Tissue Engineered Vascular Grafts: The Molecular Mechanism of Neotissue Creation

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Purpose: The development of tissue engineered vascular grafts (TEVGs) holds promise for advancing cardiovascular surgery. We performed the first trial evaluating bone marrow-seeded TEVGs in humans. Despite the successful translation of this technology, little is known about the molecular mechanisms that promote TEVG patency. Using a murine model, we investigated the role of bone marrow derived mononuclear cells (BMMNCs), macrophages, and the cytokine MCP-1 in the formation of vascular neotissue.

Methods: Biodegradable PGA-P(LA/CL) scaffolds, 0.8 mm in diameter, were implanted into the mouse inferior vena cava in the following groups: (1) Grafts seeded with BMMNCs (n=25) and unseeded controls (n=25) in wild-type mice; (2) Clodronate treated (to deplete macrophages, n=10) and untreated (n=10) mice; (3) MCP1-/- (n=5) and wild-type mice (n=5). Mice were sacrificed 14 days following implantation for histological analysis of the grafts. Additionally, qPCR was utilized to determine the relative quantity of seeded BMMCs in the graft over a two week time period following implantation.

Results: Histology demonstrated that BMMNC seeding inhibited stenosis (seeded 0.54±0.27mm, unseeded 0.36±0.28mm; p=0.031). Macrophage infiltration was significantly lower in BMMNC seeded group than the unseeded groups (seeded 420+/-93 /HPF, unseeded 554+/-89 /HPF; p=0.029). Patency was improved in macrophage depleted and MCP-/- groups, compared with the untreated and WT group. qPCR demonstrated that seeded cell number decreased rapidly with a corresponding infiltration of the graft by host-derived cells.

Conclusion: BMMNC seeding is critical to neotissue formation in TEVGs via the paracrine effect by inhibition of macrophage recruitment through a MCP-1 dependent mechanism.

Pericyte Based Human Tissue Engineered Vascular Grafts: Fabrication, Characterization and in Vivo Assessment

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Purpose: The use of human adult stem cells in tissue engineered vascular grafts (TEVGs) may represent a critical step toward clinical translation. Pericytes have recently been shown to express mesenchymal stem cell features. Their relative availability and multipotentiality make pericytes a promising cell source for TEVG applications. The objective of this study was to evaluate the behavior of human pericytes (hPs) following incorporation into biodegradable scaffolds and to assess the efficacy of the hP-seeded constructs in a xenogeneic rat model.

Methods: Bi-layered elastomeric poly(ester-urethane)urea scaffolds were bulk seeded with hPs isolated from human skeletal muscle. The constructs were cultured in spinner flasks for 2 days and then implanted into Lewis rats (n=7) as aortic interposition grafts for 8 weeks. Non-seeded scaffolds (n=8) and rat smooth muscle cell (rSMC)-seeded constructs (n=4) were implanted as control groups.

Results: After implantation, all (100%) hP-seeded TEVGs were patent and demonstrated a tissue-like appearance. In contrast, only 3 out of 8 (38%) acellular TEVGs and 3 out of 4 (75%) rSMC-seeded TEVGs were patent. The patent hP-seeded TEVGs revealed extensive tissue remodeling in the porous layer, enriched with both collagen and elastin. The remodeled tissue consisted of multiple layers of α-smooth muscle actin- and calponin-positive cells and a von Willebrand factor-positive monolayer in the lumen.

Conclusions: These results demonstrate the feasibility of a pericyte-based TEVG and suggest that the pericytes play a role in maintaining patency and remodeling the TEVG into a functional arterial conduit.

Cellular Plasticity of Inflammatory Myeloid Cells in the Peritoneal Foreign Body Response

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Purpose: Implantation of a sterile foreign body into the peritoneal cavity of an animal initiates an inflammatory response and results in its encapsulation by cells of bone marrow origin. Over 2-3 weeks, a multi-layered tissue capsule develops with abundant myofibroblasts (smooth muscle-like cells) embedded in extracellular matrix. Living tubes of this tissue have been successfully used as autologous vascular grafts. The aim of the study was to identify the cells that encapsulate the foreign body and transdifferentiate into smooth muscle-like cells.

Methods: Sterile foreign bodies (boiled blood clots or polyethylene tubing) were implanted in the peritoneal cavity of transgenic MacGreen mice in which cells of myeloid origin express EDFP driven by the CSF-1r (cfms)-promotor. The cells that encapsulated the bodies were then examined by immunofluorescence and FACS analysis.

Results: Cells comprising the capsule were EGFP+ (myeloid) and changed morphology over 14 days from round to spindle shaped and co-expressed the myofibroblast / smooth muscle marker alpha smooth muscle actin. Prior depletion of peritoneal macrophages by treatment of the mice with dichloromethylene-bisphosphonate (clodronate)-loaded liposomes revealed almost complete ablation of the EGFP+ cells and prevention of tissue capsule development.

Conclusions: These results confirm the identity of the encapsulating cells as monocyte/macrophages and demonstrate their in vivo plasticity to a mesenchymal phenotype.

Channelrhodopsin-2 Enables Optogenetic Control of Human Embryonic Stem Cell-derived Cardiomyocytes

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Purpose: Despite the use of a variety of differentiation methods, current yields of human embryonic stem cell (hESC)-derived cardiomyocytes (CM) have been low and no method exists to prospectively generate specific phenotypes such as pacemaker or ventricular cells. By introducing light-activated channelrhodopsin-2 (ChR2) into hESC, our purpose is to modulate short- and long-term optogenetic stimulation of hESC-CM in order to control their synchronization (pacing) and potentially increase their yields and specific phenotypes.

Methods: We introduced ChR2 coupled to yellow fluorescent protein (EYFP) into undifferentiated hESC via a lentiviral vector and tested for expression via PCR, flow cytometry (FC), and immunocytochemistry (ICC). $hESC^{ChR2+}$ were sorted, expanded, and tested for pluripotency. Via directed differentiation, wildtype hESC-CM (WT-CM) and ChR2-CM were produced and subjected to both electrical and optical stimulation of varying amplitudes, pulse widths, and frequencies. Electrical, biochemical (calcium), and mechanical (contractility) signals were then assessed by patch clamping, microelectrode arrays (MEAs), and video microscopy.

Results: ChR2 was stably introduced into undifferentiated hESC and $hESC^{ChR2+}$ could be differentiated into CM, all confirmed by PCR, FC, ICC, and electrophysiological methods. Both WT-CM and ChR2-CM responded to traditional electrical stimulation and produced similar calcium and contractility features but only ChR2-CM could be paced by optical stimulation.

Conclusions: Here we show for the first time that ChR2 can enable optical control of hESC-derived electrically active cells. The long-term application of optical stimulation could potentially lead to increased yields and specific phenotypes of hESC-CM, and therefore contribute significantly towards creating effective cardiovascular therapies.

Electrically Activated Cell Sorting of Induced Pluripotent Stem Cell-derived Cardiomyocytes

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Purpose: One of the major challenges in translating stem cell biology into tissue replacement therapy is the establishment of effective purification methods which specifically isolate differentiated cells and exclude cells which may hamper graft performance or lead to teratoma formation. Our purpose was to create an electrically activated cell sorter (EACS) based on evoked responses from electrical stimuli applied to flowing induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) in a microfluidic platform.

Methods: iPSC-CM were created via directed differentiation by controlling Activin, BMP, and Wnt pathways. A microfluidic platform with an electrical stimulus generator and differential amplifier was designed and fabricated having areas for cell trapping, stimulation, recording, and sorting.

Results: iPSC-CM began spontaneously contracting between days 9-14 as assessed by static microelectrode array recordings and video microscopy. iPSC-CM also expressed cardiomyocyte markers as assessed by PCR and immunocytochemistry. Finally, flowing iPSC-CM colonies could be trapped, stimulated, and their evoked potentials could be detected for downstream sorting.

Conclusions: We have engineered a continuous-flow microfluidic platform which traps colonies of iPSC-CM, electrically stimulates them, and records their resulting extracellular field potential signals. Our current efforts are aimed at using computational analysis of these signals for ultimately providing a highly specific, label-free mechanism for purification of iPSC-CM at the single-cell level. We believe that our sorting method could substantially reduce the risk of teratoma formation and that electrophysiological homogeneity of implanted CM will lead to improved graft viability, improved electromechanical coupling, and reduced incidence of arrhythmias when compared to other sorting techniques.