Journal of Colloid and Interface Science 519 (2018) 174–185

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

Regular Article

Semi-interpenetrating network hyaluronic acid microgel delivery systems in micro-flow

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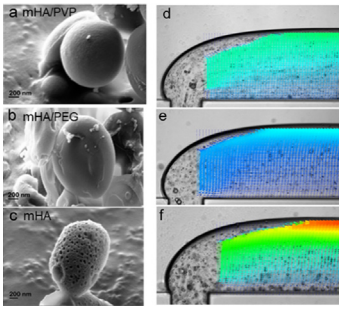
GRAPHICAL ABSTRACT

Semi-interpenetrating hyaluronic acid (HA)-based hydrogel microparticles in a form of spherical or biconcave shape have been developed, suitable for injectable therapies with high safety profile in micro-flow.



ARTICLE INFO

Article history:
Received 24 November 2017
Revised 15 February 2018
Accepted 15 February 2018
Available online 16 February 2018

Keywords:
Microgel
Semi-interpenetrating network hydrogel particles
Micro-flow
Hyaluronic acid
Injectable hydrogel
Hemocompatibility

ABSTRACT

Macroscopic hydrogels are commonly used as injectable scaffolds or fillers, however they may easily obstruct blood vessels, which poses risks and limits their clinical use. In the present study, three types of hyaluronic acid (HA)-based hydrogel micro-particles with non-covalent, covalent semi-interpenetrating and conventional 3D molecular networks, have been designed, fabricated and characterized. The microparticles are spherical, biconcave or irregular in shape and their diameter ranged between 2.5 and 3.5 μm; their suspensions exhibit a tuneable viscosity, shear-thinning behaviour, dynamic stability and dispersity in microfluidic flow as a result of their specific particulate nature, providing thus a well-controlled injectable platform. Hydrogel particle suspensions also demonstrate an enhanced safety profile, in terms of the dispersity, cell safety, and hemocompatibility. In addition, Rhodamine 6G has successfully been loaded and released from the particles as a model for drug delivery. Functionalisation of hydrogel microparticles using synthetic polymers has been proven to be a cost-effective way to achieve desirable rheological properties and flow dynamic stability with improved physicochemical properties and biocompatibility *in vitro*, showing promise as a multifunctional biomedical material for various advanced surgical devices and therapies.

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https://doi.org/10.1016/j.jcis.2018.02.049
0021-9797/

1. Introduction

Macroscopic hydrogels are commonly used in the biomedical field, including scaffolds in tissue engineering [1], vehicles for drugs or biomolecules [2], and surgical materials [3,4]. A common and attractive property of hydrogels lies in its injectability, which enables minimally invasive delivery. However, there is concern that hydrogels may block blood vessels, should the needle accidentally hit a vessel, as seen in soft tissue fillers or drug delivery systems. In some instances, such a blockage may result in serious complications, including massive tissue necrosis and blindness [5]. To make matters worse, there is no effective cure for such complications. Thus, much attention has been drawn to preventive measures, including anatomical education and introduction of blunt-tip cannulae [6,7]. Unfortunately, these measures are not sufficient to eliminate such complications. Also, they are not suitable for every clinical situation [8]. Considering the high vascular density in certain tissue areas and the anatomical varieties, it is difficult to avoid at least some level of blood vessel damage during actual procedures.

One of the most important shortcomings many hydrogels share is their complex rheological behaviour, which varies as a function of the specific composition and the structure of the hydrogel network. With a yield stress and a shear-thinning property, many injectable polymeric hydrogels may 'thin' quickly, i.e. may flow easier due to a decrease in viscosity with an increase of the pressure applied, compromising the controllability of delivery. If this happens in a blood vessel, a gel embolus may form immediately. Such emboli would not respond to thrombolytics. Furthermore, in the case of polymers with high molecular weights, a high viscosity can be reached at a low concentration and/or crosslinking degree. Thus, when the hydrogels are used as drug delivery systems, there is a trade-off between the injectability and the releasing kinetics, resulting in a compromised tunability.

As an important glycosaminoglycan existing in most of tissues of the human body, hyaluronic acid (HA) is widely used for tissue reconstruction and drug delivery due to its good biocompatibility and tuneable degradability. Meanwhile, synthetic polymers such as polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) are well known for their good hemocompatibility, molecular control and large-scale production. By combining the characteristics of natural and synthetic polymers, novel hybrid hydrogels can be developed, with improved physical/mechanical properties and biofunctions for various applications [9–12]. Since modification with hydrophilic molecules is a common method to further improve the hemocompatibility of biomaterials [13], HA microgels functionalized with PEG or PVP, as a representative model of functionalized microgel system, may demonstrate both synergistic biocompatibility and hemocompatibility [14]. In comparison with the macroscopic and nanoscale counterparts, such a degradable microgel system is envisaged to improve cytological safety [15] and to cause less extensive ischemic damage with intravascular injection [16]. Meanwhile, it is expected to have good injectability and tunability, while maintaining good biocompatibility.

In the present study, physically and chemically modified HA microgels were designed, manufactured and characterized, with formation of PVP-HA and PEG-HA hybrid network, respectively. HA microgels with no modification were fabricated as the control. As commercial HA dermal fillers are typical representatives of macroscopic HA-based hydrogels, a commercial HA filler sample was also used as an important reference in the rheological and swelling tests.

2. Material and methods

2.1. Materials

HA (average M_w 500 kDa) was purchased from Bloomage Freda Biopharm Co., Ltd. PEG methyl ether (PEGME, mw 5 kDa), PVP (mw 10 kDa), Sorbitan monooleate (Span® 80), Rhodamine 6G, Hoechst 33258, 2-isopropanol, acetone, penicillin-streptomycin, hyaluronidase from bovine testes and light mineral oil were purchased from Sigma-Aldrich. Divinyl sulfone (DVS) was purchased from VWR. The Dulbecco's Modified Eagle Medium (high glucose, L-Glut, phenol red) and the foetal bovine serum were purchased from Life Technologies. The AlamarBlue reagent was purchased from invitrogen™. Two millilitres of commercialized HA-based soft tissue fillers (BioHyalu, Freda Biopharm) was kindly provided by the Plastic Surgery Department, Nanfang Hospital, Guangzhou, China.

2.2. Preparation of microgels of mHA, mHA/PEG and mHA/PVP

Hyaluronic acid was dissolved in deionised water to make the 10 mg/mL HA solution. PEGME and PVP were dissolved in deionised water to make 150 mg/mL solutions. DVS was dissolved in deionised water to make the 50 mg/mL solution.

Several drops of 2 M NaOH were added to 3 mL of the 10 mg/mL HA solution to adjust the pH value in the range of 11 to 12. 100 μL of 150 mg/mL PEGME solution (PVP solution for mHA/PVP, deionized water for mHA, respectively) were added to the HA solution. Span® 80 was dissolved into mineral oil, with the volume ratio of 1 to 80. 12 mL of the resulting mineral oil solution were added to the previous HA/PEGME solution.

An ultrasonic processor (Model CP-750, Cole Parmer, USA) with a converter (Model CV-33) was used to prepare the w/o emulsion. During the ultrasonication, an amplification of 30% was selected, and the ultrasonication was run for three minutes. During the process, 0.6 mL of 50 mg/mL DVS solution was added. The reaction was allowed to proceed for one hour at room temperature without intervention.

Next, the oil phase was washed away with 16 mL isopropanol for mHA/PEG and mHA, and 16 mL acetone for mHA/PVP. Following agitation, the mixtures were centrifuged at 3000 rpm for 15 min. The precipitate was collected and washed several more times. The precipitate was dried under a negative pressure and re-suspended in deionised water.

2.3. Characterization

2.3.1. Fourier transform infrared spectroscopy

All FTIR measurements were carried out with Jasco FT/IR-4200 (Jasco Co. Ltd., Japan). Absorbance spectra were recorded for uncrosslinked HA, mHA/PVP, mHA/PEG, and mHA. In order to fit the baseline and to minimise the effects of the slopes on absorbance peaks, Essential FTIR™ v.3.50 was used to analyse the raw data and generate second derivative spectra, with the Savitsky-Golay algorithm as the smoothing method. The derivative spectra were further manipulated with vector normalization to correct the scattering effect and to normalize the baseline. The intensity of N-H bending peak was measured. The N-H bending intensities in mHA/PVP, mHA/PEG and mHA were compared to that of uncrosslinked HA, to determine the relative content of HA in the products.

2.3.2. Dynamic light scattering assay

In order to determine the average size of the particle units, including individual hydrogel particles and particle clumps in the suspension, dynamic light scattering (DLS) measurements and