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Engineering human renal epithelial cells for transplantation in regenerative medicine

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ABSTRACT

Cellular transplantation may treat several human diseases by replacing damaged cells and/or providing a local source of trophic factors promoting regeneration. We utilized human renal epithelial cells (hRECs) isolated from cadaveric donors as a cell model. For efficacious implementation of hRECs for treatment of kidney diseases, we evaluated a novel encapsulation strategy for immunoisolation of hRECs and lentiviral transduction of the Green Fluorescent Protein (GFP) as model gene for genetic engineering of hRECs to secrete desired trophic factors. In specific, we determined whether encapsulation through conformal coating and/or GFP transduction of hRECs allowed preservation of cell viability and of their trophic factor secretion. To that end, we optimized cultures of hRECs and showed that aggregation in three-dimensional spheroids significantly preserved cell viability, proliferation, and trophic factor secretion. We also showed that both wild type and GFP-engineered hRECs could be efficiently encapsulated within conformal hydrogel coatings through our fluid dynamic platform and that this resulted in further improvement of cell viability and trophic factors secretion. Our findings may lay the groundwork for future therapeutics based on transplantation of genetically engineered human primary cells for treatment of diseases affecting kidneys and potentially other tissues.

1. Introduction

Cellular transplantation holds the potential to treat several human diseases [1–10]. Among them, acute kidney injury and chronic kidney disease are two very prevalent medical conditions that are associated with raising healthcare costs due to high cardiovascular morbidity and mortality [11–18]. Strategies to delay the progression of chronic kidney disease or induce a faster recovery from acute kidney injury are highly needed.

Abbreviations: **2D**, Two-dimensional; **3D**, Three-dimensional; **ALG**, alginate; **CCE**, conformal coating encapsulation; **DTT**, dithiothreitol; **dVS**, divinyl sulfone; **EGF**, epidermal growth factor; **FGFb**, basic fibroblast growth factor; **GFP**, green fluorescent protein; **GM-CSF**, granulocyte macrophage colony-stimulating factor; **HBSS**, Hanks' balanced salt solution; **HGF**, hepatocyte growth factor; **hRECs**, Human Renal Epithelial Cells; **MCP-1**, monocyte chemotactic protein-1 (CCL2); **MMP-2**, matrix metalloproteinases-2; **MOI**, multiplicity of infection; **MTT**, 3-(4,5-Dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide; **PAI-1**, plasminogen activator inhibitor-1; **PBS**, phosphate-buffered saline; **PEG**, polyethylene glycol; **pHEMA**, Poly(2-hydroxyethyl methacrylate); **PPG**, polypropylene glycol; **RPM**, revolutions per minute; **SCF**, stem cell factor; **TEA**, triethanolamine; **TGFα**, transforming growth factor alpha; **TIMP**, tissue inhibitor of metalloproteinase; **UP-MVG**, ultrapure medium viscosity (> 200 mPas) sodium alginate where minimum 60% of the monomer units are guluronate; **VCAM-1**, vascular cell adhesion molecule-1; **VEGF**, vascular endothelial growth factor.

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Various therapeutic approaches are being explored. These include transplanting tissue-derived cells either alone or as helper cells in damaged/dysfunctional tissues. The latters may contribute to tissue regeneration via local secretion of cytoprotective factors and/or through the recruitment of cells with regenerative potential [11–14,19–24]. The fate of implanted cells depends on their plasticity and on phenotypical changes influenced by the microenvironment. Culture of most cells in three-dimensional (3D) scaffolds has shown to better preserve cell phenotype when compared to standard two-dimensional (2D) culture [22,25–33].

Autologous cells are not rejected by the host immune system and they could integrate into the remodeling tissue [34–39]. Unfortunately, achieving a satisfactory yield when recovering healthy cells from damaged tissue may be a limitation factor. Autologous somatic cells (i.e., skin cells) that have undergone *in vitro*'reprogramming' are another therapeutic option. These cells though need expansion and this poses a major challenge. Also, timely use might not be possible in case of acute organ failure. Furthermore, if the cause for organ dysfunction is genetically determined, autologous cells require gene therapy prior to utilization. Allogeneic cells may also represent a viable option for regenerative/tissue repair therapies, as they could be cultured and banked to be readily available to treat acute medical conditions. The major drawback of allogeneic cells is their susceptibility to immune rejection after implantation. Their use requires concurrent administration of immunosuppressive drugs that are associated with undesirable side effects [40,41]. Moreover, immunosuppression may also interfere with cell replication and function, potentially altering the regenerative properties of the implanted cells [42].

As an alternative with the potential to overcome these limitations, immunoisolation through encapsulation of allogeneic cells and cell clusters may represent an appealing strategy to prevent immune rejection without the need of life-long immunosuppression [43–46]. Conventional cell microencapsulation is based on the generation of capsules with a constant diameter that ranges between 500 and 1500 µm. Experimental and computational studies have shown that 150 µm is the maximum distance between cell and blood supply allowing for proper diffusion of nutrients and oxygen [47–50]. Conventional microcapsules can cause central hypoxia of enclosed cell clusters, accumulation of cellular waste and delayed secretion of trophic factors due to the capsule large size. Moreover, the high volume of capsule material limits the choice for transplant sites to the intraperitoneal cavity. To overcome such limitations, we have recently developed a novel method for conformal coating encapsulation (CCE) of cell clusters based on fluid dynamic principles. This process allows 'shrink-wrapping' of cell clusters with a thin (few tens of microns) layer of biocompatible polyethylene glycol (PEG) and alginate (ALG) hydrogel without affecting cell viability or functionality of the encapsulated cells [51].

In this study we used previously characterized [52] human renal epithelial cells (hRECs) as a model for showing feasibility of a cell engineering approach that allows long-term *ex vivo* culture, immunoisolation and genetic manipulation of the cells while preserving viability and secretion of trophic factors involved in tissue regeneration.

2. Materials and methods

2.1. Human renal epithelial cells

Tissue considered unsuitable for transplantation was obtained through the National Disease Research Interchange (Philadelphia, PA) following institutional protocol approvals. To remove blood cells and debris, kidneys were washed in Dulbecco's modified Eagle's medium (DMEM, Life Technologies). Tissues were dissected from the cortex region of the kidneys. The tissues were then mechanically dissociated in tissue culture plates and digested in good manufacturing practice grade enzyme mixtures containing 0.25 units 4-phenylazobenzyloxycarbonyl activity/mL collagenase (NB6, Serva Electrophoresis GmbH) and 2.5 units/mL dispase II (Roche Diagnostics Corporation). The enzyme mixture was combined with renal epithelial growth medium (REGM, Lonza). The conical tubes containing tissue, medium, and digestion enzymes were incubated at 37°C in an orbital shaker at 225 rpm for 2 h If large pieces of tissue were still present after the digestion step, they were removed by gravity sedimentation or by slow centrifugation. The supernatant containing the suspended cells was then transferred into a new 50 mL tube and centrifuged. Cells were resuspended in REGM, plated on gelatin-coated tissue culture flasks, and cultured at 37°C under normal atmospheric conditions for cytological analyses. Resulting human renal epithelial cells (hRECs), were passaged up to 6 times and demonstrated 88% viability and a normal 46 XY karyotype. Cell characterization has been already published [52]. All experiments presented here were conducted with hRECs at passage 6.

2.2. GFP labeling of hRECs

hRECs at passage 4 and ~70% confluency were transduced at multiplicity of infection (MOI) between 1 and 10 with a pRRLsinPPT-EGFP lentivirus provided by the viral vector core facility at the University of Miami. Culture media was changed after 8 h. GFP positivity was confirmed 5 days after transduction by both fluorescence microscopy and flow cytometry with LSRII machine (BD Biosciences). GFP+ cells were expanded from passage 4 to passage 6. In order to confirm that viral proteins were cleared from the cell supernatant, two weeks after transduction an HIV-1 p24 antigen ELISA was performed according to manufacturer's protocol (Zeptometrix).

2.3. Generation of three-dimensional hREC spheroids

Several methods for aggregating passage 6 hRECs into 3D spheroids were evaluated. First, we compared suspension culture in non-tissue culture-treated, 6-well plastic plates and 35 mm custom-made silicon-perfluorocarbon plates [53–55] (to improve cell oxygenation during culture) with orbital shaking at 125 revolutions per minute (RPM) during incubation at 37°C and 5% CO_2. Three seeding densities were compared: 10,000, 25,000, and 50,000 cells/cm^2 (0.2×10^6, 0.5×10^6, and 0.9×10^6 and cells/well). In an alternative approach, we fabricated biologically inert plates by coating tissue culture plates with Poly(2-hydroxyethyl methacrylate) (pHEMA; Sigma Aldrich). A stock solution of 10% was prepared by dissolving 2.4 g of pHEMA in 24 ml of 95% Ethanol. A 0.5% diluted solution was used for coating tissue culture treated and non-treated 6-well plates. Plates were dried for 48 h at 37 °C, exposed to UV light overnight and stored in sterile conditions at room temperature for future use. Cells were plated in tissue culture treated and non-tissue culture treated pHEMA-coated 6-well plates and incubated at 37 °C and 5% CO_2.

2.4. Characterization of hREC spheroid growth

For characterization of spheroid growth, hRECs at passage 6 cells were plated on pHEMA-coated non-tissue culture treated plate at three cell densities: 0.2×10^6, 0.5×10^6, and 0.9×10^6 and cells/well. Cell aggregates were imaged through phase contrast microscopy 2, 24 and 48 h after seeding and the aggregate major axes D1 and D2 (5 fields/well, 3 wells/condition) were measured for each condition and time point using Zeiss AxioVision 4.8 software. Spheroid volume was calculated according to the ellipsoid

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