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Blood-clotting mimetic behavior of biocompatible microgels

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ABSTRACT

Recent advances in hydrogel chemistry have led to the development of various soft materials capable of self-healing. Nevertheless, their self-healing capabilities are primitive compared to the responsive and adaptive strategies of blood clotting and wound healing in the human body. We developed a novel microgel system that mimics the process of blood clotting. Electrospun polylactic acid (PLA) fibers were entrapped inside the microgels of a hyaluronic acid conjugate with hesperidin side groups crosslinked by Fe ions; the resulting hydrogels showed fast self-healing and magnetic responsive properties. By applying an external magnetic field, which mimicked blood flow, the microgels successfully aggregated at target sites, like platelets. The aggregates were stable, as demonstrated by resistance to sonication for 30 min, and their moduli spanned tens to hundreds of kPa, demonstrating the mechanical integrity of the artificial clots. Like fibrin, the PLA fibers successfully strengthened the aggregates due to formation of uniform fiber-reinforced hydrogels; the artificial clots containing fibers had a 300% improved modulus and 50% increased hardness relative to the hydrogels without fibers. This unique intelligent system can be utilized in future self-healing systems, delivery systems, and devices with microfluidic channels.

Introduction

For decades, scientists and engineers have worked at developing self-healing materials that are safe, have extended lifespans, are energy efficient to produce, and have minimal environmental impact [1,2]. Examples of self-healing in nature include blood clotting and wound healing, which have inspired the recent development of sophisticated structured materials for self-healing [3–5]. For example, the vascular networks of synthetic vascular materials successfully mimic the role of blood vessels and can be used to distribute healing agents throughout a material [6,7]. The synthetic materials have been used in vascular grafting design for a variety of reasons, mainly due to the ease and flexibility of tailoring their mechanical properties. Although this approach has improved liquid-based healing technologies, the networks simply mimic the structure of vascular networks, not the complex processes that occur in blood vessels. Indeed, blood clotting and wound healing are intricate dynamic processes that have not previously been successfully mimicked using artificial materials [8].

In normal tissues, bleeding occurs immediately after an injury [4]. During hemostasis, blood vessels constrict and reduce blood flow to the damage point. Platelets express 'sticky' glycoproteins on their cell membranes, allowing them to aggregate with each other (Fig. 1a). Thrombin, a protease produced by blood platelets at sites of damage, polymerizes the soluble protein fibrinogen in the blood into an insoluble protein called fibrin, which forms a mesh. Aggregation of platelets begins minutes after damage and occurs as a result of activation of the GPIIb/IIIa receptor, which allows platelets to bind to von Willebrand factor or fibrinogen [9]. When at least nine different platelet surface receptors are activated, intra-platelet signaling pathways induce existing GpIIb/IIIa receptors to change their shape from curled to straight, allowing the receptors to bind [9]. Because fibrinogen is a rod-like protein with domains capable of attaching to GPIIb/IIIa, activated platelets with exposed GPIIb/IIIa can bind fibrinogen, resulting in aggregation. Polymerized fibrin together with platelets forms a hemostatic plug or clot over a wound site that blocks the flow of blood from the wound and traps proteins and other particles (Fig. 1a) [10,11]. Artificial materials developed for the treatment of hemorrhage simply promote clotting [12–14].

Herein, we developed a novel intelligent and biocompatible system that mimics blood clotting in nature. To mimic the aggregation of platelets, we employed microgels composed of fast self-healing materials based on mussel-inspired phenolic OH groups and hyaluronic acid. Similar materials, such as a triblock copolymers or hyaluronic acids with rapid self-healing properties

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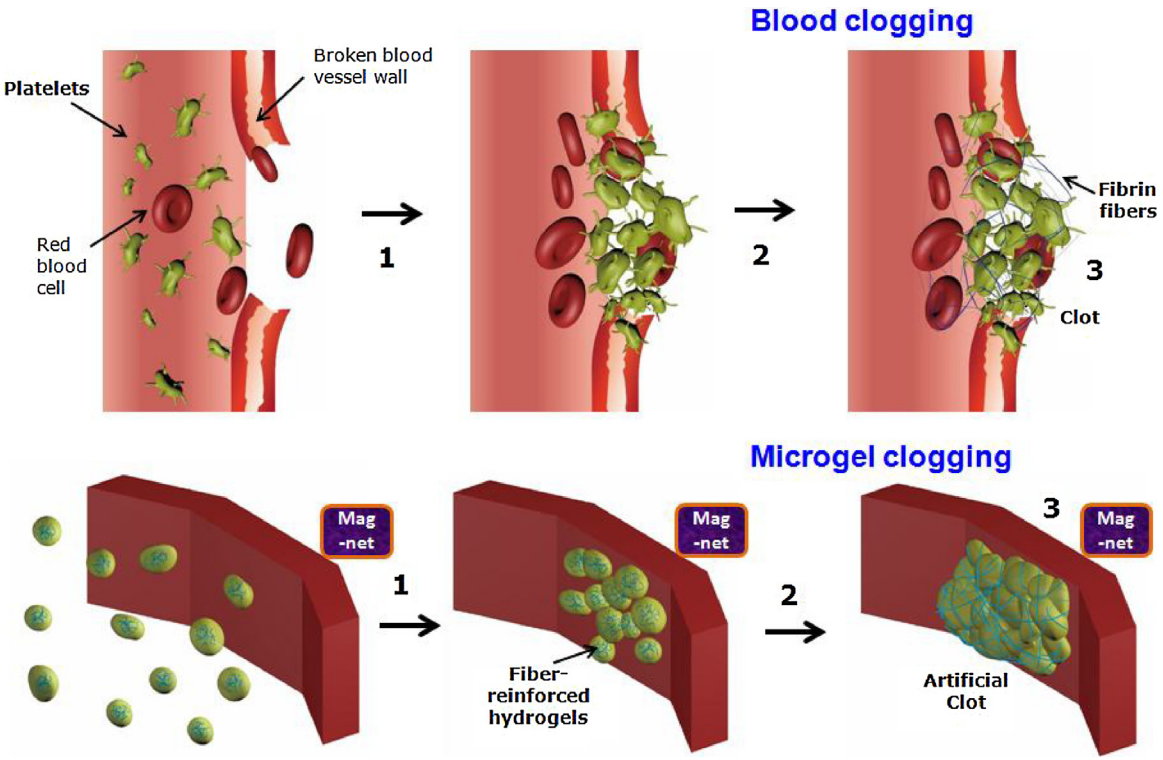

Fig. 1. Blood clogging (above): 1. Platelets attach to the endothelium. 2. Platelets start to release fibrin and begin to seal the endothelium. 3. Fibrin networks trap cells and proteins. Biomimetic aggregation of self-healing microgels by external magnetic force. 1. Microgels move to the target point following magnetic field. 2. Microgels aggregate and self-heal. 3. Reinforcement effect of PLA fibers.

through catechol-mediated specific and aromatic interactions, have been developed for injectable biodegradable hydrogels [1,15]. In this study, hyaluronic acid conjugated with hesperidin (HH) with many phenol functional groups enabled fast self-healing. Hesperidin was chosen based on our previous report on polyphenol-conjugated hyaluronic acids, because it provided fast cross-linking behavior [16–18].

The self-healing material was shaped into microgels with Fe ions, and the movement of the microgels was controlled by a magnetic field to mimic the flow of oxygen, nutrients, and blood to the area of damage in the blood vessel. Functional groups participating in crosslinking and self-healing principally mimicked the role of GPIIb/IIIa receptors. To mimic the fibrin mesh, biodegradable electrospun fibers were incorporated into the microgels using a modern preparation method involving combined electrospinning and spraying. This modern preparation method allowed us to prepare a large amount of functional fiber-containing microgels, which is not possible using photolithography [19]. During the past two decades, the simple one-step electrospinning process has fast developed along three directions; one is the production at large scale [20]. The second is the simultaneous treatments of multiple working fluids for creating complex nanostructures [21,22], and the third is the combination of electrospinning with other traditional technologies to provide new strategies for developing novel functional materials. Here, we showed an example of the third direction, i.e., electrospinning and spraying were combined to incorporate fibers into microgels to mimic the fibrin mesh.

Experimental

Materials

Poly (lactic acid) (PLA) was purchased from Green Chemical (GCS4000, Incheon, Republic of Korea). Coumarin 6 (98%, absorption λ_{max} 444 nm) and epichlorohydrin (ECH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Iron (III) chloride hexahydrate ($FeCl_3$ $6H_2O$, ferric chloride, 270.30 g/mol, extra pure 99.0%) was purchased from Acros Organics (Seoul, Republic of Korea). The sodium salt of hyaluronic acid (HA) was purchased from Bioland (Cheonan, Republic of Korea). Hesperidin (H) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Dimethyl formamide (DMF) (≥99.0%, 0.948 g/mL) was purchased from Samchun Pure Chemicals (Pyongtack, Gyeonggi, Republic of Korea). Dichloromethane (DCM) (≥99.0%), chloroform ($CHCl_3$) (≥99.0%), and ethanol (≥99.9%, 0.789–0.791 g/mL at 20 °C) were purchased from Duksan Pure Chemicals (Ansan, Gyeonggi, Republic of Korea). Deionized water was used for the preparation of HH and ferric chloride solutions. All chemicals were used as received without further purification.

Preparation of hesperidin–hyaluronic acid conjugate

The conjugation reaction of H to HA was achieved using ECH chemistry [16,17]. An aq. solution of HA (0.67 wt%) was first prepared

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