COMPOSITE INKS OF HYDROGELS AND INORGANIC BIOACTIVE FILLERS AS POTENTIAL MATERIALS FOR 3D BIOPRINTING

MONIKA BIERNAT*, ANNA WOŹNIAK, LIDIA CIOŁEK, PIOTR SZTERNER

BIOMATERIALS RESEARCH GROUP, LUKASIEWICZ RESEARCH NETWORK INSTITUTE OF CERAMICS AND BUILDING MATERIALS, CERAMIC AND CONCRETE DIVISION IN WARSAW, CEMENTOWA 8, 31-983 KRAKÓW, POLAND
*E-MAIL: MONIKA.BIERNAT@ICIMB.LUKASIEWICZ.GOV.PL

[ENGINEERING OF BIOMATERIALS 163 (2021) 62]

Introduction

One of the newest technological possibilities for regenerative medicine is 3D bio-printing. This technique makes it possible to print an artificial model of an organ or a scaffold for cells with the use of appropriate components. Typically, bioinks are used for bioprinting, consisting of low-viscosity biocompatible hydrogels. Hydrogels are characterized by a high water content, which provides an appropriate environment similar to the natural extracellular matrix (ECM) for maintaining cell function and viability [1,2]. However, pure hydrogels often exhibit relatively low mechanical and structural stability and often degrade too quickly and unpredictably, which limits their use as bioinks for long-term cell culture or their transfer to in vivo applications [3]. For this reason, composite hydrogel bioinks combining the advantages of hydrogels and various solid fillers are being developed more and more often [4,5].

The aim of the work was to develop new composite inks that would combine the positive features of biocompatible hydrogels with the properties of bioactive inorganic fillers such as bioglass or hydroxyapatite particles. The assumption was that the obtained composite inks were characterized by good printability, and the models obtained after printing had higher mechanical resistance and stiffness than models made of pure hydrogel. The hypothesis was that the use of bioactive fillers with a controlled degree of degradation would allow to obtain inks with the appropriate viscosity, and in the longer term with the appropriate concentration of released ions supporting the adhesion and proliferation of cells in bioinks.

Materials and Methods

Natural polymers were used as the basic components of the hydrogel matrix of composite inks: chitosan and carboxymethylchitosan (Heppe Medical Chitosan). Sodium tripolyphosphate and calcium chloride (Avantor) were used as crosslinkers and stabilizers. As inorganic bioactive fillers the following were used: bioglass with a controlled degree of degradation, enriched with ZnO and nanometric apatite particles.

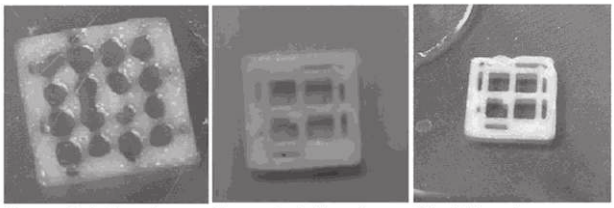
Bioglass was obtained by the sol-gel method in the SiO_2-P_2O_5-CaO-ZnO system, where the composition was 70 wt.% SiO_2, 5 wt.% P_2O_5, 23 wt.% CaO and 2 wt.% ZnO. After the heating process at 650°C for 15 hours, the obtained bioglass was crushed in a mechanical mortar and ground in a rotary-vibration mill until the grain size was: Dv (0.1) 1.578; Dv (0.5) 7.966; Dv (0.9) 29.258.

The nanoapatite particles were obtained by precipitation from calcium hydroxide and phosphoric acid (Chempur) at pH 11. The composition of the particles obtained was determined by the XRD method as hydroxyapatite.

Composite inks were obtained by mixing the ingredients with the use of two syringes and luer lock adapter. A BIO X, CELLINK printer was used to make 3D prints of the obtained composite inks.

Results and Discussion

Based on selected hydrogels and prepared bioactive fillers, composite inks with solids content of 5-15 wt.% were produced. For the developed inks, printing parameters were established for two types of print mesh for a 3D model in the form of a cuboid with dimensions of 10x10x0.3 mm (FIG. 1).



Non-stabilized printed model (Rectlinear) | Non-stabilized printed model (Grid) | Stabilized printed model (Grid)

FIG. 1. Images of printouts of composite ink of chitosan hydrogel with 5% of bioglass printed with following print parameters: nozzle diameter= 0,25 or 0,41 mm (25G or 22G), layer height= 0,05-0,1 mm; first layer height= 50-60%; infill density= 15-30%; speed = 2-10 mm/s; p= 10-30 kPa; preflow= 0-10ms

On the basis of the conducted research, it was observed that the addition of inorganic fillers results in higher strength and mechanical stability as well as better reproduction of the shape of the printout. The particle size and the proper distribution of the filler in the hydrogel matrix are important factors influencing the 3D printing process. Too high concentration of inorganic particles greatly increases the viscosity and thus leads to increased pressures during printing. Such an increase in shear forces may have a negative effect on cells in the perspective of future bioprinting, therefore the selection of an appropriate concentration of solid particles is very important.

Conclusions

The developed composite inks based on chitosan and/or chitosan derivatives hydrogels and on selected inorganic bioactive fillers are characterized by good printability and allow for obtaining of stable 3D prints with good shape reflection. The developed methods of their cross-linking and stabilization, the selection of natural hydrogel matrices and the osteogenic properties of the fillers used allow to hope for the use of these composites as potential bioinks for applications in the regeneration of bone or cartilage tissue.

Acknowledgments

Work funded by current operations funding (subvention) from the Ministry of Science and Higher Education for 2021.

References

[1] N.A. Peppas, J.Z. Hilt, *et al.*, Adv. Mater. 18 (11) (2006) 1345-1360.
[2] A.S. Hoffman, Adv. Drug Deliv. Rev. 64 (2012) 18-23.
[3] G. Turnbull, J. Clarke, *et al.*, Bioact. Mater. 3 (3) (2018) 278-314.
[4] A. Wenz, K. Borchers, *et al.*, Biofabrication. 9 (4) (2017) 44103.
[5] A. Gantar, P. Lucilia, *et al.*, Mater. Sci. Eng. C. 43 (2014) 27-36.