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Electrically conductive graphene/polyacrylamide hydrogels produced by mild chemical reduction for enhanced myoblast growth and differentiation

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ABSTRACT

Graphene and graphene derivatives, such as graphene oxide (GO) and reduced GO (rGO), have been extensively employed as novel components of biomaterials because of their unique electrical and mechanical properties. These materials have also been used to fabricate electrically conductive biomaterials that can effectively deliver electrical signals to biological systems. Recently, increasing attention has been paid to electrically conductive hydrogels that have both electrical activity and a tissue-like softness. In this study, we synthesized conductive graphene hydrogels by mild chemical reduction of graphene oxide/polyacrylamide (GO/PAAm) composite hydrogels to obtain conductive hydrogels. The reduced hydrogel, r(GO/PAAm), exhibited muscle tissue-like stiffness with a Young's modulus of approximately 50 kPa. The electrochemical impedance of r(GO/PAAm) could be decreased by more than ten times compared to that of PAAm and unreduced GO/PAAm. *In vitro* studies with C2C12 myoblasts revealed that r(GO/PAAm) significantly enhanced proliferation and myogenic differentiation compared with unreduced GO/PAAm and PAAm. Moreover, electrical stimulation of myoblasts growing on r(GO/PAAm) graphene hydrogels for 7 days significantly enhanced the myogenic gene expression compared to unstimulated controls. As results, our graphene-based conductive and soft hydrogels will be useful as skeletal muscle tissue scaffolds and can serve as a multifunctional platform that can simultaneously deliver electrical and mechanical cues to biological systems.

Statement of Significance

Graphene-based conductive hydrogels presenting electrical conductance and a soft tissue-like modulus were successfully fabricated via mild reduction of graphene oxide/polyacrylamide composite hydrogels to study their potential to skeletal tissue scaffold applications. Significantly promoted myoblast proliferation and differentiation were obtained on our hydrogels. Additionally, electrical stimulation of myoblasts via the graphene hydrogels could further upregulate myogenic gene expressions. Our graphene-incorporated conductive hydrogels will impact on the development of new materials for skeletal muscle tissue engineering scaffolds and bioelectronics devices, and also serve as novel platforms to study cellular interactions with electrical and mechanical signals.

1. Introduction

A variety of biomaterials that can actively modulate the responses of biological systems have been extensively explored to leverage the development of functional tissue engineering scaffolds [1,2]. As material-cell interactions involve multiple biological,

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chemical, electrical, and mechanical signals, the importance of multifunctional biomaterials that can deliver multiple critical signals to cells/tissues has been highlighted for various biomedical applications, such as tissue engineering scaffolds and prosthetics [1,3–6]. In particular, electrical signals play essential roles in cell communication and cellular behaviors (e.g., proliferation, differentiation, and migration) [7–9], which has strongly encouraged the development of electrically conductive biomaterials to precisely and efficiently provide electrical signals. For example, electrical stimulation via conductive biomaterials could improve the growth and differentiation of various cells, such as neurons, cardiomyocytes, myoblasts, and stem cells [10–14]. In addition, material rigidity can substantially influence cell behaviors, accompanied with changes in actin organization and gene expression via mechanotransduction [15–17]. Soft materials, such as hydrogels, have been therefore widely used to mimic the mechanical properties of the native soft tissues and study cellular behaviors [18,19].

Accordingly, increasing attention has been paid to the synthesis of electrically conductive hydrogels that simultaneously present electrical activity and a tissue-like softness. Conductive hydrogels have typically been synthesized by combining a hydrophilic polymer with conductive materials, such as conductive polymers [20–26], metal nanowires [27], and carbon materials (e.g., carbon nanotubes, graphene oxide, and graphene) [28,29]. For example, Bettinger and colleagues polymerized polyaniline in heparin/gelatin hydrogels and found that these conductive hydrogels supported the attachment, proliferation, and differentiation of myoblasts [22]. Shin et al. fabricated composite hydrogels by mixing carbon nanotubes with gelatin methacrylate to improve the mechanical and electrical properties of hydrogels for cell culture studies [24]. Yet, the electrical properties, stability under physiological conditions, and material cytocompatibility of conductive hydrogels need to be further improved.

Graphene is a 2-dimensional carbon material of atomic thickness that displays high mechanical strength and good electrical conductivity [30]. Graphene oxide (GO) is generally produced by the oxidative exfoliation of graphite by harsh oxidation processes and can be well dispersed in aqueous solutions [31]. GO, however, exhibits inherently poor electrical conductivity due to the defects in its molecular structure. In addition, GO can be reduced to form conductive reduced GO (rGO) by partly restoring sp^2 carbon bonds. Unfortunately, rGO extensively aggregates in aqueous solution due to their low water solubility. Graphene and graphene derivatives (e.g., GO and rGO) are cytocompatible and have been widely utilized as novel components of biomaterials that can promote molecular and cellular interactions [32–37]. Graphene-based conductive hydrogels can offer several advantages properties, such as improved mechanical stability (e.g., toughness and flexibility) and electrical stability because graphene derivatives strongly interact with polymer chains and do not require dopants for their electrical conductance. Especially, several studies have performed to produce graphene composite hydrogels for scaffold applications [28,29,38–40]. For example, Shin et al. produced composite hydrogels consisting of GO and methacrylated gelatin by ultraviolet crosslinking and found that these hydrogels were cytocompatible and supportive of fibroblast growth [29]. Since GO is poor in electrical conductivity, as mentioned earlier, GO-containing hydrogels can be inappropriate to efficiently deliver electrical signals to cells. On the other hand, uses conductive rGO flakes for hydrogel synthesis often lead to severe aggregation, resulting in substantial difficulties in creating homogeneous conductive rGO networks in aqueous environments [41]. Therefore, it is difficult to produce conductive hydrogels using graphene derivatives (i.e., GO and rGO) in conventional fabrication methods. Recently, we reported that mild chemical reduction of GO-incorporated calcium alginate hydrogels can convert the embedded GO to rGO with minimal aggregation within the hydrogel network [42]. These reduced GO/alginate hydrogels containing well-dispersed rGO with the matrices displayed greatly enhanced adsorption capacity of hydrophobic compounds, such as rhodamine compounds, compared to unreduced hydrogels.

In this study, we fabricated conductive hydrogels consisting of rGO and polyacrylamide (PAAm) (Fig. 1). GO/PAAm composite hydrogels were first prepared and then reduced under mild conditions to form conductive rGO networks within the gels. The resulting rGO-containing PAAm hydrogels were termed r(GO/PAAm), and different r(GO/PAAm) hydrogels were produced by varying the reduction time. The electrical and mechanical properties of the r(GO/PAAm) hydrogels were characterized. *In vitro* myoblast culture was performed to study the proliferation and differentiation of myoblasts on r(GO/PAAm) in comparison with unreduced hydrogels, GO/PAAm, and PAAm controls to explore their potential for use as skeletal muscle tissue scaffolds. Also, electrical stimulation of myoblasts was performed using the conductive r(GO/PAAm) hydrogels.

2. Materials and methods

2.1. Materials

Acrylamide solution mixed with bisacrylamide (30%, 29:1) and ammonium persulfate (APS), L-ascorbic acid (AA), bovine serum albumin (BSA), and ammonium persulfate (APS) were purchased from Sigma-Aldrich (St. Louis, MO). GO aqueous solution (6 mg/mL) was purchased from Graphene Supermarket Inc. (Calverton, NY). Cell culture reagents including Dulbecco's phosphate-buffered saline (DPBS), antibiotic–antimycotic solution, and horse serum were obtained from Invitrogen (Carlsbad, CA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Hyclone (Logan, UT). Mouse anti-human monoclonal antibody of myosin heavy chain (MHC), was purchased from R&D systems (R&D Systems, Abington, United Kingdom). 4, 6–Diamidino-2-phenylindole (DAPI), live/dead staining kit, and Alexa Fluor® 555-labeled goat anti-rabbit IgG were purchased from Life Technologies (Carlsbad, CA). A cDNA synthesis kit was obtained from TaKaRa Bio (Otsu, Japan). Power SYBR® green PCR master mix was obtained from Thermo Fisher Scientific (Waltham, MA).

2.2. Synthesis of GO/PAAM and r(GO/PAAM) hydrogels

For the preparation of GO-embedded polyacrylamide (GO/PAAm) hydrogels, 1.6 mL acrylamide/bisacrylamide solution (premixed at a 29:1 ratio of acrylamide and bisacrylamide), 3 mL aqueous GO solution, 1.4 mL double deionized (DDI) water, and 60 μL APS (10% w/v) were mixed for 1 h at 4 °C. The final mixture solution contained 8% (v/v) acrylamide and 0.3% (w/v) GO. Note that the GO used in this study was in the form of single GO sheets with an average size of ~3 μm (Supporting Information Fig. S1) [42]. After mixing, the solution was transferred to the casting frame (Mini-PROTEAN® Casting Frame, Bio-Rad Laboratories, Hercules, CA) with a short plate and spacer plate (1.0 mm gap) and polymerized in an oven at 60 °C for 4 h. A pristine polyacrylamide (PAAm) hydrogel that did not contain GO was also produced as controls via the same processes as the GO/PAAm hydrogel, except that DDI water was added instead of GO solution. For further studies, the hydrogel films were punched to produce discs (12 mm in diameters). For the synthesis of reduced composite hydrogels, i.e., r(GO/PAAm), the GO/PAAm hydrogel disc was incubated in a reduction solution containing 2 mg/mL L-ascorbic acid solution at 37 °C. Various r(GO/PAAm) hydrogels were prepared by varying the