Materials Chemistry and Physics 164 (2015) 51–62

Contents lists available at ScienceDirect

Materials Chemistry and Physics

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

A high efficacy antimicrobial acrylate based hydrogels with incorporated copper for wound healing application

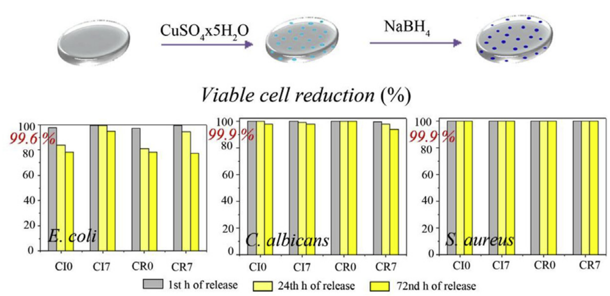
Jovana S. Vuković, Marija M. Babić, Katarina M. Antić, Miona G. Miljković, Aleksandra A. Perić-Grujić, Jovanka M. Filipović, Simonida Lj. Tomić*

Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia

HIGHLIGHTS

- Design and evaluation of novel pH responsive hydrogel series.
- Structural, morphological, thermal characterization and controlled copper release.
- Antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* over 99%.
- Antifungal activity against *Candida albicans* over 99%.
- In vitro evaluation studies revealed great potential for wound healing application.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 February 2015
Received in revised form
22 July 2015
Accepted 9 August 2015
Available online 24 August 2015

Keywords:
Polymers
Thermal properties
Transport properties
Biomaterials

ABSTRACT

In this study, three series of hydrogels based on 2-hydroxyethyl acrylate and itaconic acid, unloaded, with incorporated copper(II) ions and reduced copper, were successfully prepared, characterized and evaluated as novel wound healing materials. Fourier transform infrared spectroscopy (FTIR) confirmed the expected structure of obtained hydrogels. Scanning electron microscopy (SEM) revealed porous morphology of unloaded hydrogels, and the morphological modifications in case of loaded hydrogels. Thermal characteristics were examined by differential scanning calorimetry (DSC) and the glass transition temperatures were observed in range of 12–50 °C. Swelling study was conducted in wide range of pHs at 37 °C, confirming pH sensitive behaviour for all three series of hydrogels. The *in vitro* copper release was investigated and the experimental data were analysed using several models in order to elucidate the transport mechanism. The antimicrobial assay revealed excellent antimicrobial activity, over 99% against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, as well as good correlation with the copper release experiments. In accordance with potential application, water vapour transmission rate, oxygen penetration, dispersion characteristics, fluid retention were observed and the suitability of the hydrogels for wound healing application was discussed.

1. Introduction

Hydrogels are cross-linked hydrophilic polymers that can imbibe water or biological fluids [1]. Since Wichterle and Lim developed hydrogels based on 2-hydroxyethyl methacrylate

* Corresponding author.
E-mail address: simonida@tmf.bg.ac.rs (S.Lj. Tomić).

http://dx.doi.org/10.1016/j.matchemphys.2015.08.022
0254-0584/

(HEMA) for contact lence manufacturing, hydrogels are continuously involved in biomedical researches. Therefore, they have been classified in group of materials suitable for a range of biomedical and pharmaceutical applications [2,3].

Adequate wound care, wether in case of surgical cuts, diabetic ulcers or burns, is crucial part in a pacient recovery process. Since the healing is dynamic process which involves several phases such as haemostasis, inflammation, migration, proliferation and maturation [4], efficient wound dressing must fulfil certain requests in order to accelerate regeneration and tissue growth. It has to provide moist environment in wound area, at the same time preventing infections (sepsis) which complicate and prolong healing process.

In recent years, extended research was conducted on polymeric hydrogel dressings [5], due to resemblance to the natural living tissue more than any other class of synthetic materials because of their high water content and soft consistency [6]. Also they combine the features of moist wound healing with good fluid absorbance and transparence, allowing the monitoring of healing [7].

Distinguishing feature which makes hydrogels superior compared to other wound dressing candidates is the ability to release entrapped active agent in controlled terms. The controlled release of antimicrobial agent additionally accelerates wound healing. Wide use of antibiotics as the most common antimicrobial agents led to antibiotic-resistance of bacteria. Consequently the need for alternatives is raised. For a very long period, antimicrobial activity of copper against various microorganisms has been investigated and published [8–10]. The exact mechanism is unknown, but it is shown that copper ions destroy the bacterial cell wall, which becomes thick and coarse, the cytoplasm is then degraded and disappears, leading finally to cell death [11].

Copper as an essential trace element exists in human organism within numerous proteins, and it is included in many biochemical processes. Angiogenesis plays a central role in wound healing. Among many known growth factors, vascular endothelial growth factor (VEGF) is believed to be the most efficacious signal that is known to stimulate angiogenesis in wounds [12].

It is shown that copper is not only nontoxic at the concentration used (10^{-4} M); in fact, it stimulates proliferation of human endothelial cells [13]. Human skin is not sensitive to copper and the risk of adverse reactions due to dermal exposure to copper is extremely low [14].

Hydrogel dressings are classified according to natural [15–17] or synthetic nature [18,19] of polymer used for its manufacturing. 2-Hydroxyethyl acrylate (HEA) is a monomer highly attractive and suitable for the synthesis of polymeric biomaterials [20]. Presence of hydroxyl groups, analogous to HEMA, makes it hydrophilic, as an additional asset, in term of biomedical implementation [21].

The aim of our research was to design and evaluate three series of hydrogels based on 2-hydroxyethyl acrylate and itaconic acid, unloaded, with incorporated copper(II) ions and with reduced copper, as wound healing materials. In the first series, hydrogels were prepared with different HEA/IA ratios. The second series was obtained by incorporation of copper(II) ions in the previously synthesized hydrogels, and the third series was prepared by treating the hydrogels of the second series with a reducing agent, sodium borohydride ($NaBH_4$). The hydrogel properties were examined by structural, morphological, thermal and swelling studies. In addition, copper release studies were conducted in order to evaluate the potential of hydrogels as controlled release systems for wound healing. Analysis of the release data was performed using several models for the interpretation of copper transport mechanism. Finally, evaluation studies, typical testing for wound dressing material, water vapour transmission rate (WVTR), oxygen penetration, dispersion characteristics (wet integrity test), fluid retention and antimicrobial activity against Gram-negative bacteria *Escherichia coli* (ATCC25922), Gram-positive bacteria *Staphylococcus aureus* (ATCC25923) and fungus *Candida albicans* (ATCC10259) were conducted *in vitro*.

2. Experimental

2.1. Materials

2-Hydroxyethyl acrylate (HEA) (Aldrich, USA) and itaconic acid (IA) (Aldrich, USA) were used as reactants in this work. Ethylene glycol dimethacrylate (EGDMA) (Aldrich, USA) as crosslinker, potassium persulfate (PPS) (Aldrich, USA) as initiator and N, N, N′, N′-tetramethylethylene diamine (TEMED) (Aldrich, USA) as activator were used in hydrogel synthesis, which was obtained in a solution of ethanol/water mixture. Source of copper(II) ions was solution of copper sulfate pentahydrate ($CuSO_4 \times 5H_2O$) (Aldrich, USA). Sodium borohydride ($NaBH_4$) (Aldrich, USA) was used as the reducing agent. Phosphate and acetate buffers were used in experiments. All the chemicals were used without further purification.

2.2. Preparation of hydrogels

The hydrogels were prepared by free radical copolymerization. HEA and IA were used as monomers. IA content was varied (0, 2, 3.5, 5 and 7 mol%), which resulted in five hydrogel samples: H0, H2, H3.5, H5 and H7, respectively (Table S1, Supplementary material). All reactants were dissolved in water/ethanol mixture, and reaction mixture was subsequently degassed in nitrogen atmosphere for 20 min, in order to eliminate oxygen, and later placed between two glass plates sealed with a rubber spacer (2 mm thick). The weights of PPS, EGDMA and TEMED were 0.015, 0.05 and 0.01 g, respectively. Copolymerization reaction was carried out at 55 °C, for 24 h. When the reaction was completed, hydrogels were cut into the form of discs (diameter 10 mm). The samples were later immersed in deionized water, which was changed daily, for seven days in order to remove unreacted chemicals. The discs were dried at room temperature. The copolymer yield of the hydrogels was determined and presented in Supplementary Material.

2.3. Copper incorporation

In order to obtain the hydrogels with incorporated copper(II) ions, dry H0, H2, H3.5, H5 and H7 samples were immersed in $CuSO_4 \times 5H_2O$ solution, pH 5.50 [22] and left for 24 h to swell and imbibe copper(II) ions. Afterwards CI0, CI2, CI3.5, CI5 and CI7 samples were dried at room temperature.

The copper loading (CL) and entrapment efficiency (EE %) of the hydrogels were calculated by the following equations:

$$CL(mg/g\ hydrogel) = \frac{weight\ of\ copper\ in\ hydrogel}{weight\ of\ xerogel} \quad (1)$$

and

$$EE(\%) = \frac{content\ of\ copper\ in\ hydrogel}{theoretical\ content\ of\ copper} \times 100 \quad (2)$$

Using Atomic absorbing spectrophotometer (Phillips PYE UNICAM SP9) the initial amount of copper (theoretical content of copper) in $CuSO_4 \times 5H_2O$ solution was found 5.71 ± 0.01 ppm. The amount of unloaded copper was measured from the copper solutions after the hydrogels were taken out. Comparing these values with initial copper amount, the content of copper in hydrogel samples was determined and used for the calculation of