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Mussel-inspired electroactive chitosan/graphene oxide composite hydrogel with rapid self-healing and recovery behavior for tissue engineering

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ABSTRACT

Hydrogels currently used in electroactive tissues (cardiac tissues, skeletal muscles, and nerves) have certain shortcomings such as a lack of electrical conductivity and adhesiveness, both of which play a key role in the success of hydrogels in biomedical applications. In this study, chitosan (CS)/graphene oxide (GO) composite hydrogels with self-adhesive and self-healing properties, as well as electrical conductivity, were prepared by the incorporation of the mussel-inspired protein polydopamine (PDA). During the oxidizing process of dopamine (DA), graphene oxide was reduced by PDA and dispersed into the hydrogel network to form electric pathways. The covalent bonds, supramolecular interactions, hydrogen bonding, and π-π stacking gave the CS/GO composite hydrogels high stability, strong mechanical behaviors, good adhesiveness, self-healing properties, and a fast recovery ability. The electrical conductivity of the CS/GO reached 1.22 mS/cm and the adhesive strength of the composite hydrogel increased by 300% compared to CS-DA hydrogels. Cell culture results demonstrated that the conductive CS/GO hydrogels enhanced the cell viability and proliferation of human embryonic stem cell-derived fibroblasts (HEF1) and cardiomyocytes (CMs) compared to CS-DA hydrogels. Moreover, CMs showed a faster spontaneous beating rate than those in the control groups. Our work demonstrates a simple approach to fabricating polydopamine-based, adhesive, conductive, self-healing, and fast-recovering hydrogels that have great potential in electroactive tissue engineering applications.

1. Introduction

Hydrogels have attracted much attention in tissue engineering due to their similarity to natural soft tissues and the extracellular matrix, as well as their tunability [1–6]. Injectable hydrogels have been employed to encapsulate cells or drugs for *in situ* applications and to match the shape of damaged tissues. However, during injection, hydrogels typically experience unrecoverable deformation and detrimental biofouling. To solve this problem, self-healing and injectable hydrogels based on various mechanisms, such as reversible covalent bonds [7–11], hydrogen bonds [12], electrostatic interactions [13], ionic interactions [14], and so on, have been proposed. Compared with covalently bonded hydrogels, hydrogels based on non-covalently bonded networks—such as supramolecular bonding—tend to suffer from their fast degradation behavior, thereby hindering their long-term functioning.

In addition to the need to address the degradation shortcomings of physically cross-linked hydrogels, it has been reported that conductive polymers can promote the proliferation and

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differentiation of electrical-stimuli-responsive cells, such as stem cells [15–18], nerve cells [9,19,20], and cardiac cells [21]. Although traditional conductive hydrogels have shown many advantages on the growth of cells [22], they are not suitable for long-term use for tissues under electrophysiological conditions, such as in cardiac applications. This is because, during the delivery process, the hydrogel will encounter some mechanical load due to the contraction behavior of the heart [23], which might cause hydrogel breakage during its service period. As a result, these kinds of hydrogels cannot provide stable support for cell growth due to their non-self-healing properties. To address these problems, a series of self-healing, conductive, injectable hydrogels based on chitosan have been developed and were found to be ideal candidates for cardiac repairs and wound dressings [7,24]. The self-healing properties of these hydrogels makes it easy for them to recover upon deformation, which further prolongs their lifetime [25].

Besides being self-healing, conductive, and injectable, self-adhesiveness is also a desired property for hydrogels used in tissue engineering, as self-adhesiveness eliminates the need for additional adhesive tapes, such as polyacrylate adhesives, scotch tape, and bandages, while enhancing adhesion to the heart, skin, or other tissues [26–28]. The adhesive proteins of mussels, which are rich in catechol and amine functional groups, exhibit a great affinity for most organic and inorganic surfaces and have inspired people to develop a unique molecule, dopamine, to mimic its adhesive proteins. It has been found that dopamine very easily self-polymerizes to produce an adherent polydopamine (PDA), which also shows a great binding ability to a variety of materials including nanofillers. This functionality has facilitated the uniform distribution of nanofillers in the hydrogel network and has consequently led to novel hydrogel systems [29]. Furthermore, there are a series of non-covalent interactions—including hydrogen bonding and π-π stacking—between the PDA chains that endow hydrogels with self-healability [30,31]. In addition, Han et al. designed PDA/GO composite hydrogels by employing dopamine as a reducing agent, and these hydrogels can be used as implantable and wearable devices [32]. The fascinating properties (i.e., self-polymerization, adhesiveness, and reduction capability) of PDA make it a suitable reducing agent for GO and a capping agent to stabilize the resulting reduced GO [33].

Chitosan, a partially de-acetylated derivative of naturally occurring polysaccharide chitin, is a biocompatible and biodegradable polymer [34]. The polycationic character and the presence of reactive functional groups, including hydroxyl and amine groups, as well as an extensive hydrogel bonding capacity, endow chitosan with various beneficial properties, such as biocompatibility, biodegradability, renewability, film forming, etc. For these reasons, chitosan is widely used in many applications including as artificial skin, drug delivery vehicles, and tissue engineering scaffolds [35–37]. However, pure chitosan scaffolds are very brittle, which greatly limits their application. To address this problem, using nanofillers or compounding chitosan with the second phase of polymers could effectively improve the physicochemical properties of chitosan. Moreover, the multifunctional groups on the chitosan backbone enable it to be easily attached to filler materials [38–40]. Furthermore, the intermolecular and intramolecular hydrogen bonds in chitosan molecules strongly stabilize the packing structure of chitosan, increase its melting point, and cause it to only dissolve in acidic conditions [41].

Graphene is a single layer of two-dimensional carbon materials arranged in a honeycomb lattice [42] that is regarded as an ideal nanofiller in polymers due to its excellent mechanical properties, large aspect ratio, and excellent conductivity and optical properties [43–45]. Recent reports have also demonstrated that graphene is beneficial for cell adhesion and proliferation [2,46–49]. Graphene oxide (GO), one of the most important derivatives of graphene, has also attracted considerable attention in recent years. Monomolecular sheets of GO can be obtained by dispersing the oxidized graphite in aqueous or organic solutions. The large number of hydrophilic groups on its surface, such as carboxyl, hydroxyl, and epoxy, endow it with stable dispersion in solutions via electrostatic repulsion [50,51]. Moreover, the oxygenated surface functionalities in the GO nanosheets give it the potential to react with many polymers [52]. It has been reported to be a very effective reinforcement for chitosan films due to their strong electrostatic interactions [53,54]. However, there are few reports about chitosan/graphene composite hydrogels with desirable self-healability and adhesiveness.

Inspired by natural mussel chemistry, we proposed a simple approach to prepare chitosan hydrogels with conductive, self-adhesive, and rapid self-healing and recovery properties at room temperature. For the hydrogel system, the biocompatible polymer chitosan was chosen as a skeleton to provide the primary amine groups; GO was used as reinforcing nanofillers; and PDA was used as a crosslinker and partially converted the GO to a conductive graphene during the PDA reduction process. The hydrogels showed a fast self-healing ability, good adhesiveness, and enhanced biodegradability. The electrical conductivity of the hydrogels achieved 1.22 mS/cm, which matches that of the native myocardium. Cell culture results demonstrated that the conductive hydrogels enhanced stem cell-derived fibroblast and cardiomyocyte (CM) adhesion. Furthermore, the spontaneous beating rate of CMs on hydrogels was two times higher than the control group. All of these results suggest that the hydrogels proposed in this study could be suitable candidates for electroactive tissue engineering applications.

2. Materials and methods

2.1. Materials

Dopamine hydrochloride, low molecular weight chitosan (M_n = 50,000–190,000) with a viscosity of 20–300 cP (1 wt% in 1% acetic acid), and acetic acid (ACS reagent) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Other chemicals involved in this study were also purchased from Sigma–Aldrich unless otherwise stated. All reagents were used as received.

2.2. Graphite oxide synthesis and purification

Graphite oxide was synthesized according to an improved Hummer's method reported by Marcano [55]. Briefly, a mixture of graphite flakes (2 g) and $KMnO_4$ (12 g) were added into a mixture of concentrated H_2SO_4/H_3PO_4 (360:40 ml). The resulting mixture was stirred at 50 °C for 12 h. Afterwards, the mixture was cooled to room temperature and then poured onto a mixture of ice (~300 g) with H_2O_2 (5 ml, 30 wt %). The mixture was centrifuged (8000 rpm for 30 min) and the supernatant was decanted away. The remaining solid was then washed in succession with deionized water, 30% HCl, ethanol, and deionized water. For each wash, the filtrate was centrifuged (8000 rpm for 30 min) and the supernatant was decanted away. The resulting solid was further purified by dialysis against deionized water for 3 days. After that, the solution was freeze-dried to obtain the graphite oxide powder. The graphite oxide powder was then dispersed in water via ultrasonication for 1 h to obtain a uniform graphene oxide (GO) solution for future use.

2.3. Hydrogel preparation

Using a typical process for the preparation of hydrogel, 300 mg