Reduced Graphene Oxide Incorporated Myocardial Matrix as a Functional Scaffold for Cardiac Tissue Engineering

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Statement of Purpose: The key limitation of current porous matrices used for cardiac tissue engineering is their poor conductivity, which hampers signal propagation and cell to cell electrical communication between pores[1]. One particular nanomaterial with increasing potential for cardiac engineering is graphene. Due to its unique single-atom thick structure, graphene possesses many unusual physicochemical properties including high electrical and thermal conductivities, high specific surface area, and high mechanical strength. In native tissue, the extracellular matrix (ECM) provides the physical and chemical conditions that enable the development of all biological tissues. It is a complex nano-to-microscale structure made up of protein fibers and serves as a dynamic substrate that supports tissue repair and regeneration. This study examined the hypothesis that a composite biomaterial composed of reduced graphene oxide (rGO) integrated with decellularized extracellular matrix (dECM) can be used as an electroactive three-dimensional scaffold with tunable electrical and mechanical properties for cardiac tissue engineering.

Methods:

Fabrication of rGO-dECM Composite Scaffolds

Porcine ventricular tissue was sectioned into 100 µm thick fragments and undergo lysis in a solution of 10 mM Tris buffer and 0.1% w/v EDTA for 2 h, followed by 12 h of solubilization in 1% SDS and washed in 1% Triton-X100 and PBS to remove excess detergent and cell debris. Single-layer graphene oxide (GO) stock solution (Graphene Laboratories, Calverton, NY) was diluted to an initial concentration of 0.5 mg/mL and sonicated for 1 h. Sonicated GO solution is then centrifuged, and the supernatant is collected to obtain a GO solution that contains a high percentage of non-aggregated GO. This GO solution is then reduced by $NaBH_4$ for 1 hour before solid reduced graphene oxide (rGO) is filtered out. Lyophilized rGO and dECM was combined together with sonication in a acetic acid , pepsin, and riboflavin solution at various rGO concentrations and at 2% w/v dECM. Composite gel solutions were then crosslinked at 37ºC overnight. Solutions containing riboflavin were further crosslinked with long-wavelength UV light for 30 mins.

Scaffold Characterization

Scaffold structure was examined using scanning electron microscopy (SEM) using a beam voltage of 5kV and with samples sputtered with AuPd. Conductivity of scaffolds were measured using a four-point probe method, and mechanical properties were measured using an Instron compressive tester.

Results: SEM imaging of composite scaffolds revealed a porous 3D structure with pores approximately 50-200µm in size. Higher magnification images of the pore walls showed a deposition of rGO flakes throughout. The collagen and GAG content of scaffolds was found to be comparable to that of native tissue, confirming that the ECM composition was unaffected by the decellularization process. Conductivity measurements showed an increasing trend in conductivity corresponding both with an increase in rGO content and an increase in the degree of reduction of GO. The addition of rGO and the reduction of GO also led to an increase in modulus, and it was found that further crosslinking of the collagen in the dECM with riboflavin and UV exposure led to scaffolds with moduli comparable to that of native cardiac tissue. Scaffolds seeded with RUES2-derived cardiomyocytes showed good cell viability, and cardiomyocyte maturation was assessed with immunohistological and qRT-PCR analysis of Cx43 and cTnI expression. Additionally, live-cell calcium dye imaging was used to examine the ability of the composite scaffolds to enhance cell signal propagation.

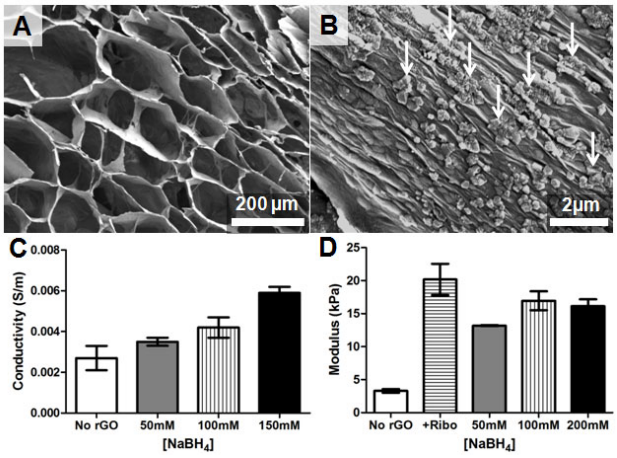

Figure 1. (A) SEM images showing the porous structure of the composite hydrogels and (B) the presence of rGO flakes (arrows) on pore surfaces. (C) Conductivity increases as a function of GO reduction. (D) Compressive modulus also increases as a function of GO reduction, as well as with riboflavin-assisted crosslinking.

Conclusions: In this study we have developed an electrically conductive 3D scaffold with tunable properties, and demonstrated the ability for these composite functional scaffolds to promote cardiomyocyte maturation and effectively mimic the cell-cell signaling seen in native cardiac tissue. Future studies will involve examining whether this enhanced maturation and signal propagation translates to improved cardiac function in an *in vivo* infarct model, possibly leading to the development of a treatment for this pathology. While the benefits of a conductive 3D scaffold were demonstrated here with cardiomyocytes, it can be reasoned that such composite scaffolds could lead to similar advances in the engineering of tissues derived from other electrically-sensitive cell types such as nervous tissue and skeletal muscle.

References: 1) Bursac N. Biochem Biophys Res Commun. 2007; 361:847–853.