European Polymer Journal 77 (2016) 114–123

Contents lists available at ScienceDirect

European Polymer Journal

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

Gellan gum methacrylate and laponite as an innovative nanocomposite hydrogel for biomedical applications

Settimio Pacelli [a,b,*], Patrizia Paolicelli [a], Giuseppe Moretti [a], Stefania Petralito [a], Silvia Di Giacomo [c], Annabella Vitalone [c], Maria Antonietta Casadei [a]

[a] Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
[b] BioIntel Research Laboratory, Department of Chemical and Petroleum Engineering, Bioengineering Graduate Program, School of Engineering, University of Kansas, Lawrence, USA
[c] Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy

ARTICLE INFO

Article history:
Received 27 October 2015
Received in revised form 4 February 2016
Accepted 8 February 2016
Available online 10 February 2016

Keywords:
Gellan gum methacrylate
Nanoclays
Laponite
Nano-composite hydrogel
Rheological properties
Wound dressing

ABSTRACT

Successful treatment of infected wounds still represents an open challenge in the biomedical field. Nanocomposite hydrogels (NC) are a valid solution to this problem as they can be used as wound dressing materials for the delivery of therapeutic agents at the site of the injury. In addition, their improved mechanical properties make possible a sterile treatment, which is not generally possible using conventional hydrogels. Here in this work, gellan gum methacrylate (GG-MA), a biocompatible polymer recently proposed for the fabrication of injectable and photocrosslinkable hydrogels, was combined with laponite® XLG to form novel NC hydrogels as innovative wound dressing material. Clay concentration was varied in the range of 0.1 up to 1% w/v to study the possible clay–polymer interactions prior and after UV irradiation, showing the formation of weak gels having storage modulus G' varying from 1 up to 100 Pa, even prior photocrosslinking. As expected, the highest value of G' (over 1000 Pa) was found for the NC hydrogel containing 1% w/v of laponite after UV irradiation. The same system showed unaltered mechanical properties after steam sterilization compared to the one composed of 0.5% of clay, which showed a decrease in the stress failure after steam treatment. Moreover, the effect of laponite on the swelling and release capability was studied to test these new nanocomposite systems as a carrier of the model drug ofloxacin. Laponite was able to decrease the amount of antibiotic released over the first eight hours compared to the GG-MA hydrogels, although the amount of clay did not influence the diffusion coefficient of ofloxacin. Finally, preliminary cytotoxicity tests on human fibroblasts were carried out to evaluate the NC hydrogel biocompatibility. Overall, we anticipate the possible use of this novel NC hydrogel as wound dressing material for the treatment of burn wounds, which are subjected to chronic infections.

1. Introduction

The design of advanced hydrogels, as carriers of therapeutic agents or as scaffolds to promote healing of soft tissues, is still an open challenge [1,2]. To address all the requirements necessary to obtain a multifunctional system, innovative com-

* Corresponding author at: Department of Drug Chemistry and Technologies, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy.
E-mail address: settimio.pacelli@uniroma1.it (S. Pacelli).

http://dx.doi.org/10.1016/j.eurpolymj.2016.02.007
0014-3057/

binations of known organic and inorganic biomaterials are required, as each component plays a key role in enhancing the properties of the composite material [3,4]. In this sense, a range of nano-fillers like clay, hydroxyapatite, carbon nanotubes, and metallic nanoparticles have been combined with polymeric networks to obtain nanocomposite hydrogels [5,6]. These hybrid systems offer the possibility to have the advantages of both nanoparticles and hydrogel matrices within a unique engineered structure [7,8]. In this scenario, clay hydrogel nanocomposites (NC) have been widely investigated in both drug delivery and tissue engineering applications, showing promising properties as biomaterials mainly because of the interactions at the clay–polymer interface [9,10]. Specifically, clay nanoparticles can be uniformly dispersed within the polymeric matrix, acting as both fillers and cross-linkers during gel formation.

The most commonly used clay minerals to produce innovative NC hydrogels belong to the smectite family including montmorillonite, hectorite, and laponite; each one of them showing specific surface area, adsorptive and cation exchange capacity [11]. All these characteristics are fundamental in defining the degree of clay dispersion in the polymer matrix as well as the extent of polymer–clay interactions, which can affect the mechanical properties of the corresponding NC hydrogels [12]. Among the smectite family, laponite $Na_{0.7}[(Si_8Mg_{5.5}Li_{0.3})O_{20}(OH)_4]_{0.7}$ is a synthetic clay composed of a layered structure (~25 nm diameter, ~1 nm thickness) that has been used over the past decade to synthesize a wide range of NC hydrogels [13]. In fact, the biocompatible nature, absence of heavy metals along with its high degree of cationic exchange make this clay a suitable candidate for the enhancement of several characteristics in conventional hydrogels.

Laponite has been shown to improve the mechanical toughness and control the elasticity, tensile strength, stiffness and swelling properties of NC hydrogels [14–16]. Moreover, the effect of laponite on all these properties can be tailored by varying different parameters such as concentration of clay and type of crosslinking obtained during the gel synthesis. All these variables must be taken into consideration while designing a novel NC biomaterial with potential biomedical applications [17]. Regarding the type of polymer used to create NC matrices, polysaccharides represent a valid alternative to synthetic materials due to their higher biocompatibility and availability from renewable natural sources. In this sense, several polysaccharides such as alginate, methylcellulose and chitosan have been recently combined with laponite to overcome the extreme fragility of their single-network hydrogels, adding versatility to these biomaterials. [18,19].

In our work, gellan gum, a biocompatible polysaccharide which is receiving increasing attention due to its wide applicability ranging from drug delivery to cartilage tissue engineering applications [20], has been mixed with laponite to form a novel NC hydrogel. Specifically, gellan gum has been functionalized with methacrylic groups to form a derivate gellan gum methacrylate (GG-MA), which can form photochemical hydrogels after UV irradiation in the presence of a photoinitiator. GG-MA has been already investigated in our research group as a promising biopolymer for the preparation of biocompatible injectable hydrogels in combination with polyethylene glycol dimethacrylate (PEG-DMA). The corresponding interpenetrating networks showed an increase in the viscosity prior to irradiation, as well as enhanced mechanical properties compared to the fragile matrix made only of GG-MA [21]. To further highlight the versatility of GG-MA for biomedical applications particularly as wound dressing material, here we report its use in combination with laponite as an alternative strategy to reinforce GG-MA photochemical hydrogels, which are too fragile to be of any practical use. In specific, laponite will serve as linker among the rigid polymeric chains of GG-MA to provide enough strength and the necessary amount of flexibility required for this hydrogel to be used as wound dressing coating.

To address this point, several concentrations of laponite have been tested (0.1 up to 1% w/v) to study how the different amounts of clay were able to influence the mechanical properties of the NC hydrogels GG-MA. Specifically, the influence of clay/polymer molecular interactions was investigated through rheological studies on the solution prior and after gel formation. In specific, frequency and strain sweep were carried out prior and after sterilization treatment to evaluate the effect of the thermal treatment over the mechanical properties of the gels. In addition, it was important to verify whether the presence of laponite in the polymeric network was able to modulate and control the swelling and release behavior, thereby influencing the drug delivery capability of GG-MA. For this reason, the ability of the new NC hydrogels to swell in different media, were evaluated through swelling and release studies using ofloxacin as a model drug. This molecule was chosen to investigate the possible use of this NC hydrogel as wound dressing material for the delivery of antibiotic in the treatment of burn wounds subjected to chronic infections as in the ones caused by *Pseudomonas Aeruginosa*, which occur several weeks after the initial burn [22]. Finally, a neutral red assay was carried out on human fibroblast cell line (WI-38) in order to assess the *in vitro* biocompatibility of NC hydrogels and estimate their biological safety.

2. Materials and methods

2.1. Materials

All used reagents were of analytical grade. Low acetyl gellan gum (GG) with Mn 1×10^6, methacrylic anhydride (MA), potassium bromide (KBr), deuterium oxide (D_2O), Irgacure 2959 (2-hydroxy-4-(2-hydroxy-ethoxy)-2-methylpropiophenone), ofloxacin, citric acid, methanol, acetonitrile, anhydrous dimethyl sulfoxide (DMSO), 4-dimethylaminopyridine (4-DMAP), triethylamine (TEA) and N-methyl-pyrrolidone were obtained from Sigma-Aldrich. Laponite® XLG was kindly gifted by Rockwood. Laponite® XLG is one of the purity grades available for laponite®, certified for low heavy metal and low microbiological content. Dialysis tubes (cut-off 12,000–14,000) were purchased from Medicell International.