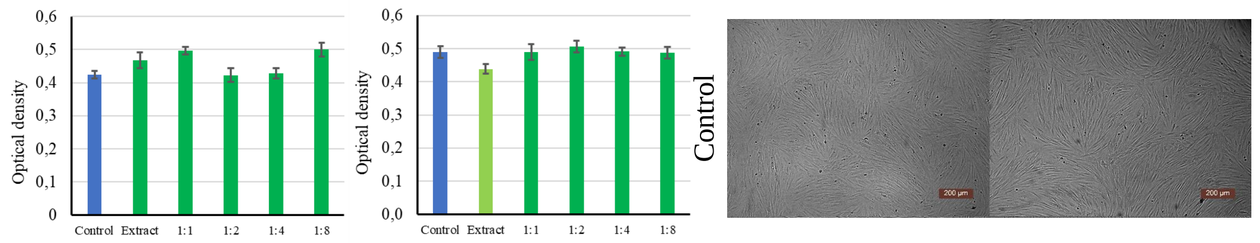


Cytotoxicity study for $Ca_{10-2x}Bi_xNa_x(PO_4)_6F_2$

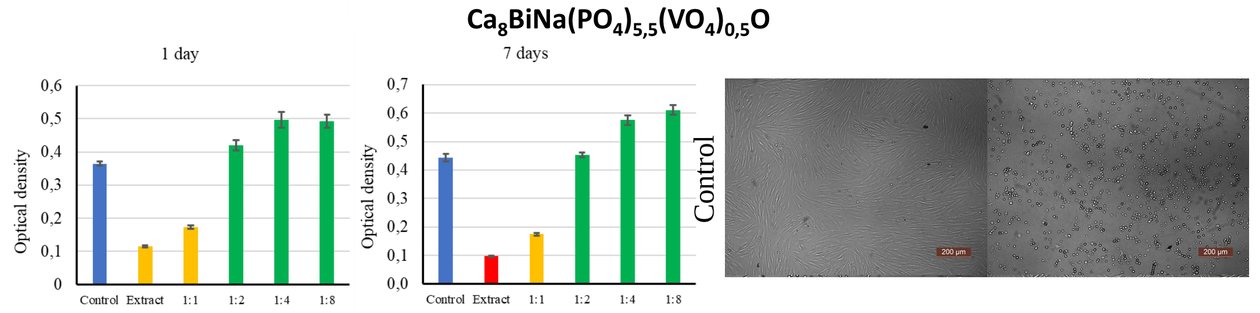
The data obtained in the MTT-test demonstrate the absence of cytotoxicity and a slightly increased values of relative cell growth intensity in the case of the composition with maximum calcium content are also noteworthy. According to the studies of powder morphology, these compositions are characterized by a greater spheroidality of particles, which facilitates, as follows from a number of works, the penetration of individual smallest representatives through the cell membrane, which is probably the cause of the observed phenomena.

RESULTS & DISCUSSION

$Ca_8BiNa(PO_4)_6O$



$Ca_8BiNa(PO_4)_{5,5}(VO_4)_{0,5}O$

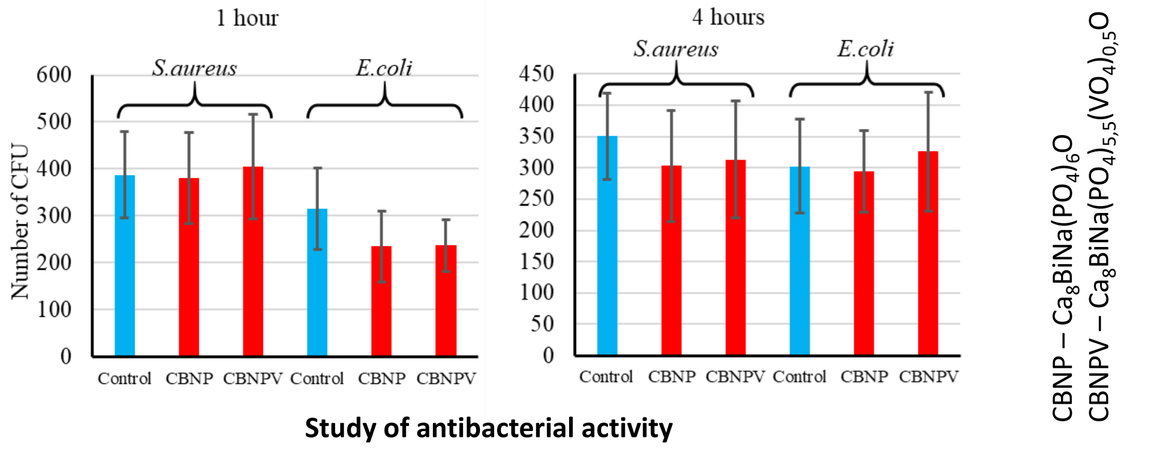


Cytotoxicity study for $Ca_8BiNa(PO_4)_6O$ and $Ca_8BiNa(PO_4)_{5,5}(VO_4)_{0,5}O$

From the X-ray radiographs and elemental analysis results, it is evident that the structural type during the study as well as the chemical composition is preserved. Moreover, the absence of shift in the X-ray radiographs also indicates the absence of crystallochemical changes and intercalation processes in the studied substance.

Cells both in the control and in the series with $Ca_8BiNa(PO_4)_6O$ extract and its dilutions, retained typical morphology, in the absence of pronounced cell death, so it can be recommended for the manufacture of medical devices.

In the case of $Ca_8BiNa(PO_4)_{5,5}(VO_4)_{0.5}O$, undiluted extracts and extracts diluted at a ratio of 1 : 1 showed pronounced toxicity, which increased slightly with increasing extraction duration. The toxic effect was evidenced by a significant decrease in cell number and cell damage compared to the control. When the extract was diluted, the negative effect decreased and no cytotoxicity was observed, suggesting that the toxicity exhibited at high concentrations of the substance in the culture medium, which was not achieved in the phase stability study, is presumably due to leaching of vanadium ions during prolonged extraction in trypsin.



Study of antibacterial activity

During 4 hours of the standard experiment, the microorganisms remained viable on both the glass and the test samples, i.e., the apatites did not exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria. This result led, in fact, to the refutation of a series of works devoted to the antibacterial activity of apatites with bismuth, suggesting that it is due to the secondary phase and not isomorphically bound in the structure of apatite bismuth.

CONCLUSION

Based on the results of the study, we can say that bismuth-apatites can be considered as promising biocompatible materials that stimulate cell proliferation, but do not have the expected antibacterial properties. Bismuth ions are firmly fixed in the crystalline structure, which excludes bactericidal activity of the substance, at least due to leaching of bismuth ions from the material. It is obvious that further studies should include systematic complex analysis and characterization, starting from chemical composition, crystallographic description, morphology study and ending with in vitro and in vivo experiments on objects from the same synthesis series.

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REFERENCES

- Bulanov E.N., *et al.* // Dalton Transactions. 51 (2022) 969. DOI 10.1039/D1DT03558D
- Bulanov E.N., *et al.* // Ceramics International. 50 (2024) 11150. DOI 10.1016/j.ceramint.2024.01.016
- Bulanov E.N., *et al.* // Solid State Sciences. 151 (2024) 107527. DOI 10.1016/j.solidstatesciences.2024.107527