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Viability and functionality of cells delivered from peptide conjugated scaffolds

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ABSTRACT

Many cell-based therapies aim to transplant functional cells to revascularize damaged tissues and ischemic areas. However, conventional cell therapy is not optimally efficient: massive cell death, damage, and non-localization of cells both spatially and temporally all likely contribute to poor tissue functionality. An alginate cell depot system has been proposed as an alternative means to deliver outgrowth endothelial cells (OECs) in a spatiotemporally controllable manner while protecting them in the early stages of tissue re-integration. Here OECs exiting the alginate scaffold were measured for viability, functionality, and migration speed and characterized for cytokine and surface marker profiles. OECs were highly viable in the alginate and were depleted from the scaffold via migration at a speed of 21 ± 6 μm/h following release. Prolonged interaction with the alginate scaffold microenvironment did not detrimentally change OECs; they retained high functionality, displayed a similar angiogenic cytokine profile as control OECs, and did not have significantly altered surface markers. These results suggest that alginate-OEC interactions do not adversely affect these cells, validating control of cellular migration as a means to control the cell delivery profile from the material system, and supporting usage of the alginate scaffold as an efficient cell delivery vehicle.

1. Introduction

One of the major challenges facing cell therapies is that of delivery: cells transplanted into patients via conventional approaches (IV infusion and direct tissue injection) typically die en masse (>90% within the first days) [1,2], and frequently have poor localization [3]. Together these factors likely lead to a suboptimal return of functionality to the target tissue in clinical trials using cell therapy to treat ischemia to date [4,5]. Importantly, even when delivery is not the limiting factor, many natural regenerative processes require a sequential series of events to occur in precise space and time [6]. With conventional cell therapy, such fine-tuned spatiotemporal control over the transplanted cells is given up once they enter the body. This limits what can be done clinically to reproduce some of the more complex cascade of events that may be required for regenerative medicine. For example, angiogenesis, the process of blood vessel formation, requires sequential endothelial cell activation, proliferation, sprouting, and migration in response to spatiotemporally constrained chemical cues [7,8]. It has been proposed that controlling when and where the vasculature forms is the singular most important issue to be explored in tissue engineering and regenerative medicine [9], and that this control could provide a valuable treatment option for patients with diseases such as peripheral artery disease (PAD) [10]. However, cell therapy intended to drive angiogenesis has yielded inconsistent results and suboptimal efficiency to date [11].

An alginate-based cell delivery vehicle has previously been proposed as a novel means to deliver cells to patients while maintaining their viability and potential to repopulate ischemic tissues [12]. Briefly, an alginate carrier containing cells and bioactive factors that enhances that cell population's survival is transplanted. The material microenvironment protects the cells from loss and damage, and allows for the sustained and controlled release of viable cells outwards to repopulate and regenerate the damaged tissue at the desired target location. Alginate, an FDA-approved copolymer of α-L-guluronic and β-D-mannuronic acid sugar residues derived from brown algae, is an attractive material candidate thanks to its low immunogenicity [13], tunable biodegradability [14], and gentle gelation procedure [15]. It can be formulated into a macroporous scaffold [16] on which cells to be transplanted can be seeded and will reside. However, because the

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alginate itself is inherently non cell-adhesive, it is necessary to couple cell-adhesion molecules such as arginine–glycine–aspartic acid (RGD) containing peptides to the alginate polymer. The RGD tri-peptide sequence is known to affect cell viability, proliferation, migration, and cell fate [17,18] and is found on many natural extracellular matrix proteins. Outgrowth endothelial cells (OECs), a population of endothelial progenitor cells isolated from cord blood mononuclear cells or peripheral blood [12], were chosen as the cell system for this application due to their high proliferation rate and therapeutic potential [19]. Vascular Endothelial Growth Factor isoform 121 ($VEGF_{121}$), a fast-diffusing isoform of the VEGF-A family known to be highly pro-angiogenic and important in the initial stages of angiogenesis, was also placed in the scaffold to activate OECs to a more migratory state so that they would exit the scaffold [12]. OECs released from this multi-factorial alginate delivery system have been shown to be significantly more effective in rescuing mouse ischemic hindlimbs than any component of this system delivered alone [12]. However, the cells exiting the alginate scaffolds have not been characterized. Thus, the OECs delivered from alginate scaffolds were characterized in the present study, and the impact of cell–material interaction on the cells upon exiting the material was analyzed.

2. Materials and methods

2.1. Macroporous RGD-coupled alginate scaffold fabrication

High molecular weight (~250 kDa) ultrapure sodium alginate powder (Novamatrix Pronova UP MVG alginate) enriched (≥60%) in G blocks was used to make alginate scaffolds. As previously described [20], a 2% w/v alginate solution in dH_2O was oxidized by 1% with sodium periodate [14] to create hydrolytically labile bonds. The oxidized alginate solution was reconstituted in a MES solution and covalently conjugated to the cyclic RGD peptide (GGGGCRGDSPC–OH, Commonwealth Biotechnologies) using carbodiimide chemistry [21]. Briefly, sulfo-NHS (Fisher), EDC (Sigma), and 123.4 mg of the RGD peptide (per 100 mL alginate solution) were added sequentially. After 20 h, hydroxylamine hydrochloride (Sigma) was added to quench the reaction and allowed to stir for 30 min. This resulted in 20 RGD peptides covalently conjugated per chain alginate (degree of substitution 20, abbreviated as DS 20). The alginate solution was then dialyzed (MWCO 3500 dialysis membrane, Spectrumlabs), sterile filtered, and lyophilized. To make scaffolds, the sterile alginate stock was reconstituted to a 2% w/v solution in basal EBM media (Lonza) which was mixed with $VEGF_{121}$ (0.3 μg per scaffold), and a 0.21 g/mL calcium sulfate slurry (25:1 alginate to calcium solution) via a syringe connector. The mixture was left at room temperature between two sigma-coated glass plates with a 1 mm separation to gel. Discs were obtained using an 8 mm disc-punch (Acuderm), frozen overnight at −20 °C to create macropores [16], and lyophilized until completely dry.

2.2. Outgrowth endothelial cell culture and scaffold seeding

OECs were isolated from cord blood as previously described [12] and cultured in T75 flasks with daily media changes (EGM-2MV – Lonza). Cells were passaged and/or collected for alginate scaffold incorporation by incubation with 0.05% Trypsin-EDTA (Invitrogen) for 5 min at 37 °C. All cells seeded onto alginate scaffolds or used as a 2D control were in passage 6. To seed cells onto the alginate scaffolds, OECs were trypsinized, centrifuged at 700 rpm for 6 min, and resuspended at a concentration of 500,000 cells per 40 μL. A volume of 20 μL of the cell solution was added to the top of each scaffold, which was incubated for 20 min at 37 °C, after which the scaffolds were flipped over and the process repeated on the opposite side.

2.3. Migration assay and LiveDead staining

To mimic the *in vivo* microenvironment, OEC-seeded alginate scaffolds were placed on a fibrin gel formed with a volume of 57 μL of fully supplemented EGM-2MV media added to 170.5 μL of fibrinogen (Sigma) (4 mg/mL dissolved in 0.09% NaCl solution) and 22.7 μL of aprotinin (500 μg/mL) (Sigma). This solution was mixed with 200 μL of thrombin (2.08 U/mL) (Sigma) and was incubated at 37 °C for 30 min to allow for gel formation. A volume of 300 μL of EGM-2MV fully supplemented media was added to maintain the cells while keeping the scaffold in direct contact with the fibrin gel. Scaffolds were moved to a fresh fibrin gel after 12 h to distinguish migrated cells from those that were washed out of the scaffold when media was initially added. OECs that migrated out after 12 h onto the new gel (24-h time point) were LiveDead stained (Molecular Probes) according to suggested manufacturer protocol after washing the fibrin gel twice with PBS. Pictures were taken using an Olympus-IX81 light microscope

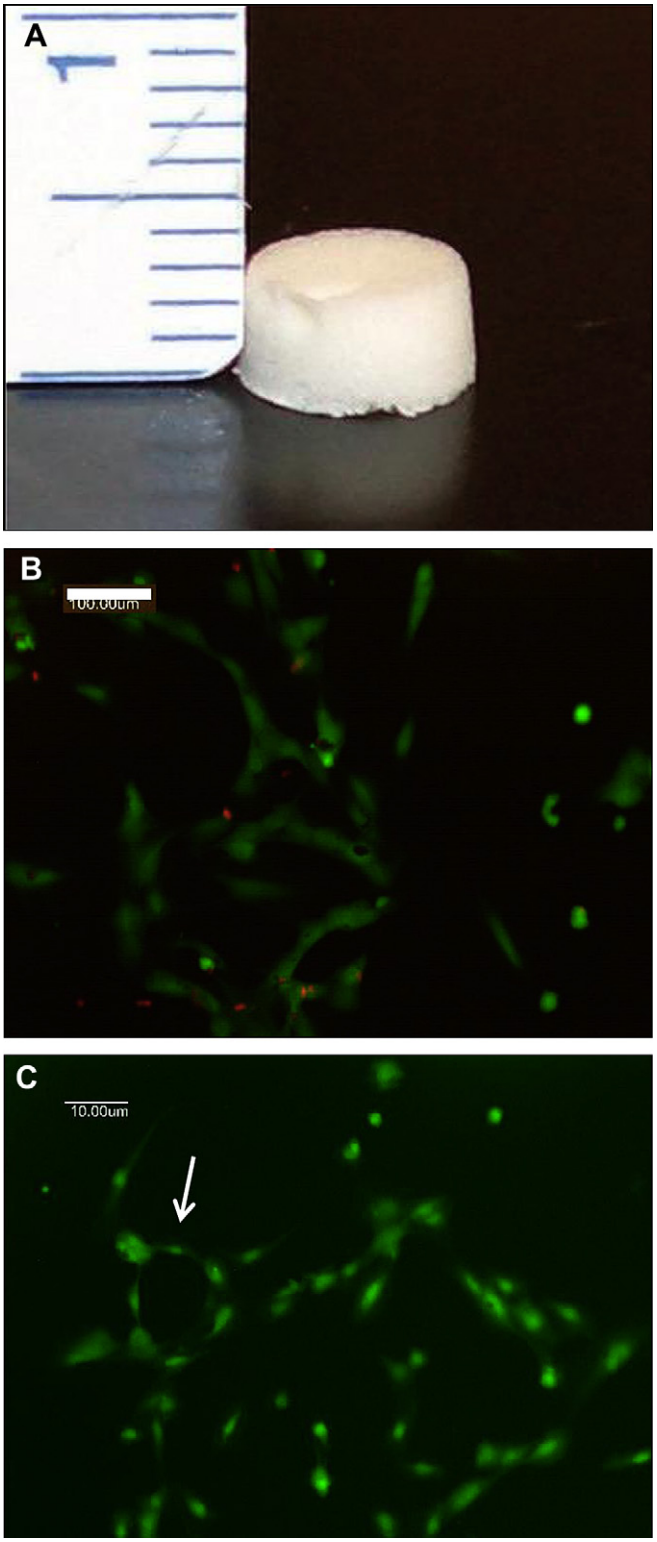

Fig. 1. A) Photograph of a macroporous alginate scaffold. B) Photomicrograph of OECs residing in a scaffold 11 days after seeding stained with LiveDead. Viable cells are green and non-viable cells are red. Image is a superimposition of the FITC (green) and Cy3 (red) signals. C) OECs that migrated from scaffolds onto the fibrin gel at the 24 h time point stained with LiveDead. Note the self-assembly of cells into tubular structures denoted by the arrow. Scale bar denotes 100 μm in B and denotes 10 μm in C.