Materials Letters 212 (2018) 32–36

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/mlblue

Bimetallic alginate nanocomposites: New antimicrobial biomaterials for biomedical application

Ivana Malagurski [a,*], Steva Levic [b], Miodrag Mitric [c], Vladimir Pavlovic [b], Suzana Dimitrijevic-Brankovic [a]

[a] University of Belgrade, Faculty of Technology and Metallurgy, 11000 Belgrade, Serbia
[b] University of Belgrade, Faculty of Agriculture, 11081 Belgrade, Serbia
[c] University of Belgrade, Vinca Institute of Nuclear Science, P.O. Box 522, 11001 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 2 June 2017
Received in revised form 17 September 2017
Accepted 15 October 2017
Available online 16 October 2017

Keywords:
Zinc
Copper
Alginate
Biomaterials
Nanocomposites
Antimicrobial activity

ABSTRACT

Two bimetallic (Zn/Cu) alginate based nanocomposites, impregnated with carbonate or phosphate mineral phase, were prepared by a facile procedure. Mineralized samples exhibited different morphologies and properties when compared to the non-mineralized sample. Antimicrobial testing against *Escherichia coli, Staphylococcus aureus* and *Candida albicans* showed that mineralized samples are more efficient than non-mineralized in elimination of microorganisms. The results of this study suggest that bimetallic mineralized alginates could be potentially used as affordable, easy to produce antimicrobial materials.

1. Introduction

Alginates are biocompatible, hydrophilic biomaterials with numerous biomedical applications [1]. However, lack of antibacterial activity and limited stability in biological environment impose certain drawbacks in their application. A possible solution for improving alginate-based biomaterials is to combine them with other components in order to make composites with desirable properties. Structural similarity of alginate hydrogel to extracellular matrix makes it a suitable platform for designing composite biomaterials through biomineralization process [2,3]. The concept of organic/inorganic composites represents a promising approach in production of bioactive, biomimetic materials. A good example of such biomaterials are alginate/bioglass composites used in bone tissue engineering. The addition of osteoinductive bioactive glasses into alginate, improved the mechanical properties, cytocompatibility and mineralization potential of the obtained scaffolds [4,5].

In the presence of divalent metallic cations, alginate chains are cross-linked producing in turn hydrogels. By adding suitable mineral precursors into alginate solution prior to gelation, it is expected that two parallel processes, both mediated by metallic cations, take place: hydrogel formation and mineral deposition within hydrogel.

Zinc and copper are transitional metals with strong antimicrobial activity, and as such they have been used as functional components in many biomaterials with antimicrobial action [6,7]. Taking into account that their biocidal action is mediated through different mechanisms (*i.e.* Zn – inhibition of essential metabolic pathways and antioxidants depletion; and Cu – oxidative stress) and that microorganisms have different sensitivity towards different metals, the optimal strategy for designing improved biomaterials with wide spectrum of antimicrobial action would be to employ both metals as structural and functional components.

Here, we tested the hypothesis that incorporation of essential metal-based minerals (*e.g.* bimetallic Zn/Cu mineral phase) into an alginate network, could result in new nanocomposites with antimicrobial properties.

2. Experimental

2.1. Synthesis of bimetallic-mineralized alginate nanocomposites

Mineralized alginates were made by electrostatic extrusion. Extrusion solution (1.5% w/v medium viscosity Na-alginate, 100 mM Na_2HPO_4 or 100 mM Na_2CO_3) was extruded through a positively charged needle into grounded, constantly stirred gelling

* Corresponding author.
E-mail address: madzovska@tmf.bg.ac.rs (I. Malagurski).

https://doi.org/10.1016/j.matlet.2017.10.046
0167-577X/

solution with essential metallic cations (70 mM $ZnCl_2$ and 70 mM $CuSO_4$). The operational parameters were: 20 G needle size, 40 ml/h flow rate, 7.5 kV applied voltage and 2.5 cm electrode distance.

The obtained samples, in the form of microbeads, were left for 24 h in gelling solution to provide optimal cross-linking and mineral phase formation. Non-mineralized samples were made using 1.5% w/v Na-alginate solution for extrusion. Prior further characterization, the microbeads were washed in distilled water and saline solution and dried at room temperature. Free minerals were made by precipitation in solution (*i.e.* by adding saturated solution of Na_2HPO_4 or Na_2CO_3 to gelling solution). The samples were denoted as A (non-mineralized Zn/Cu-alginate), MinC-A (Zn/Cu-carbonate mineralized Zn/Cu-alginate), MinP-A (Zn/Cu-phosphate mineralized Zn/Cu-alginate), C (free Zn/Cu-carbonate minerals) and P (free Zn/Cu-phosphate minerals).

2.2. Characterization

Microbeads morphology was investigated using scanning electron microscopy (JEOL JSM-6390LV SEM). Elemental analysis of the microbeads surface was examined using EDX analysis (JEOL JSM-6390LV) and Oxford Instruments detector. The Fourier-transformed infrared (FTIR) spectra were taken with a FTIR spectrometer IRAffinity-1 (SHIMADZU) in the spectral range 4.000–500 cm^{-1} and resolution of 4 cm^{-1}. Raman spectra were collected with a XploRA Raman spectrometer from Horiba Jobin Yvon, using a laser at 532 nm. X-ray diffraction (XRD) analysis was performed using a Philips PW 1050 diffractometer with Cu-K$\alpha_{1,2}$ radiation (Ni filter). Measurements were done in 2θ range of 6–90° with scanning step width of 0.05° and 4 s/step. The X-ray Line Profile Fitting Program (XFIT) with a Fundamental Parameters convolution approach to generating line profiles was used for the calculation of the mean crystallite size. Thermogravimetric analysis (TGA) was performed on a SDT 2960 TGA/DSC Analyzer (TA INSTRUMENTS), in a dynamic air atmosphere (flow rate 20 ml/min), 30–1000 °C temperature range and heating rate of 20 °C/min.

2.3. Antimicrobial assay

Antimicrobial potential of dry, non-mineralized and bimetallic-mineralized alginate samples was evaluated against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 10231) using modified Broth macrodilution method. Tested samples were added to microbial cultures to reach the final concentration of 15 mg/ml. During incubation, serial dilutions of media samples, taken at different time points (0, 1, 4 and 24 h) were plated on agar. The antimicrobial activity of tested samples was established by decrease in $\log_{10}$ CFU/ml of the test culture during incubation.

3. Results and discussion

3.1. Synthesis and characterization

Two-phase composites were successfully produced using electrostatic extrusion method. Microbeads morphology was highly affected by the presence and type of mineral precursor used for synthesis. Non-mineralized microbeads were light-blue (Fig. 1a inset) with smooth surface (Fig. 1a), while the mineralized appeared greenish (MinC-A, Fig. 1b inset) and turquoise (MinP-A, Fig. 1c inset) with mineral precipitates clearly visible on microbeads surface (Fig. 1b and c). In addition, EDX analysis confirmed different elemental composition of samples with different formulations (Fig. 1d–f).

The results of XRD, FTIR and Raman analyses are shown in Fig. 2.

The structural and chemical properties of the non-mineralized alginate hydrogel samples (A) are generally in agreement with the literature data for non-mineralized alginate hydrogel [3]. Free

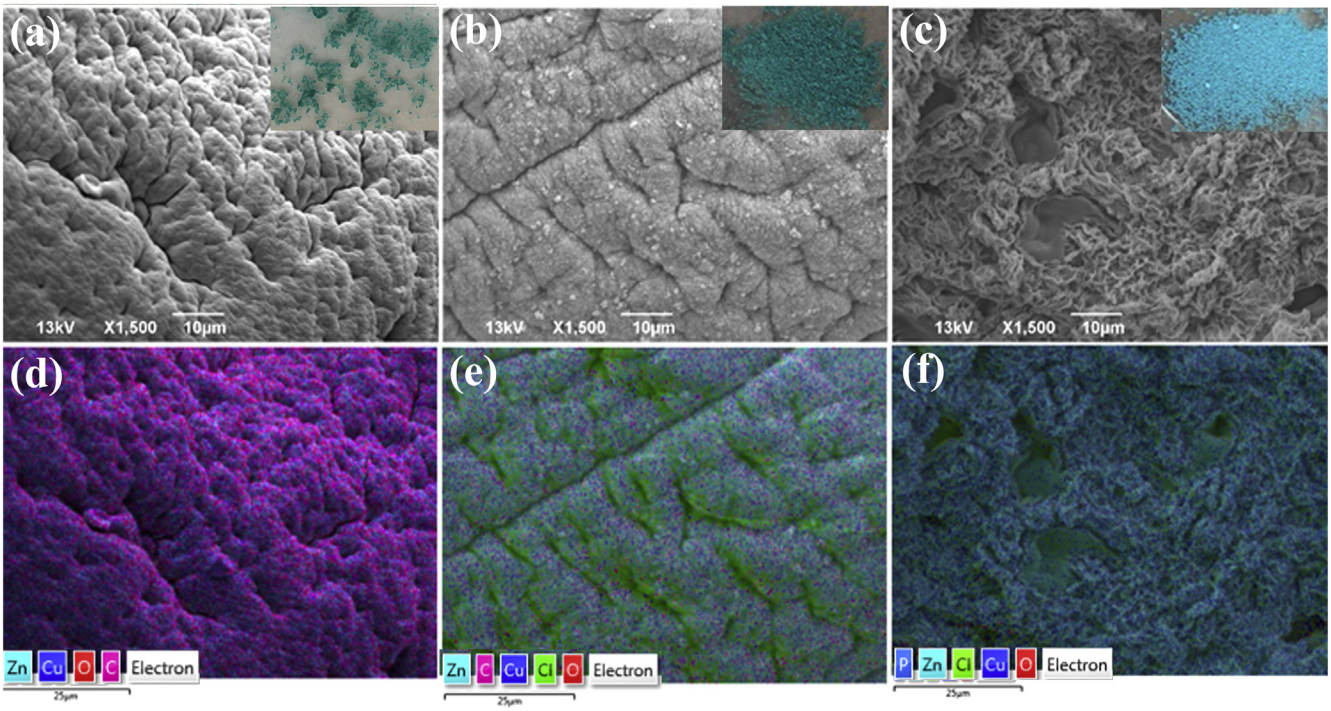

Fig. 1. SEM micrographs of samples surface: (a) A; (b) MinC-A; and (c) MinP-A. Insets represent overall appearance of dry samples. EDX analysis of: (d) A; (e) MinC-A; and (f) MinP-A.