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In situ assembly of fibrinogen/hyaluronic acid hydrogel via knob-hole interaction for 3D cellular engineering

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ABSTRACT

Hyaluronic acid (HA)-based hydrogels have applied widely for biomedical applications due to its biocompatibility and biodegradability. However, the use of initiators or crosslinkers during the hydrogel formation may cause cytotoxicity and thereby impair the biocompatibility. Inspired by the crosslinking mechanism of fibrin gel, a novel HA-based hydrogel was developed via the *in situ* supramolecular assembly based on knob-hole interactions between fibrinogen and knob-grafted HA (knob-g-HA) in this study. The knob-grafted HA was synthesized by coupling knob peptides (GPRPAAC, a mimic peptide of fibrin knob A) to HA via Michael addition. Then the translucent fibrinogen/knob-g-HA hydrogels were prepared by simply mixing the solutions of knob-g-HA and fibrinogen at the knob/hole ratio of 1.2. The rheological behaviors of the fibrinogen/knob-g-HA hydrogels with the fibrinogen concentrations of 50, 100 and 200 mg/mL were evaluated, and it was found that the dynamic storage moduli (G′) were higher than the loss moduli (G″) over the whole frequency range for all the groups. The SEM results showed that fibrinogen/knob-g-HA hydrogels presented the heterogeneous mesh-like structures which were different from the honeycomb-like structures of fibrinogen/MA-HA hydrogels. Correspondingly, a higher swelling ratio was obtained in the groups of fibrinogen/knob-g-HA hydrogel. Finally, the cytocompatibility of fibrinogen/knob-g-HA hydrogels was proved by live/dead stainings and MTT assays in the 293T cells encapsulation test. All these results highlight the biological potential of the fibrinogen/knob-g-HA hydrogels for 3D cellular engineering.

1. Introduction

With the exquisite nano- and micro-scale features of extracellular matrix (ECM), hydrogels have become a class of biomaterials for cell encapsulation, tissue engineering and regenerative medicine in recent years. Currently, hydrogels fabricated from the natural materials including collagen, hyaluronic acid (HA), and alginate are attracted much attention. Among them, HA, which is abundant in many tissues of the body [1], plays vital roles in cellular behaviors and physiological processes such as proliferation [2,3] morphogenesis [4,5] migration [6] inflammation and wound healing [7–9]. HA has been used widely in the fields of surface coating, drug/gene delivery [10], and particularly tissue engineering [11]. The biocompatibility and biodegradability make HA hydrogel to be an attractive matrix for regenerating a wide variety of tissues and organs such as cartilage [12,13], adipose [14,15], brain [16,17], and blood vessel [18].

To obtain the robust mechanical property, HA hydrogels are normally formed by a covalent crosslinking by the addition of crosslinkers, catalysts or photoinitiators, which may cause cytotoxicity [19–23]. Currently, several strategies were reported to avoid these disadvantages. For example, HA hydrogels were crosslinked via Diels-Alder reaction to avoid impairing the biocompatibility [24]. In our previous work, Xing et al. prepared a bioactive HA-based hydrogel by Diels-Alder reaction between the CQAASIKVAV peptide-modified furan-HA and the bismaleimide-functionalized poly(ethylene glycol) (PEG) for promoting neurite outgrowth of PC12 cells [25]. Crescenzi et al. also developed a novel crosslinking procedure based on copper-catalyzed azide-alkyne cycloaddition to form the HA-based gels *in situ*, which did not show toxic for both yeast cells and red blood cells [26]. Recently, Broguiere et al. demonstrated the specific transglutaminase activity of the activated blood coagulation factor XIII (FXIIIa) on accelerating

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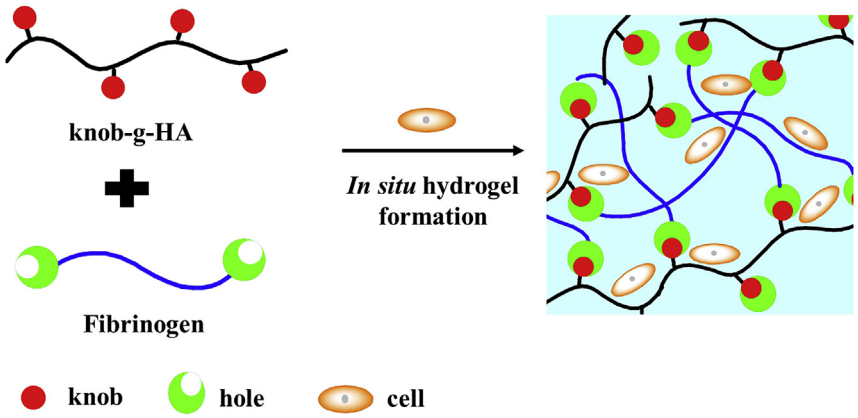

Scheme 1. Schematic illustration of the formation of fibrinogen/hyaluronic acid hydrogel via knob-hole interaction for cells encapsulation.

the reaction between lysine and glutamine of HA derivatives, and thus the formation of HA hydrogels [27].

Supramolecular assembly, based on natural host-guest pairs such as biotin and avidin or synthetic pairs like cyclodextrin and PEG, has been employed in the fields of surface modifications [28], molecular separation [29], drug delivery and hydrogels formation [30,31]. Park et al. prepared a supramolecular hydrogel of cucurbit [6]uril (CB[6])-conjugated HA via the interaction between CB[6] and diaminohexane (DAH). The CB[6]/DAH-HA hydrogels were feasible to deliver proper cues for cellular proliferation and differentiation [32]. Thompson et al. reported a series of noncovalent hydrogels crosslinked by receptor-ligand interactions between biotinylated PEG and avidin. Furthermore, the hydrogels based RGD- and metal matrix proteinase (MMP)-functionalized biotinylated PEGs were proved to be biocompatible with the improved spreading of mesenchymal stem cells (MSCs) [33].

As one of the main components in plasma [34], fibrinogen avoids eliciting an inflammatory response, foreign body reaction and other adverse reactions and has been an attractive material for regenerative medicine. With the development of molecular biology, the molecular mechanism of the conversion of fibrinogen into fibrin was disclosed gradually [35,36]. Fibrinogen is a 45 nm long and 340 kD large glycoprotein consisting of three pairs of polypeptide chains (Aα, Bβ, and γ) [37,38]. Cleaved by thrombin, a pair of binding sites (knob A/B) was generated at the fibrinopeptide A (FpA) and B (FpB) in the central N-terminal part of E-domain. Under a specific interaction between “knob” and “hole” (the site in the D-domains of fibrinogen), the fibrin fibers are aggregated and further covalently crosslinked into stable clots by FXIIIa. It was found that the yield force between “knob” and “hole” is comparable with the strongest noncovalent interaction between streptavidin and biotin [35]. It gives the potentials to use “knob”-“hole” interaction for engineering a hydrogel.

Inspired by the crosslinking mechanism of fibrin gel, a novel HA-based hydrogel with no decaying in biocompatibility was developed via the “knob”-“hole” interactions between fibrinogen and the knob-grafted HA (knob-g-HA) in this study (Scheme 1). Briefly, knob-g-HA was synthesized by coupling GPRPAAC peptides (knob) to methacrylated HA (MA-HA) via Michael addition reaction. Then the fibrinogen/knob-g-HA hydrogels were prepared by mixing fibrinogen and knob-g-HA solutions. The influences of the fibrinogen concentration and the “knob”/“hole” ratio on gel formation were investigated. The rheological behaviors, swelling properties, and microstructures were also studied. Finally, the application of the fibrinogen/knob-g-HA hydrogels for cells encapsulation was investigated.

2. Material and method

2.1. Materials

Hyaluronic acid (HA, Mw 1.1 × 10^5 Da) was purchased from Zhenjiang Dong Yuan Biotech Co., Ltd. Methacrylic anhydride was bought from Alfa Aesar (Tianjin) chemical Co., Ltd. Cysteine terminating fibrin knob peptide GPRPAAC (knob A) was synthesized by Sangon Biotech (Shanghai) Co., Ltd. Triethanolamine (TEA) was purchased from Sinopharm Chemical Reagent Co., Ltd. 5, 5′-Dithio bis-(2-nitrobenzoic acid) (DTNB) was the product of Aladdin Chemistry Co., Ltd. Human fibrinogen was purchased from Green Cross (China) Bio-products Co., Ltd. Tris(hydroxymethyl)aminomethane (Tris) was obtained from Amresco. Propidium iodide (PI) and 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. Calcein AM(Ca-AM) was purchased from US Everbright Inc., and 2-hydroxy-4’-(2-hydroxyethoxy)-2-methylpropiophenone (2959) was obtained from Tokyo Chemical industry Co., Ltd. All other reagents were analytical grade and used without further treatment if not specially mentioned. Water used in the experiments was purified by Milli-Q water system (Millipore, USA).

2.2. Synthesis of methacrylated HA (MA-HA)

HA was methacrylated according to the method reported previously [39]. Briefly, HA sodium salt (1 g) was dissolved in Milli-Q water (100 mL) at 4 °C under agitation overnight, into which five-fold methacrylic anhydride was added dropwise. The pH value was maintained between 8 and 10 by adding 5 M NaOH. The reaction was carried out in ice bath for 24 h under agitation. Then, NaCl was added to the mixture to a concentration of 0.5 M. MA-HA was collected by precipitating the solution in 5-fold volume of ethanol twice. MA-HA was then dialyzed (MWCO 3500 Da) against deionized water for 4 days, and the final product was obtained by lyophilization and stored at 4 °C. The purified MA-HA was characterized by ^{1}H NMR, from which the methacrylation degree of MA-HA was quantified.

2.3. Synthesis of knob-g-HA

Knob-g-HA was synthesized via Michael addition reaction between the methacrylate groups of MA-HA and the thiol groups of knob peptide. In brief, MA-HA (450 mg) was dissolved in Milli-Q water (15 mL) at room temperature. After complete dissolution, 30 mL nucleophilic buffering reagent TEA (30 mM) was added to

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