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The self-crosslinking smart hyaluronic acid hydrogels as injectable three-dimensional scaffolds for cells culture

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ABSTRACT

Although the disulfide bond crosslinked hyaluronic acid hydrogels have been reported by many research groups, the major researches were focused on effectively forming hydrogels. However, few researchers paid attention to the potential significance of controlling the hydrogel formation and degradation, improving biocompatibility, reducing the toxicity of exogenous and providing convenience to the clinical operations later on. In this research, the novel controllable self-crosslinking smart hydrogels with *in-situ* gelation property was prepared by a single component, the thiolated hyaluronic acid derivative (HA-SH), and applied as a three-dimensional scaffold to mimic native extracellular matrix (ECM) for the culture of fibroblasts cells (L929) and chondrocytes. A series of HA-SH hydrogels were prepared depending on different degrees of thiol substitution (ranging from 10 to 60%) and molecule weights of HA (0.1, 0.3 and 1.0M Da). The gelation time, swelling property and smart degradation behavior of HA-SH hydrogel were evaluated. The results showed that the gelation and degradation time of hydrogels could be controlled by adjusting the component of HA-SH polymers. The storage modulus of HA-SH hydrogels obtained by dynamic modulus analysis (DMA) could be up to 44.6 kPa. In addition, HA-SH hydrogels were investigated as a three-dimensional scaffold for the culture of fibroblasts cells (L929) and chondrocytes cells *in vitro* and as an injectable hydrogel for delivering chondrocytes cells *in vivo*. These results illustrated that HA-SH hydrogels with controllable gelation process, intelligent degradation behavior, excellent biocompatibility and convenient operational characteristics supplied potential clinical application capacity for tissue engineering and regenerative medicine.

1. Introduction

Hyaluronic acid (HA), or hyaluronan, is a kind of glycosaminoglycan (GAG) with repeating disaccharide units (α-1, 4-D-glucuronic acid and β-1, 3-*N*-acetyl-D-glucosamine) [1–4]. HA plays an essential role in the composition and structure of the extracellular matrix (ECM), which is mainly composed of GAGs and collagens through covalent and non-covalent interactions [5–7], could regulate cells adhesion, migration and morphogenesis, adjust cells proliferation and differentiation [8,9] and has important effects on the development, organization or remodeling of tissue, angiogenesis, modulation of inflammation and wound healing [10–12]. Because of its unique features, HA has been widely investigated and applied as biomaterials for drug and protein delivery [13–15], three-dimensional (3D) scaffold for cells culture [16–18], cartilage and bone regeneration [12,19,20], wound healing [21,22], bio-printing [23,24], and so on. However, as a natural polymer, rapid degradation *in vivo* and poor biomechanical properties limited its biomedical applications to some extent [2,12]. Furthermore, HA-based biomaterials are reported to inhibit cell attachment due to its hydrophilic and polyanionic nature, since cells were more willing to selectively adhere to neutral, hydrophobic or polycationic surfaces of materials [6,9,25].

Hydrogels are desirable biomaterials for tissue engineering because of high water content, good mechanical property, promising biocompatibility and high permeability to oxygen, nutrients and other water-soluble metabolites [26,27]. For obtaining appropriate mechanical properties and stable metabolic characterizations, a variety of chemical crosslinking methods and crosslinking agents have been developed to produce amphiphilic

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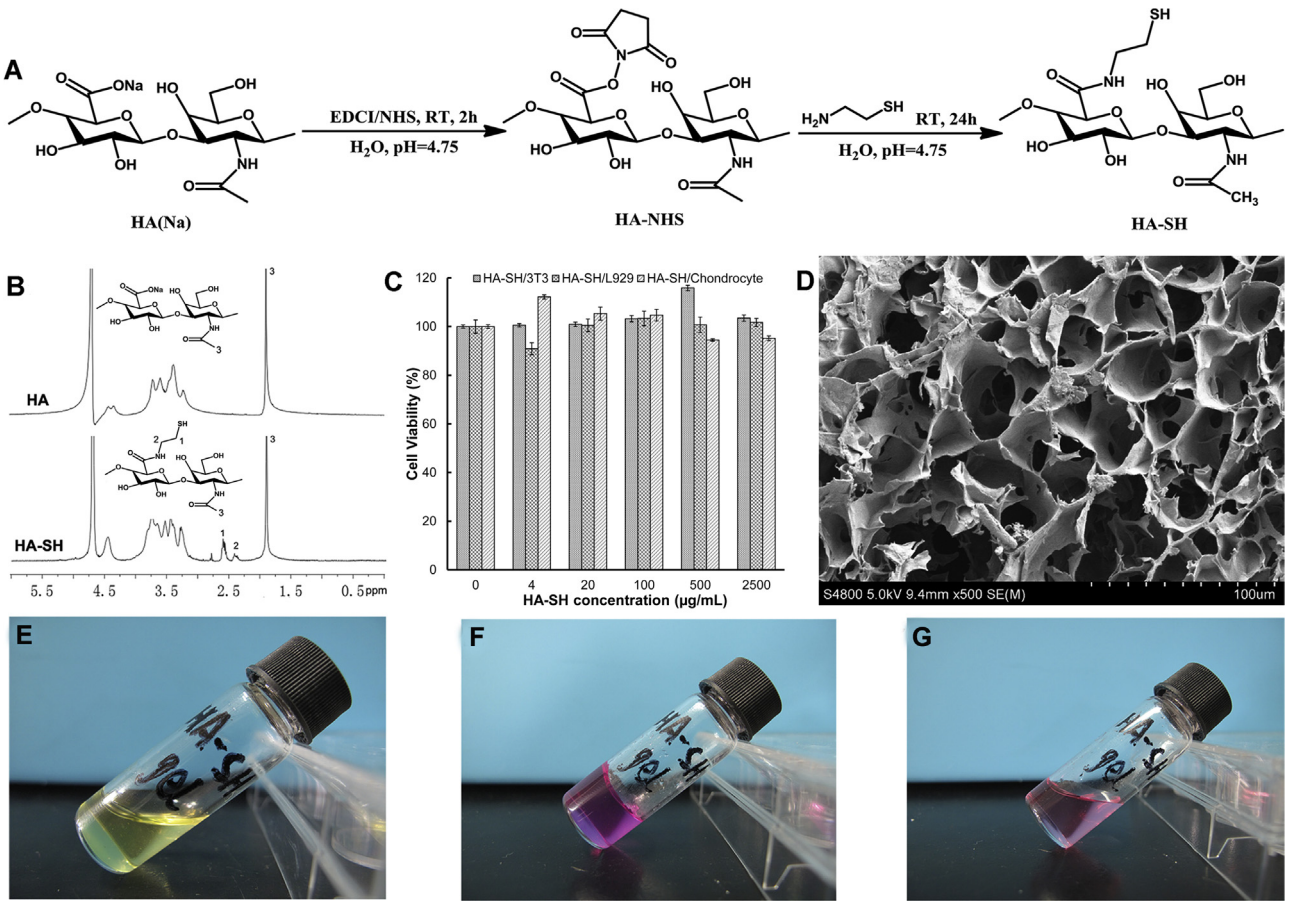

Fig. 1. (A) Synthesis protocol of thiol modified hyaluronic acid (HA-SH). (B) ^{1}H-NMR (D_2O) spectra of HA and HA-SH (Mw = 0.1M Da, Ds = 37.47%). (C) Biocompatibility of HA-SH polymers (Mw = 0.3M Da, Ds = 55.44%) for chondrocytes, 3T3 cells and L929 cells after 48 h of co-culture (n = 3). (D) The SEM micrograph of HA-SH hydrogel (Mw = 0.3M Da, Ds = 55.44%, 3.0% (w/v)). (E) Picture of HA-SH solution (Mw = 0.3M Da, Ds = 55.44%, 3.0% (w/v)). (F) Picture of HA-SH hydrogel. (G) Picture of HA-SH solution derived from the decomposition of HA-SH hydrogel with DTT (100 mM).

and gel-like HA materials by forming covalent networks [1], such as photo polymerization [28], Michael-type addition reaction [29], Schiff-base reaction [30], thiol-ene reaction [31], oxidizing reaction of tyramine [32,33] and other reactive groups [34,35]. Although these methods could effectively prepare HA hydrogels for application in biomedicine field, it still remained some problems for researchers to dissolve, such as the exogenous cytotoxicity derived from the additive initiator, crosslinking agents or byproducts and poor gelation efficiency due to use of light, radiation to initiate the crosslinking reaction. Moreover, uncontrollable gelation process and lack of responses to stimuli resulted in many limitations for reasonable clinical applications [36,37].

In recent years, disulfide bond crosslinked hydrogel has attracted many researchers' interests because of its simple synthesis protocol, convenient gelation method, *in-situ* gelation property, stimulating response to reductant and low-risk of crosslinking agent and byproduct. Prestwich's groups developed disulfide crosslinked HA-gelatin hydrogel film and sponge, and then co-cultured with cells to investigate the biocompatibility. The results showed that addition of gelatin into the HA-DTPH hydrogel could obviously increase the attachment and spreading of BALB/c 3T3 murine fibroblasts, which was seeded on the surface of the materials. Meanwhile, the subcutaneous implantation of thiolated HA films in rat peritoneal cavity showed that the films were tolerated with modest inflammatory response [38]. Wu et al. synthesized thiol-modified chitosan and prepared disulfide bond crosslinked hydrogel with pore size ranging from 5 to 30 μm to simulate the native extracellular matrix (ECM). The co-culture of cells and the hydrogels indicated that disulfide bond crosslinked chitosan hydrogels exhibited good potential for encapsulating cells and delivering protein *in vitro* [39]. Palumbo et al. synthesized a thiolated HA derivative, and then obtained a disulfide bond crosslinked hydrogel through the auto-oxidization of free thiol groups. Human derm fibroblasts were encapsulated into the hydrogel. The results suggested that the cells could proliferate effectively, which showed the potential application for tissue engineering [40]. Although these reported disulfide bond crosslinked hydrogels had good biocompatibility and were responsive to reductive condition, HA hydrogels with controllable characters and injectable properties have always been intense requirements as biomaterials applied in different biomedical fields.

In this research, a novel self-crosslinking smart hydrogel with *in-situ* gelation property was prepared from a single component, thiolated hyaluronic acid derivatives (HA-SH), which was obtained through simple chemically modification of HA. HA-SH hydrogels have excellent biocompatibility because HA was a kind of natural polymer and sulfhydryl compounds were widely distributed in animal tissues [41,42]. The disulfide bond of HA-SH hydrogels could be dissociated by reductant glutathione (GSH), which could be synthesized in cells and secreted in and out of cells. Disulfide bond crosslinked HA-SH hydrogels with encapsulated cells could be decomposed *via* a stepwise cells proliferation process because of the cleavage of the disulfide bond, triggered by GSH secreted in cells. Based on these characteristics, a series of HA-SH hydrogels were prepared by regulating molecule weight of HA, degrees of thiol substitution and gelation concentrations of HA-SH