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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 $21 \pm 6 \mu\text{m/hr}$ 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.

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 events require a sequential series of events to occur in precise space and time. [6] With conventional cell therapy, such fine-tuned

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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 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 to act 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 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 (VEGF₁₂₁), 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.

Materials & Methods

Macroporous RGD-Coupled Alginate Scaffold Fabrication

High molecular weight (~250 kDa) ultrapure sodium alginate powder (Novamatrix Pronova UP MVG alginate) enriched ($\geq 60\%$) in G blocks was used to make alginate scaffolds. As previously described,[20] a 2 % w/v alginate solution in dH₂O 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 123.4mg of the RGD peptide (per 100mL alginate solution) were added sequentially. After 20 hours, hydroxylamine hydrochloride (Sigma) was added to quench the reaction and allowed to stir for 30 minutes. 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 VEGF₁₂₁ (0.3µg per scaffold), and a 0.21g/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 1mm separation to gel. Discs were obtained using an 8mm disc-punch (Acuderm), frozen overnight at -20°C to create macropores,[16] and lyophilized until completely dry.

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 minutes 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 700rpm for 6 minutes, and resuspended at a concentration of 500,000 cells per 40µL. A volume of 20µL of the cell solution was added to the top of each scaffold, which was incubated for 20 minutes at 37°C, after which the scaffolds were flipped over and the process repeated on the opposite side.

Migration Assay and Live-Dead 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) (4mg/mL dissolved in 0.09% NaCl solution) and 22.7 µL of aprotinin (500µg/mL) (Sigma). This solution was mixed with 200µL of thrombin (2.08U/mL) (Sigma) and was incubated at 37°C for 30 minutes to allow for gel formation. A volume of 300µL 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 hours to distinguish migrated cells from those that were washed out of the scaffold when media was initially added. OECs that migrated out after 12 hours onto the new gel (24-hour time-point) were Live-Dead 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 connected to an Olympus DP70 digital image capture system such that cells fluorescing under the FITC and the Cy3 filters were co-localized. At the end of 11 days, cells in the alginate scaffold were similarly stained, imaged, fixed on a slide, and counted to quantify cells remaining within.

3D *in vitro* Sprouting Assay

To determine whether OECs that migrated out of alginate scaffolds retain angiogenic properties, namely, the ability to form tubular structures, alginate scaffolds were embedded between two layers of fibrin gel to create a three-dimensional microenvironment for the migrated cells. A volume of 1mL of EGM-2MV fully supplemented media was added to the cells and changed every day for three days, during which representative pictures were taken.

Time-lapse Microscopy

Images of the alginate scaffolds sandwiched between two layers of fibrin were taken every 30 minutes, for a period of 13 hours, at 26 depths at the same position (Z stack) with 20 positions per scaffold using an Olympus-IX81 light microscope with IPLab 3.7 (Scanalytics) connected to an Olympus DP70 digital image capture system. Videos of cell migration were exported as Quicktime files playing at 7 frames per second (each frame corresponds to 30 minutes in real-time). The cell migration speed was quantified using the video analysis tool in the Logger Pro 3.5.0 software by tracking the leading edge of the cell in each frame.

Profile of Secreted Cytokines

To characterize the angiogenic profile of cells before and after interaction with the alginate scaffold, four different conditions were tested using a cytokine expression array as described in the results section (cell-seeded scaffolds were never in the presence of fibrin gels so as to reduce the variables that could potentially affect the readout). As in all previous experiments, cells were maintained in EGM-2MV media with all of the supplements except VEGF, except when the conditioned media was collected for the array, where cells were incubated with 1mL EGM-2MV media containing all the supplements except for the growth factors for 20 hours. This conditioned media was collected and stored at -20°C until analyzed following manufacturer protocol for the proteome profiler human angiogenesis array (R&D Systems). The streptavidin-HRP complexes were detected with the chemiluminescent detection reagents SuperSignal West Pico Luminol/Enhancer Solution (Thermo Scientific) and SuperSignal West Pico Stable Peroxide Solution (Thermo Scientific).

Isolation of Cells from Scaffold

The alginate scaffolds were degraded to isolate the cells by incubating for 30 minutes at 37°C with 500 μL of a 250 $\mu\text{g}/\text{mL}$ alginate lyase solution (Sigma). The resulting solution was centrifuged, passed through a 70 μm cell strainer (BD), plated in a 24 well-plate, and fed with fully supplemented EGM-2MV media until confluence was reached, at which point the cells were passaged to a 6-well plate, then a T25, and finally, a T75 flask.

Fluorescence-Activated Cell Sorting (FACS)

A total number of $1.0 - 1.5 \times 10^6$ OECs - isolated from the DS 20 alginate scaffolds - were trypsinized, centrifuged, washed twice with Stain Buffer BSA (BD Pharmingen), and resuspended in fresh stain buffer. A minimum of 1×10^5 cells were pipetted into each eppendorf tube and exposed to human Fc-blockers for 5 minutes at room temperature (1 μg Sigma human IgG per 10^5 cells). The Fc-blocked cells were then treated with 10 μL of either an isotype control (PE mouse IgG1, PE mouse IgG2a, and FITC mouse IgG1 from BD Biosciences) or an antibody against cell surface markers of interest ($\alpha\text{V}\beta 5$, $\alpha\text{V}\beta 3$, VEGFR2, and CXCR4 from R&D Systems and CD31 and CD146 from BD Biosciences) and incubated on ice for 30–45 minutes. The cells were washed twice with 1mL stain buffer to remove any unreacted antibodies, resuspended in 400 μL stain buffer, and analyzed using a FACS LSRII (BD) machine.

Statistical Analysis

Statistical analyses were carried out using InStat 2.01 (Graphpad) software. Differences between the means of different samples were first determined using the One-Way Analysis of Variance (ANOVA) calculation; thereafter, if the means were significantly different, the Tukey-Kramer Multiple Comparisons Test was applied as a post-test to determine the level of significance and p-value. Differences between conditions were considered significant if $p < 0.05$.

Results

Cell viability and outward migration

The effect of the alginate scaffolds on resident cells was examined by seeding OECs onto the alginate macroporous scaffolds (Figure 1A). OECs residing in the scaffolds were 91% viable at Day 11 and were well adherent, as evidenced by a representative image of viable spindle-shaped OECs lining the scaffold macropores (Figure 1B). Cells migrated outwards from the alginate over time (Movie, S1), and could self-assemble into tubular structures in

the fibrin gel (Figure 1C). The distribution of OECs remaining in the scaffolds after 11 days was then quantified. The highest cell densities were found at the outside edges of the scaffolds, suggesting outward cell migration was depleting the cells in the interior of the scaffold by this time-point (Fig. 2A).

Outward cell migration into a tissue mimic was further analyzed to characterize cell dispersion from the scaffolds. Time-lapse microscopy revealed that OECs migrated within the scaffold by extending and retracting filopodia (Movie, S2), with a migration speed of $21 \mu\text{m/hr} \pm 6 \mu\text{m/hr}$. This migration speed was maintained even as the cells exited the scaffold into the tissue mimic, as there was no statistically significant difference between the speed in the scaffold, at the boundary between the alginate scaffold and the fibrin gel, and in the fibrin gel (Figure 2B).

Maintenance of angiogenic potential

A series of comparative functional studies were next carried out to assess the angiogenic potential of the OECs before and after interaction with the scaffolds. Scaffolds loaded with OECs were embedded between two layers of fibrin gel and examined for OEC sprout formation, using this three-dimensional sprouting assay as a more stringent assessment of the retention of angiogenic potential of the migrated OECs. At Day 1, OECs were already observed to form sprouts from the scaffold into the fibrin gel (Figure 2C) and even self-assembled into interconnected tubes at the boundary between the alginate scaffold and the fibrin gel, as would normally be observed in a conventional sprouting assay using OECs on microcarrier beads (see S3).

Although the qualitative sprouting assay suggested that OECs retained their angiogenic potential even after interaction with the alginate carrier, to better pinpoint whether and how the material microenvironment alters the OECs, cytokine expression was analyzed. Cytokine secretions from OECs grown under four different conditions were examined: *i*) OECs grown inside scaffolds for 11 days after initial seeding *ii*) The same number and passage number OECs as in condition *i*, but maintained on 2D tissue culture plastic *iii*) OECs grown in scaffolds for 11 days then isolated and re-plated on 2D tissue culture plastic (cytokine expression measured 6 days after re-plating) *iv*) OECs that migrated off the scaffold 24hrs after the initial seeding.

OECs secreted Endothelin-1, IL-8, Pentraxin-3, and Serpin E1 at a high level in all of the experimental conditions (Figures 3A–D). Interestingly, the interaction of OECs with the scaffolds caused some changes in the cytokine expression pattern (Table 1). For instance, Epidermal growth factor (EGF) was only expressed when the OECs were in the scaffold. Conversely, certain proteins were down-regulated to undetectable levels when cells were in the scaffolds or freshly migrated out of the scaffolds, such as Angiogenin and some isoforms of the Platelet-derived growth factor (PDGF-AB/PDGF-BB, which are strong mitogens). Interestingly, PDGF-AA was slightly down-regulated in the scaffold, undetectable in cells that recently migrated off the scaffold, but then up-regulated again to original control levels once re-plated on tissue culture plastic. This same pattern of down-regulation of the cytokine (compared to the control) when OECs were in the scaffold, further down-regulation - often to undetectable levels - shortly after cell migration, then up-regulation, often to original control levels once OECs were isolated and re-plated on tissue culture plastic was also observed for the CXCL16, MCP-1, PIGF, and uPA cytokines. Endoglin, Endostatin, and IGFBP-2 were undetectable in OECs that had recently migrated, whereas for these cells TIMP-1 and Thrombospondin-1 were only slightly down-regulated from levels found in other conditions. Many tested cytokines were not expressed at a detectable level for all experimental conditions (for a complete cytokine listing and key, see S4).

Characterization of cell-surface markers post OEC-alginate interaction

To further characterize and compare OECs after prolonged exposure to the alginate carrier vis-a-vis those grown in normal tissue culture conditions, fluorescence-activated cell sorting (FACS) was carried out to quantitatively profile the cell surface markers of cells in both experimental conditions (Table 2). Interestingly, OECs isolated from the alginate scaffolds show a marked elevation of the $\alpha V\beta 5$ integrin, as compared to OECs of the same passage number grown on tissue culture plastic (74% positive expression as compared to 46 % positive expression in the control). On the other hand, CD31 (PECAM-1) expression was markedly decreased in cells isolated from the scaffold (53 % positive compared to 97 % positive in normal tissue culture conditions). There was also a 10 % decrease in CD146 expression, a 16 % decrease in expression of VEGFR2, and a slight increase (3 %) in the expression level of the $\alpha V\beta 3$ integrin (Figure 4 A–F) following residence in the scaffold.

Discussion

The results of this study suggest that cell-material interactions in this scaffold-based cellular depot system do not adversely influence the viability, functionality, or angiogenic characteristics of the cells to be transplanted. OECs both within the scaffold and those freshly migrated into the surrounding fibrin tissue mimic were highly viable and show a similar morphology to what one expects *in vivo*. OECs are adhesion-dependent primary cells and their survival within the scaffold suggests that the density of the cell-adhesion motif RGD (DS 20) was sufficient to ensure viability of these cells. These RGD ligands were also sufficient to enable outward cell migration. Starting from a uniform distribution, OECs moved progressively towards the extremities of the scaffold, depleting the interior of the scaffold in a manner consistent with previous observations that cells seeded into the carrier repopulate the surrounding tissue mimic.[12] The direct observation of the OECs' migration both within the scaffold and as they exit revealed the typical extension and retraction of successive edges of the cell body that are found in migrating cells, and the speed of migration was in a range consistent with that seen when cells migrate in the presence of adhesion ligands in a 2-dimensional system[22] and when they migrate in a fibrin tissue mimic.[23]

The finding that outwardly migrating cells maintain their ability to self-assemble into 3-dimensional tubular structures suggests that the scaffold microenvironment does not reduce OECs' angiogenic potential. Analysis of cytokine expression indicated that prolonged residence in the scaffold does not dramatically alter OECs angiogenic protein secretion when compared to control OECs cultured on a 2D cell culture surface. Notably, Platelet-derived growth factor (PDGF; a strong mitogen that promotes the maturation of blood vessels by recruiting smooth muscle cells to the nascent blood vessel)[24] was down-regulated to undetectable levels when OECs were in the scaffold, but up-regulated once the cells were out of the scaffold. Since these scaffolds are designed to allow outward OEC migration, not inward smooth muscle cell migration,[25] this suggests that exiting OECs may be capable of orchestrating vessel maturation via smooth muscle cell recruitment after they have left the scaffold and formed new vessels in the surrounding tissue. This reoccurring pattern of down-regulation of expression in the scaffold, further down-regulation during migration, then up-regulation once OECs were isolated and replated was found for numerous cytokines, most of which are pro-angiogenic (CXCL16,[26] MCP-1, [27] PlGF,[28] and uPA[29]), and could be explained by the higher effective concentration of cells per volume when they were residing in the scaffolds, or lower gene transcription during migration, respectively. Furthermore, the finding that EGF, a strong mitogen that regulates endothelial cell survival and promotes cellular growth, proliferation, and differentiation, was only expressed when OECs were in the scaffold is consistent with prior observations that high RGD densities such as that in the scaffold increase cell proliferation

and differentiation[17] and could be desirable in allowing for the expansion of the cells *in vivo*. In any case, though, most changes in expression levels were reversed once OECs left the scaffolds suggesting that any changes would not be permanent and could revert once cells are released from these scaffolds.

Finally, a significant increase in the fraction of cells positive for $\alpha V\beta 5$ integrin expression was observed for cells resident in the scaffolds. Since there was a high density of RGD ligands within the scaffold and these ligands interact directly with the integrin, this likely is the cause of the up-regulation of the $\alpha V\beta 5$ expression. That being said, it is also possible that the scaffold environment provides a survival or proliferation advantage to cells either stochastically expressing a higher level of the integrin or a subpopulation of cells within the OEC population with this trait. On the other hand, the $\alpha V\beta 3$ integrin, which was already expressed at a high level in the experimental control, was only slightly increased. Interestingly, decreases in the VEGFR2 and CD146 marker expressions were also observed, which could result from a scaffold selection for cells with lower expression, or a down-regulation of these receptors in response to the RGD ligands or VEGF₁₂₁ in the scaffolds.

Conclusions

This study demonstrates the maintenance of cellular viability and functionality of endothelial progenitors placed in an alginate delivery system and lends proof-of-concept to support the usage of scaffolds as depot systems to reliably and consistently deliver viable and functional cells via the well characterized and tunable mechanism of cellular migration. The adhesive ligands and morphogen/chemokine presentation can readily be tuned in the future to optimize this system for a variety of cell types. This model system could also be used to address important biological questions, including those related to the differences in cellular behavior in 3-dimensional as opposed to 2-dimensional culture, the development of *in vivo* cellular niches and cell niche mimicry, and the effects of individual ECM components and morphogens on cellular behavior and functionality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 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 24hr 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.

Figure 2.

A) Quantification of the cell density within the scaffolds (representative of n=6 scaffolds). The numbers on the x-axis reflect the increments (every other 540 μ m) by which the field of view was moved for quantification. B) Average speed of OEC migration in scaffolds as determined by time-lapse microscopy. Scaffolds were embedded between two layers of

fibrin and the rates of cell migration in the scaffold, at the boundary between the scaffold and the fibrin gel, and in the fibrin after the cells migrated out of the scaffold were quantified. The sample number was 42, 9, and 14 for each respective condition, and differences between conditions were not statistically significant. C) Photomicrograph of cells at the interface between the tissue mimic (fibrin gel) and embedded scaffold. Solid arrows denote OECs sprouting from the alginate scaffold into the fibrin gel; dashed arrows point to cells still in the alginate scaffold.

Figure 3. Cytokine expression array for A) OECs grown on 2D tissue culture plastic B) OECs that migrated off of scaffolds at the 24-hour time point C) OECs isolated from scaffolds after 11 days residence and re-plated on 2D tissue culture plastic. Cytokine expression was measured 6 days after re-plating. D) OECs in scaffolds at Day 11.

Figure 4.

Cell surface marker expression of OECs isolated from alginate scaffolds after 11 days of residence. Markers tested: A) $\alpha V\beta 3$ integrin (93% positive) B) $\alpha V\beta 5$ integrin (74% positive) C) CD31 (53% positive) D) CD146 (84% positive) E) VEGFR2 (57% positive) F) CXCR4 (42% positive). Grey curves denote the isotype control, while red curves denote the sample.

TABLE 1

Summary of angiogenic factor expression for OECs grown on 2D tissue culture plates (control), cells migrated out of the scaffold at the 24hr time point, cells isolated from the scaffolds and re-plated on 2D tissue culture plastic, and cells resident in the scaffolds (Day 11). Cytokines with a high expression (compared to the base-line positive controls) are denoted with a +++, while those with medium positive expression, low expression, or no detectable expression are denoted with ++, +, and –, respectively.

Cytokine	OECs on tissue culture plastic	OECs that migrated off scaffolds after 24 hours	OECs isolated from scaffolds after 11 days inside, expression 6 days after re-plating	OECs inside scaffolds at Day 11
Angiogenin	+	-	+	-
CXCL16	++	-	++	+
EGF	-	-	-	++
Endoglin	+	-	+	+
Endostatin/Collagen XVIII	++	-	++	++
Endothelin-1 (ET-1)	+++	+++	+++	+++
IGFBP-2	+	-	++	+
IL-8	+++	+++	+++	+++
MCP-1	+++	-	+	-
MMP-9	+	-	-	-
Pentraxin-3 (PTX-3)	+++	+++	+++	+++
PDGF-AA	++	-	++	+
PDGF-AB/ PDGF-BB	+	-	++	-
PIGF	+++	-	++	+
Serpin E1 (PAI-1)	+++	+++	+++	+++
TIMP-1	+++	++	+++	+++
Thrombospondin-1 (TSP-1)	++	+	++	++
uPA	+++	+	+++	++

TABLE 2

Comparison between cell surface marker expression of OECs grown on 2D tissue culture plastic at different passages (control) and OECs isolated from scaffolds (comparable to P11 OECs in 2D culture).

	OEC (P3)	OEC (P5)	OEC (P11)	OEC re-plated in 2D after 11 days in scaffold
AlphaVB3	95	97	91	93
alphaVB5	65	53	46	74
CD34	2	neg	neg	neg
CD31	94	96	97	53
CD146	97	99	95	84
CXCR4				57
VEGFR2	62	65	58	42
CD144	97	98	97	26