Evaluation of the antibacterial activity of some doped phosphocalcic glasses with silver and copper

Avram Daniela[1], Ungureanu Dan Nicolae[2], Ioniță Ionica[3]*, Popescu Elena Corina[1], Avram Cosmina Cristiana[4]

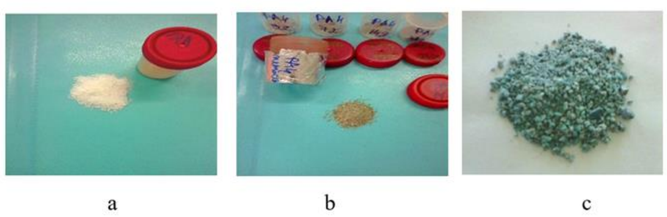
1 Department of Food Engineering, Valahia University of Târgoviște, Faculty of Environmental Engineering and Food Science, Aleea Sinaia Street, nr. 13,130004 Târgoviște, România
2 Department of Material Engineering, Valahia University of Târgoviște, Faculty of Materials Engineering and Mechanics, Aleea Sinaia Street, nr. 13,130004 Târgoviște, România
3 Department of Sciences and Advanced Technologies, Valahia University of Târgoviște, Faculty of Science and Arts, Aleea Sinaia Street, nr. 13,130004 Târgoviște, România
4 Diamed Center SRL, Str.Zagazului, Nr. 7, Sector 1, 14261, București

Doping of calcium phosphate glasses from the ternary system SiO_2-CaO-P_2O_5, with transition metals ions makes them to acquire additional properties, of a prophylactic nature (anti-inflammatory, healing, antimicrobial). This paper presents the microbiological study performed of three glass compositions synthesized by sol-gel method, one doped with silver, the other doped with copper II, compared to a control sample, with the same compositional matrix, but not doped, in order to highlight their antimicrobial character and the determination of the minimum bactericidal dose of each type of glass on two strains of bacteria with high pathogenic potential.

The bacteriostatic activity of phosphocalcic glass is a great bonus in the general economics in oral and maxillofacial orthopedic bone reconstruction surgery, given the fairly high risk of postoperative nosocomial infection.
The analyzed glasses in this study have the chemical composition shown in Table 1 (expressed as% by mass). Sample P is the control sample, not doped with metals, which is the basic composition, from which were obtained by partial substitution of 5% SiO_2, the other two compositions, PA – the composition doped with silver, respectively PC – the one doped with copper ions (Figure 1).

Table 1. The chemical composition of glasses

Sample code	SiO_2	CaO	P_2O_5	Ag_2O	Cu_2O
P	55	40	5	-	
PA	50	40	5	5	-
PC	50	38*	7	-	5

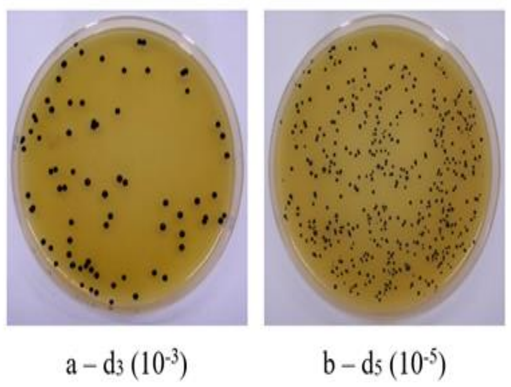


a b c

Figure 1. Undoped glass powder (a), silver doped (b) and doped with copper (c)

Before being introduced into the study, the glass powders synthesized by the sol-gel technique were finely ground and subsequently sterilized at 125^0C. The working protocol requires strict aseptic conditions to avoid infection of the samples and misleading results. For this purpose, the culture media used are also sterilized beforehand and the glassware used is sterile and disposable.
For this study were used pure freeze-dried cultures of bacteria, which were reconstituted (returned in liquid medium as a suspension form); a bacteria inoculum of constant concentration - $3x10^8$ microbial cells (CFU - colony forming units)/mL physiological serum was used for the analyses presented in this paper.

The working method described below for the actual testing of the analyzed bioactive glasses is the same for all types of glasses. Thus, 0.1 g of each glass (P, PA, PC) was dispersed in Petri dishes.
Figure 2 shows the bacteriostatic effect of 5% silver - PA doped glass on Staphylococcus aureus bacteria as a function of the concentration of the bioactive glass (of the metal ions) in the decimal dilutions made. The images in Figure 2 a and b show that as the dilution of the bioactive glass increases (from d3 to d5), i.e. the concentration of silver ions in the medium decreases, the number of specific Staphylococcus aureus colonies increases considerably. Therefore, the bacteria grow optimally the less they are "stressed" by the toxic action of the doping metal, i.e. their multiplication is less and less inhibited.



a – d_3 (10^{-3}) b – d_5 (10^{-5})

Figure 2. Staphylococcus aureus plates at different dilutions of PA glass

CONCLUSIONS

As a general conclusion of this study, it can be said that the silver-doped bioglasses has a more effective antimicrobial activity than the copper-doped one, but in the long term, in the general economy of the exploitation duration of a graft or implant (a few weeks, months, years) this aspect may be insignificant.
Given the dilutions performed and the amounts of bioactive glass with which the study was started at the base dose, it can also be concluded that the glasses could be synthesized with a doping metal content much lower than 5%, as both compositions have been shown to be bactericidal at low doses, much lower than in the bioactive glass (MBCs of 0.1, 0.05 and 0.03g/mL/24h were determined). A percentage of 1% (wt%), or even lower by an order of magnitude, can ensure the bactericidal property of all compositions.
Since the copper compounds with which the doping of bioactive glasses can be achieved are on average ten times cheaper than the silver salts used for doping, it can be concluded that copper-doped biomaterials are considerably cheaper and can provide, in the long term, an antimicrobial effect comparable to silver-doped ones.

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Corresponding author:
ioana67@gmail.com