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Glucose-sensitive self-healing hydrogel as sacrificial materials to fabricate vascularized constructs

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ABSTRACT

A major challenge in tissue engineering is the lack of proper vascularization. Although various approaches have been used to build vascular network in a tissue engineering construct, there remain some drawbacks. Herein, a glucose-sensitive self-healing hydrogel are employed as sacrificial materials to fabricate branched tubular channels within a construct. The hydrogel composes of mainly reversibly crosslinked poly(ethylene glycol) diacrylate and dithiothreitol with borax as the glucose-sensitive motif. The hydrogel is injectable and mechanically strong after injection. Moreover, it can be rapidly removed by immersion in the cell culture medium. To show the feasibility in building a vascularized tissue construct, the designed branching vascular patterns of the glucose-sensitive hydrogel are extruded and embedded in a non glucose-sensitive hydrogel containing neural stem cells. Vascular endothelial cells seeded in the lumen of the channels by perfusion can line the channel wall and migrate into the non-sacrificial hydrogel after 3 days. In long-term (~14 days), the endothelial cells form capillary-like structure (vascular network) while neural stem cells form neurosphere-like structure (neural development) in the construct, revealing the morphology of "a vascularized neural tissue". The novel sacrificial materials can create complicated but easily removable structure for building a vascularized tissue construct particularly a neurovascular unit.

1. Introduction

Thousands of people each year are waiting for tissue or organ donations. The unavailability of adequate organs has led to a focus of studies on regenerative medicine. Because host-capillary invasion upon implantation of man-made tissues require several weeks for complete vascularization [1], insufficient nutrient and oxygen supply may give rise to cell death in the core of the implant, which causes problems in tissue integration. Therefore, building vascular networks within a tissue construct is a very important issue.

There are several strategies to generate vascularized constructs in vitro including cell-based approaches and fabrication of interconnected channels within scaffolds [2–4]. Literature have demonstrated that co-culture of endothelial cells with the other types of cells such as mesenchymal stem cells or progenitors could provide functional vasculature throughout the constructs [5,6]. However, this strategy is time consuming and restricted to relatively thin constructs [7]. Meanwhile, recent studies have developed artificial microchannels to form organized vascular networks. For instance, hydrogels can be cast around non-sacrificial and sacrificial templates. Upon removal of the sacrificial template by mechanical force or liquefaction followed with flushing [8–10], the defined channel structures are left behind. Although these designs seem to work well, sacrificial structures with distinct physical properties (e.g. melting point and solubility) used in the system should be tugged or suctioned very carefully. Otherwise, the integrity of the construct with tunnels may be destroyed. Moreover, some of these designs only display unbranched structures within the construct, not fully resembling the *in vivo* situation. Thus, there exists a need to develop novel techniques to engineer functional vascular networks within hydrogel constructs.

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Self-healing hydrogels are a class of smart materials that have drawn much recent attention [11,12]. Multi-stimuli responsive (e.g. enzymes, glucose, and pH) self-healing hydrogels are highly desirable and have the potential in a broad range of applications [13–15]. Owing to the self-healing ability, the complicated and interconnected patterns such as vasculature can be designed. Furthermore, the designed patterns within the constructs may be removable according to their responses to specific stimuli. Therefore, self-healing hydrogel served as a sacrificial structure within the other hydrogel is a promising strategy to generate vascularized constructs. For instance, guest-host hydrogel with self-healing property has been used to build such a construct [16]. However, the construct lacks enough mechanical properties for perfusion and for long-term stability of the channel structure.

In this study, we developed a novel convenient fabrication method to build tubular channels within a construct and showed the capability of the construct to form a vascularized neural tissue ("AVANTI"). We prepared a glucose-sensitive self-healing hydrogel as the sacrificial layer of branched tubular channels inside another hydrogel containing neural stem cells (NSCs). When the construct was soaked in the culture medium, the glucose-sensitive self-healing hydrogel was rapidly dissolved and spontaneously formed the channels inside the other non-glucose-sensitive hydrogel. Moreover, vascular endothelial cells (ECs) seeded in the channels could proliferate on the channel wall, further migrate in the non-sacrificial hydrogel, and form capillary network interacting with NSCs in the construct. We proposed that this glucose-sensitive hydrogel may be used to create complicated but easily removable structure in tissue engineering constructs for vascularization.

2. Materials and methods

2.1. Preparation of glucose-sensitive self-healing hydrogel

Glucose-sensitive self-healing hydrogel was prepared as the sacrificial component by thiol-ene Michael polyaddition chemistry. For the purpose, 100 mg poly(ethylene glycol) diacrylate (PEGDA) and 22 mg dithiothreitol (DTT) were first dissolved in 0.5 ml phosphate buffered saline (PBS) and then 0.5 ml of 0.1 M borax solution was added to the mixture under vigorous stirring. The glucose-sensitive self-healing hydrogel formed within a few minutes of stirring. Non-sacrificial hydrogel could be either fibrin or other types of gel suitable for 3D cell culture.

2.2. Characterization of the glucose-sensitive self-healing hydrogel

The mechanical properties of the hydrogel was measured by a rheometer (HR-2, TA Instruments) with a cone and plate geometry at 1 Hz and 1% strain after preparation. The 40 mm diameter cone with 2° angle was used in this study. To measure the self-healing hydrogel, the mixture of PEGDA and DTT solution was first loaded on the plate. Then, the borax solution was added onto the mixture. The temperature was adjusted to 25 °C and 37 °C during the measurement. The self-healing properties were evaluated by rheology. Continuous step change of oscillatory strain between 1% and 150% at 1.0 Hz was given to test the strain-induced destruction and recovery of hydrogel. Structure destruction was induced via application of 150% strain for 2 min and structure recovery was evaluated by decreasing strain to 1% for 2 min. The hydrogel with shear-thinning properties for injectability was evaluated by the steady shear experiment. The viscosities of the hydrogel were measured as a function of shear rates.

A Fourier-Transform infrared spectrometer (FT-IR; Perkin Elmer, USA) was used to confirm the borax-catalyzed thiol–ene Michael addition reaction. The hydrogel was lyophilized prior to FT-IR analysis. The lyophilized hydrogel was ground with KBr for measurement. The absorbance was collected at a range of 450–4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.3. Culture of endothelial cells (ECs) and neural stem cells (NSCs)

Endothelial cells (ECs) were obtained from bovine carotid arterial and maintained in Dulbecco's Modified Eagle's Medium-low glucose (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), and 100 U/ml penicillin–streptomycin (Caisson Labs, USA). Mouse neural stem cells (NSCs) were cultured in Dulbecco's Modified Eagle's Medium-high glucose and Ham's F-12 (DMEM/F12, Gibco, USA) supplemented with 100 U/ml penicillin–streptomycin, 400 μg/ml G418 (Invitrogen), and 10% FBS. Cultures were incubated in a humidified incubator with 5% CO_2 at 37 °C. After reaching 80% cell confluency, cells were subcultured using 0.25% trypsin/EDTA solution (Gibco). The medium was refreshed once every 2–3 days. For better observation, ECs and NSCs were labeled by a red fluorescent cell tracker PKH26 and a green fluorescent cell tracker PKH67, respectively.

2.4. Preparation of vascularized constructs

The cell-laden vascularized construct was prepared by the sacrificial gel (glucose-sensitive self-healing hydrogel) and the non-sacrificial gel (e.g. fibrin gel or chitosan gel, and etc.) selected to encapsulate cells. First, NSCs (2×10^6 cells/ml) were embedded in non-sacrificial gel and then cast on the culture plates. The sacrificial gel was then extruded by a syringe on the non-sacrificial gel. After that, non-sacrificial gel encapsulated NSCs was covered on the top. The combined hydrogels were immersed in the culture medium, the sacrificial gel would dissolve due to their glucose sensitive property and the tunnels formed. ECs were injected in the tunnels and the cell-laden vascularized construct was achieved. For better observation, ECs and NSCs were labeled by a red fluorescent cell tracker PKH26 and a green fluorescent cell tracker PKH67, respectively.

2.5. Cell proliferation in constructs

The cell-laden constructs were incubated for 72 h. The culture medium was replaced every 2 days. Before the cell number analysis, the cell-laden constructs were washed in PBS and then digested in papain (Sigma) solution at 60 °C for overnight. Cell number was analyzed after reaction with Hoechst 33528 dye (Sigma-Aldrich) [17]. The fluorescence intensity was determined by the microplate reader (fluorescence mode, SpectraMax M5, Molecular Devices) with excitation at 365 nm and emission at 458 nm. Cell number was obtained from the value of intensity using a calibration curve.

2.6. Gene expressions for cells encapsulated in hydrogels

Because ECs and NSCs were obtained from different animal species, the expression of the same genes from different cells can be distinguished by primer specificity. To determine the neuro-vascular communication of cells encapsulated in hydrogels, the expression level of neural-related and angiogenesis-related genes was analyzed using the real-time reverse transcription polymerase chain reaction (RT-PCR) in vitro. Total RNA was extracted by the Trizol® reagent (Invitrogen, USA) from cell-laden constructs (ECs, NSCs, and co-culture of ECs and NSCs) at 72 h. RNA strand was first reverse transcribed into cDNA and amplified by the RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, St. Leon-Rot, Germany). The real-time RT-PCR was performed using the Dy-NAmo Flash SYBR Green qPCR Kit (Finnzymes Oy, Espoo, Finland).