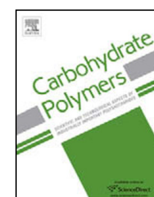
Carbohydrate Polymers 112 (2014) 480–485

Contents lists available at ScienceDirect

Carbohydrate Polymers

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



Effect of hyaluronic acid on the thermogelation and biocompatibility of its blends with methyl cellulose

Laura Mayol[a,b], Daniela De Stefano[a], Francesca De Falco[a], Rosa Carnuccio[a], Maria Chiara Maiuri[a,c], Giuseppe De Rosa[a,b,*]

[a] Dipartimento di Farmacia, Università degli Studi di Napoli Federico II, Via D. Montesano 49, 80131 Napoli, Italy
[b] Interdisciplinary Research Centre on Biomaterials, CRIB, Università di Napoli Federico II, P.le Tecchio 80, Napoli, Italy
[c] INSERM, U848, IGR, 39 rue C. Desmoulins, 94805 Villejuif, France

ARTICLE INFO

Article history:
Received 4 April 2014
Received in revised form 16 May 2014
Accepted 4 June 2014
Available online 17 June 2014

Keywords:
Hyaluronic acid
Methylcellulose
Drug delivery
Wound healing
Thermogelation
Polymeric blend

ABSTRACT

Aim of this work was to investigate the influence of hyaluronic acid (HA) molecular weight on the thermogelation and biocompatibility of its blends with methyl cellulose in view of a possible application in drug delivery and/or wound healing.

We found out that it was possible to obtain MC/HA blends showing a rheological behavior typical of a viscous solution at 20 °C and of a weak gel at 37 °C only when blending MC with low molecular weight HA. Moreover, the blends containing low molecular weight HA did not affect human foreskin fetal fibroblasts viability, proliferation and migration. On the contrary, the cell incubation with high molecular weight HA resulted in a marked and significant reduction of cell viability, compared to control cells. Finally, the optimized blends, in terms of rheological properties and biocompatibility, proved to be able to control and prolong bovine serum albumin release by a combined mechanism of platform dissolution and drug diffusion.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Temperature-sensitive polymers hold great potential as drug delivery systems, mainly for their simple formulation, their ease of administration and the possibility to avoid the use of organic solvents (He, Kim, & Lee, 2008; Huynh et al., 2008; Klouda & Mikos, 2008; Mayol, Quaglia, Borzacchiello, Ambrosio, & La Rotonda, 2008; Shim, Yoo, Bae, & Lee, 2005). In particular, if the transition temperature is properly designed to be close to the physiologic temperature, these systems can undergo a sol-to-gel transition under physiological conditions without any additional external stimuli. Therefore, thermo-responsive gels can be simply administered into the body as a liquid drug dosage form and are able to generate *in situ* an entangled network structure which allows a sustained and controlled release of the active molecule(s). This is of special interest for the controlled release of biotechnological drugs, such as protein and peptides, which can be administered basically by parenteral routes due their poor biopharmaceutical profile. Moreover, these kind of matrices offer several advantages over systems shaped into their final form before implantation since, for example, injectable materials do not require a surgical procedure for placement and withdrawal if biodegradable. Finally, if they are used to fill a cavity of a wound or a defect, their flowing nature enables a good fit with a further advantage to release active macromolecules, such as grow factors, *in situ* with a controlled kinetic.

Cellulose derivatives are examples of thermosensitive polymers and, in particular, aqueous solutions of methylcellulose (MC), at low concentrations (1–10 wt%), are known to be liquid at low temperature but able to gel upon heating up to 40 and 50 °C (Desbrieres, Hirrien, & Rinaudo, 1998). It is possible to lower MC gelation temperature, up to the physiological one, by adding mono and divalent salts (Almeida, Rakesh, & Zhao, 2014) or by blending it with other polymers such as poly(acrylic acid) or polyethylen glycol, carboxymethyl cellulose and chitosan or hyaluronic acid (HA) (Caicco et al., 2013; Negim et al., 2014; Zhang et al., 2014). HA is a natural mucoadhesive polysaccharide, widely used in drug delivery and biomedical field, composed of alternating D-glucuronic acid (GlcA) and *N*-acetyl-D-glucosamine (GlcNAc) repeating units linked together via β-(1,4) and β-(1,3) glycosidic junctions (Borzacchiello, Mayol, Garskog, Dahlqvist, & Ambrosio, 2005; Borzacchiello et al., 2007; Mayol et al., 2014a; Borzacchiello, Mayol, Schiavinato, & Ambrosio, 2010). Polymeric blends represent an emerging class

* Corresponding author at: Università degli Studi di Napoli Federico II, Department of Pharmacy, Via Domenico Montesano, 49, 80131 Naples, Italy.
Tel.: +39 081 678666; fax: +39 081 678630.
E-mail address: gderosa@unina.it (G. De Rosa).

http://dx.doi.org/10.1016/j.carbpol.2014.06.020
0144-8617/© 2014 Elsevier Ltd. All rights reserved.

of biomaterials since they can combine the advantages of the polymers utilized. In particular, in the case of MC/HA blends, the thermosensitive properties of MC can be implemented with the well-known biocompatibility and mucoadhesivity properties of HA. In particular, a recent paper investigated the influence of polymers composition on MC/HA blend mechanical properties and cell survival (Caicco et al., 2013). However, in view of a possible application in the field of controlled macromolecules delivery, it is crucial the understanding of how the polymers interact each other leading to rheological synergism which determines their ability to control and sustain the release of macromolecular active molecules (Mayol et al., 2011).

In this context, the aim of this work was to investigate the influence of HA molecular weight (MW) on the rheological, thermogelation and biocompatibility properties of its blends with MC. In particular, we aimed to formulate a thermosensitive and biocompatible blend, with a phase transition temperature near to body temperature, able to release macromolecular active ingredients with a controlled kinetics. To this aim, here we analyzed the rheological properties of MC/HA blends, both with low molecular weight (LMW) and high molecular weight (HMW) HA, as a function of temperature. Furthermore, the human foreskin fetal fibroblasts (HFFF2) were used to exploit the biocompatibility of this system by cell viability, proliferation and migration assays. Finally, in order to explore the feasibility of these platforms for the sustained delivery of macromolecules, the optimized system was loaded with a model protein such as bovine serum albumin (BSA) and its release properties studied *in vitro*.

2. Materials and methods

2.1. Materials

Low molecular weight (150 kDa) HA was supplied by Fab (Abano Terme, Italy). High molecular weight HA (1×10^6 Da), MC, BSA, and phosphate buffer salts were purchased from Sigma (Milano, Italy).

2.2. Gel preparation

MC/HA-based formulations were prepared as follows. Half the solvent (PBS) was placed in a beaker at 0 °C, the other half heated up to boiling. The right amount of MC was dissolved in the hot solvent in which the cold solvent was then added. The obtained solution was placed in an ice bath under stirring. For a complete solubilization, the solution was left at 4 °C overnight, afterward HA at 1 and 2% w/v was added to MC solutions. For *in vitro* release tests, BSA was simply dispersed into the gel.

2.3. Rheological experiments

Rheological tests were performed by small-amplitude oscillatory shear experiments using a rotational rheometer (Malvern Kinexus) as previously reported (Maltese et al., 2006). Briefly, experiments were performed at 20 °C and 37 °C in the 0.1 to 10 Hz oscillation frequency range, and a strain amplitude at which linear viscoelastity is attained. The shear storage or elastic modulus (G') as well as the shear loss or viscous modulus (G'') were measured as a function of frequency.

2.4. Cell culture

The human fetal foreskin fibroblast (HFFF2) cell line was cultured at 37 °C in humidified 5% CO_2/95% air in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. The cells were plated in 24 culture wells at a density of 2.5×10^5 cells/ml per well and allowed to adhere upon the samples for 2 h.

2.5. Cell viability

The cell viability was determined by using 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) conversion assay (De Stefano et al., 2009). Briefly, 100 μl MTT (5 mg/ml in complete DMEM) were added and the cells were incubated for additional 3 h. After this time point the cells were lysed and the dark blue crystals solubilized with 500 μl of a solution containing 50% (v:v) *N*,*N*-dimethylformamide, 20% (w:v) SDS with an adjusted pH of 4.5. The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCCC/340) equipped with a 620 nm filter. The cell viability in response to treatment with test compounds was calculated as % dead cells = 100 − (OD treated/OD control) × 100.

2.6. Cell proliferation assay

Proliferation was evaluated by incorporation of ^{3}H-thymidine (1 μCi/well) in HFFF-2 cells. Briefly, 2.5×10^5 cells were seeded in the wells upon the samples in a 24 well plate in the presence of ^{3}H-thymidine. The cells were then incubated for 24 h at 37 °C. After incubation, the cells were harvested, scraped in 1 M NaOH (100 μl/well) and collected on plastic miniature vials (PerkinElmer) automatic cell harvester prior to liquid scintillation counting (UltimaGold®, PerkinElmer). The effect on cell proliferation was expressed as count per minute per microgram of protein (CPM/μg protein) of incorporating ^{3}H-thymidine cells.

2.7. Cell migration: Scratch assay

Cells were seeded on the samples into 6 well cell culture plates as previously reported (Mayol et al., 2014b). Once at confluence, cells were serum-starved in medium containing 0.5% FBS over night, and then scratch injury was applied using a disposable pipette tip. After injury, the monolayer was gently washed with PBS, and the medium was replaced. Cell migration from the edges of the injured monolayer was examined and the area of population was measured 24 h after scratching by a computerized analysis system (LAS, Leica).

2.8. Statistics

Results are expressed as the means ± S.D. or S.E.M. of n experiments. Normally, the experiments were carried out in triplicate and three independent experiments were performed. Statistical significance was calculated by one-way analysis of variance (ANOVA) and Bonferroni-corrected *p*-value for multiple comparison test. The level of statistically significant difference was defined as $p < 0.05$.

2.9. In vitro release study

The optimized MC/HA blend was loaded with BSA and drug release profile was evaluated in phosphate buffered solution (PBS; 120 mM NaCl, 2.7 mM KCl, 10 mM phosphate salts; pH = 7.4), in a thermostatic bath at 37 °C in sink conditions as previously reported (Mayol et al., 2008). Briefly, drug delivery tests were carried out in glass cells where, at the bottom, a covered lid containing the polymer vehicle and drug, was placed. The cell was then filled with PBS and immersed in a thermostatic bath at 37 °C. When the temperature inside the cell reached 37 °C the lid cover was removed to allow the contact between the gel and the release medium. A magnetic stirrer into the cell provided a continuous agitation. At regular time intervals, 1 ml of solution was withdrawn from the cell and replaced

Download English Version:

https://daneshyari.com/en/article/7791423

Download Persian Version:

https://daneshyari.com/article/7791423

Daneshyari.com