sufficient stimulus for T cell proliferation within the breast microenvironment, and imply that co-stimulatory factors are required. Further investigation into possible co-stimulatory factors are currently underway using our high-fidelity ex vivo model of the breast microenvironment.

Pre-clinical Application Of Tissue-engineered Human Induced Pluripotent Stem Cell-derived Epithelial Grafts In A Porcine Airway Defect Model

Ratna Varma1,2, Alba E. Marin-Araujo2,3, Sara Rostami2, Thomas K. Waddell1,2, Golnaz Karoubi1,2, Siba Haykal5,2

1Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada, 2Latner Thoracic Surgery Research Laboratories, Toronto General Hospital Research Institute, University Health Network, Toronto, ON, Canada, 3Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, ON, Canada, 4Latner Thoracic Surgery Research Laboratories, Toronto General Hospital Research Institute, Toronto, ON, Canada, 5Division of Plastic & Reconstructive Surgery, Department of Surgery, University of Toronto, Toronto, ON, Canada.

Purpose: Tracheal injury, stenosis, and malignancy demand surgical management or transplantation, however, the latter fails due to the lack of a functioning epithelium. This issue also encompasses tissue engineering approaches such as decellularized matrices and synthetic biomaterials, wherein the absence of pseudostratified, mucociliary epithelia results in airway obstruction and life-long tracheostomies for patients. Our group has developed a composite biomaterial of Silk Fibroin and Collagen Vitrigel Membrane (SF-CVM), which provides high tensile strength for surgical manipulation and allows differentiation of primary human tracheal epithelial cells (HTECs) into functional ciliated and goblet cells. While HTECs are an endogenous source for recipient-derived grafts, they are limited in their ability to expand and differentiate reliably. Therefore, we differentiated human induced pluripotent stem cells (hiPSCs), a promising alternative, to generate functional SF-CVM-based airway epithelial grafts. We further developed a porcine airway defect model to determine graft integration and survival across 3 days.

Methods: We sequentially differentiated hiPSCs based on an established protocol towards definitive endoderm, lung progenitors, and airway progenitors, and analyzed them via flow cytometry. These airway progenitors were seeded on SF-CVM grafts under air-liquid interface culture for formation of tight junctions (ZO-1) and differentiation into ciliated (acetylated α-tubulin) and goblet cells (mucin 5AC), as assessed by immunocytochemistry.

We created a 2x4 cm tracheal defect and manipulated it according to the following four groups prior to defect closure: 1) no manipulation; 2) mucosa stripped; 3) mucosa stripped and replaced with bare SF-CVM graft; and 4) mucosa stripped and replaced with hiPSC-derived SF-CVM graft labeled with CMTMR dye. Post defect closure, all groups were wrapped in sternocleidomastoid muscle for vascularization. On post-operative day (POD) 3, all defects were assessed macroscopically, while Groups 2 to 4 were assessed for cell viability and death via calcein-AM and ethidium homodimer 1 staining, respectively.

Results: We produced $92.6\pm2.1\%$ CKIT$^+$CXCR4$^+$ definitive endoderm, $38.1\pm3.9\%$ GFP-NKX2.1$^+$ lung progenitors, and $65.6\pm2.4\%$ P63$^+$ airway progenitors, which differentiated into $64.6\pm7.8\%$ ciliated and $2.1\pm1.4\%$ goblet cells on SF-CVM. This differentiation into ciliated cells on SF-CVM was significantly higher than that of HTECs ($17.2\pm5.0\%$; $P<0.05$) and physiologically relevant, being well within the 48-70% range present in human tracheae.

There were no respiratory complications or animal mortality. Group 1 resembled native epithelium, Group 2 had granulation tissue overgrowth, while Group 3 and 4 demonstrated SF-CVM integration with the surrounding tracheal tissue. Groups 2 and 3 demonstrated epithelial infiltration with high cell mortality. Group 4 retained the CMTMR label (magenta) which co-localized with calcein-AM (green), indicating that the hiPSC-derived epithelium was intact and alive on POD3.

Conclusions: We developed functional hiPSC-derived SF-CVM epithelial grafts which survived and integrated within porcine airway defects across 3 days. This is the first pre-clinical application of biomaterials-based airway grafts generated from hiPSCs that has significant clinical...
impllications for treatment of small airway defects and full-length tracheal transplants.

127

Combined Local Delivery Of Tacrolimus And Stem Cells In Fibrin Gel Is A Viable Potential Treatment For Enhancing Peripheral Nerve Regeneration

Tiam M. Saffari, MD1, Katelyn Chan, B.Eng BioSci2, Kevin J. Zuo, MD2, Gregory H. Borschel, Prof, MD2, Alexander Y. Shin, Prof, MD1

1Mayo Clinic, Rochester, MN, USA, 2SickKids Hospital, Toronto, ON, Canada.

Purpose: The outcome of scaffold-based stem cell transplantation in peripheral nerve injury remains unsatisfactory due to poor survival of transplanted cells, most likely associated with the immune rejection. Systemic immunosuppression could overcome rejection, but causes various undesired side effects. Recently, the neuroregenerative potential and bimodal dose effects of tacrolimus (an FDA approved immunosuppressant) have been explored in addition to its immunosuppressive role. The aim of this study was to determine the feasibility and effectiveness of combining local, sustained delivery of tacrolimus with stem cells.

Methods: First, the drug release profile from the local tacrolimus delivery system was validated. Tacrolimus was incorporated into fibrin gel in poly(lactic-co-glycolic) acid (PLGA) microspheres in concentrations of 0.01 ng/ml and 100 ng/ml, as previously described by Tajdaran et al. (2015). Microspheres were prepared and characterized using the validated protocol by Tajdaran et al. (2015). Drug release from the tacrolimus-delivery system was analyzed by incubating the gels in phosphate-buffered saline (PBS) at physiological temperature, replenishing and collecting release media for every 3 days up to 35 days. Release media was characterized using liquid chromatography mass spectrometry to plot drug release profiles. Secondly, adipose derived mesenchymal stem cells (MSCs) were cultured in the fibrin gel to evaluate their interaction with tacrolimus and the gel. 10^6 MSCs were seeded in (i) gel only, (ii) PLGA empty microspheres+gel, (iii) 0.01, and (iv) 100 ng/ml of tacrolimus encapsulated in PLGA microspheres+gel. MSCs were cultured in drug release media collected at days 7, 15 and 28 to mimic systemic exposure representing released concentrations at these days from both concentrations microspheres. After 72h, cytotoxicity assays and immunofluorescence staining against MSC surface marker CD90 were conducted to confirm stem cell viability and characterization, respectively. Live/dead staining was performed at 24h, 48h, 72h, and seven days.

Results: From tacrolimus microspheres containing 100 ng/ml, a sustained release of up to 35 days was detected, with a total of 200 µg tacrolimus released for the duration of the study. Microspheres containing 0.01 ng/ml showed depletion of tacrolimus by 13 days. Drug release profiles were consistent with those of Tajdaran et al. (2015), in which an inflection point is observed around 15 days due to almost complete erosion of fibrin gel, which results in an increase in the rate of tacrolimus release. For MSCs cultured in the gel groups, and in the tacrolimus drug release media from 7, 15 and 28 days release, cell viability was approximately 80% and 100% respectively, and insignificantly different across groups, when compared to MSCs cultured in untreated media (negative control). CD90 staining confirmed stem cell characterization and live/dead staining confirmed viability up to seven days.

Conclusion: Encapsulation of 100 ng/ml tacrolimus in PLGA microspheres and MSCs in fibrin gel is feasible and has strong potential to enhance survival of transplanted cells, which may ultimately lead to improved nerve regeneration.

128

Engineering Lymphatic Vessels For Secondary Lymphedema Treatment

Qixu Zhang, Yewen Wu, Mark V. Schaverien, Summer E. Hanson, Edward I. Chang, Charles E. Butler

MD Anderson Cancer Center, Houston, TX, USA.

Purpose: Lymphedema has been shown to be one of the most significant survivorship issues following the treatment of many solid tumor cancers, most publicized in breast cancer but also impacting patients with melanoma, gynecologic and urologic cancer. Recent advances in microsurgery, specifically lymphovenous bypass and vascularized lymph node transfers, have provided the closest chance at a cure for lymph flow disorders. However, the availability of qualitative autologous flaps and donor site morbidity significantly limit its application. Engineered lymphatic vessel tissues may offer a clinically alternative to autologous flaps. This study aimed to engineer lymphatic vessels with