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Stem cell differentiation to epidermal lineages on electrospun nanofibrous substrates for skin tissue engineering

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ABSTRACT

Bone marrow (BM) mesenchymal stem cells (MSC) capable of differentiating along the epidermal lineage on engineered nanofibrous scaffolds have great potential for bionanomaterial–cell transplantation therapy of skin wounds. MSC have been the focus of many tissue engineering studies, mainly because of their multipotential properties. We investigated the potential of human BM-derived MSC for epidermal cell differentiation in vitro on electrospun collagen/poly(L-lactic acid)–co-poly(3-caprolactone) (Coll/PLLCL) nanofibrous scaffolds. PLLCL and Coll/PLLCL nanofibrous scaffolds were fabricated by an electrospinning process and their chemical and mechanical characterization carried out by scanning electron microscopy (SEM), water contact angle determination, Fourier transform infrared spectroscopy, and tensile testing. The differentiation of MSC was carried out using epidermis inducing factors, including epidermal growth factor (EGF) and 1,25-dihydroxyvitamin D_3, in culture medium. The proliferation of MSC evaluated by cell proliferation assay showed that the number of cells grown on Coll/PLLCL nanofibrous scaffolds was significantly higher than those on PLLCL scaffolds. The SEM results showed that MSC differentiated on Coll/PLLCL nanofibrous scaffolds showed a round keratinocyte morphology and expressed keratin 10, filaggrin and partial involucrin protein by immunofluorescent microscopic studies. The interaction of MSC and nanofibers was studied and we concluded that the electrospun Coll/PLLCL nanofibers could mimic the native skin extracellular matrix environment and are promising substrates for advanced skin tissue engineering. Our studies on the differentiation of MSC along the epidermal lineage on nanofibrous scaffolds suggest their potential application in skin regeneration without regional differentiation.

1. Introduction

Bone marrow (BM)-derived stem cells have the capacity to give rise to several specialized cell types and it has been shown that a single BM-derived stem cell is able to differentiate into epithelial cells of the liver, lungs, gastrointestinal tract, and skin [1]. Systemic transplantation and local implantation of mesenchymal stem cells (MSC) are promising treatment methods for skin wounds [2], especially for chronic wounds [3,4]. The mechanism by which BM–MSC participate in cutaneous wound healing of the epidermis has been studied and it is postulated that the MSC could either differentiate into the various types of damaged cells [5] and/or enhance repair by creating a microenvironment that promotes the local regeneration of cells endogenous to the tissue [6]. Wu et al. also proved that BM-derived MSC treated wounds exhibited significantly accelerated wound closure with increased re-epithelialization, cellularity and angiogenesis [7]. Păunescu et al. examined whether human BM-derived MSC are able to differentiate in vitro into functional epithelial-like cells. To induce epithelial differentiation they cultured MSC using epidermal growth factor (EGF), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF) and insulin-like growth factor-II (IGF). Their results demonstrated that MSC isolated from human BM can differentiate into epithelial-like cells and may serve as a cell source for tissue engineering of epithelial tissue. They also found that only cells cultured on tissue culture plates (TCP) from the 'inner zone' of cobblestone pattern clusters differentiated towards the epithelial lineage, probably due to paracrine effects (when proteins synthesized by one cell diffuse over small distances to induce changes in a neighboring cell) [8,9]. This indicates that TCP is unsuitable as a substrate to support the differentiation of MSC along the epidermal lineage and a better scaffold which can guide MSC differentiation towards the epidermal lineage over the whole surface area of the scaffold is required.

To regenerate new biological material to replace diseased or damaged tissues or organs an artificial extracellular matrix (ECM) suitable for cell adhesion, proliferation and differentiation is essential [10]. Stem cells (SC) interact with and respond to a myriad of signals emanating from their extracellular micro-environment.

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Electrospun fibrous scaffolds can be prepared with a high degree of control over their structure, creating highly porous meshes of ultrafine fibers that resemble the ECM topography, which are amenable to various functional modifications targeted at SC survival and proliferation, directing specific SC fates and promoting tissue organization [11]. The fibrillar structure was found to promote cell attachment, proliferation and colony-forming capacity of stem cells in vitro in comparison with (non-fibrillar) tropocollagen layers [12]. Biomaterial scaffolds provide physical and chemical cues matching the surrounding tissues, providing the specific cell type to support specific tissue engineering applications [13]. Poly(L-lactic acid–co-ε-caprolactone) is a synthetic, biodegradable and non-toxic co-polymer of poly-L-lactic acid (PLLA) and polycaprolactone (PCL), investigated as a biomaterial for surgery and drug delivery systems. It is also a biodegradable elastomer and the biodegradation rate of PLLCL can be adjusted by changing the molar ratio of lactic acid and caprolactone in the co-polymer [14,15]. Collagen, on the other hand, is a natural ECM protein with high cell adhesion properties but weak mechanical strength. Blending of collagen with PLLCL and electrospinning produces a biocomposite scaffold that might improve the biocompatibility of PLLCL while preserving the mechanical strength and providing a hydrophilic mesh with high porosity and small fiber diameters desirable for skin tissue engineering. PLLCL/collagen (PLLCL/Coll) nanofibers has been electrospun and applied as a biocompatible substrate for vascular and nerve tissue engineering. He et al. found that collagen-coated PLLCL nanofibers enhanced the spreading, viability and attachment of human coronary artery endothelial cells (HCAEC) and preserved the HCAEC phenotype [16]. Prabhakaran et al. investigated the potential of MSC differentiation to neuronal cells on PLLCL/Coll nanofibrous scaffolds and their results showed that MSC differentiated on PLLCL/Coll nanofibrous scaffolds had a neuronal cell morphology and expressed neuron-specific proteins [17]. Both studies demonstrated that the performance of composite PLLCL/Coll scaffolds was better than on PLLCL nanofibers. Collagen makes up approximately 70% of the dry weight of skin and in order to provide an environment as similar to native ECM of human skin as possible we fabricated Coll/PLLCL nanofibrous scaffolds with a collagen:PLLCL ratio of 70:30 by electrospinning and studied the epidermal differentiation of MSC on these scaffolds. The scaffolds were characterized by scanning electron microscopy (SEM), water contact angle determination, and Fourier transform infrared (FTIR) spectroscopy and further utilized the scaffolds for differentiation of MSC to the epidermal lineage. Reports are available on the treatment of skin wounds using stem cells (cell therapy) [7,18,19] and on the differentiation of MSC on gels and TCP towards the epidermal lineage [8,20]. However, to the best of our knowledge there have been very few studies that focused on the differentiation of MSC towards the epidermal lineage on electrospun nanofibrous substrates. Interest in nanoengineering of MSC is mainly due to their potential for transplantation, regeneration and treatment of degenerative and autoimmune diseases of skin tissue. Our study was aimed at the fabrication of a biocompatible nanofibrous scaffold with ECM morphology and composition, which can be utilized for the differentiation of MSC to keratinocytes, in an attempt to tissue engineer a cell–scaffold construct for transplantation after skin injury.

2. Materials and methods

2.1. Materials

Human BM-derived MSC were obtained from Lonza (Portsmouth, NH). Dulbecco's modified Eagle's medium/nutrient mixture F12 (DMEM/F12), fetal bovine serum (FBS), penicillin–streptomycin solution, 4,6-diamidino-2-phenylindole, dihydrochloride (DAPI) and Alexa Fluor 594 were all purchased from Invitrogen (Carlsbad, CA). Collagen type I, ascorbic acid, 1,1,1,3,3,3-hexafluor-2-propanol (HFP), epidermal growth factor (EGF), 1,25-dihydroxyvitamin D_3 (VD_3), hydrocortisone, insulin, 3,3′,5-triiodo-L-thyronine sodium (T3) and anti-involucrin antibody (Ab), and goat anti-mouse IgG fluorescein isothiocyanate (FITC)-conjugated secondary Ab were all purchased from Sigma–Aldrich (Singapore). Mouse anti-human keratin 10 (Ker 10) and filaggrin antibodies were ordered from Thermo Fisher Scientific. CD 105 was purchased from Abcam (Cambridge, MA. Poly(L-lactic acid)–co-poly-(ε-caprolactone) (70:30, molecular weight 150 kDa) was purchased from Boehringer Ingelheim Pharma GmbH (Ingelheim, Germany).

2.2. Electrospinning of nanofibers

PLLCL was dissolved in HFP to obtain an 8% (w/v) solution. Collagen and PLLCL were dissolved in HFP at a ratio of 70:30 (wt.%) and stirred overnight. The polymer solutions were separately fed into a 3 ml standard syringe attached to a 27G blunted stainless steel needle using a syringe pump (KDS 100, KD Scientific, Holliston, MA) at a flow rate of 1.0 ml h^{-1}. A high voltage of 15 kV (Gamma High Voltage Research, Ormond Beach, FL) was applied when the polymer solution was drawn into fibers and collected on an aluminum foil wrapped collector at a distance of 12 cm from the needle tip. Nanofibers collected on 15 mm coverslips and the aluminum foil were dried overnight under vacuum and used for the characterization and cell culture experiments.

2.3. Morphology and characterization of electrospun nanofibers

The morphology of the electrospun nanofibers was studied using a field emission scanning electron microscope (FEI-Quanta 200F, Eindhoven, The Netherlands) at an accelerating voltage of 15 keV, after sputter coating with gold (JFC-1200 fine coater, JEOL, Tokyo, Japan). The diameters of the electrospun fibers were determined from the SEM images using image analysis software (Image J, National Institutes of Health, Bethesda, VA). The hydrophilic/hydrophobic properties of the electrospun nanofibrous scaffolds were measured by sessile drop water contact angle measurement using a VCA Optima Surface Analysis system (AST products, Billerica, MA). Distilled water was used for drop formation. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopic analysis of the electrospun nanofibrous scaffolds was performed in an Avatar 380 (Thermo Nicolet, Waltham, MA) over the range 600–3800 cm^{-1} at a resolution of 4 cm^{-1}. The tensile properties of the electrospun nanofibrous scaffolds were determined using a tabletop tensile tester (Instron 3345, Canton, MA) at a load cell capacity of 10 N. Test specimens of dimensions 10 mm breadth × 20 mm length, with a thickness of 70–80 μm, were tested at a crosshead speed of 10 mm min^{-1} and gauge length of 20 mm under ambient conditions [21]. A minimum of six specimens of individual scaffolds was tested and the results obtained were plotted to obtain the stress–strain curve of the scaffolds.

2.4. Mesenchymal stem cell culture

MSC were cultured in DMEM supplemented with 10% FBS and 1% antibiotic and antimycotic solutions (termed normal growth medium) in a 75 cm^2 cell culture flask. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 6 days and the culture medium was changed every 3 days. The 15 mm coverslips with electrospun nanofibers were placed in 24-well plates and pressed with a stainless steel ring to ensure complete contact of the scaffolds with the wells. The specimens were sterilized under UV light, washed thrice with phosphate-buffered saline