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Short Communication

Manipulating Mesenchymal Stem Cells for Vascular Tissue Engineering

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Human mesenchymal stem cells (hMSCs) have generated great attention in tissue engineering applications due to their extensive proliferative capacity *in vitro*, multilineage differentiation potential, and immune response modulation ability *in vivo* [1-9]. hMSCs also secrete a variety of "trophic factors", including growth factors, cytokines, and adhesion molecules, that can alter the tissue microenvironment and thereby rejuvenate or repair diseased tissues and cells [2,7,10,11]. Therefore, the hMSC-induced *in vivo* repair of dysfunctional tissues can be a result of either differentiation or secretion of trophic factors, which alter the milieu so as to regenerate the dysfunctional tissue or cells.

The hMSCs have been proven to be an excellent starting material when combined with biodegradable scaffolds, either in their undifferentiated or differentiated states, for the regeneration of damaged vascular tissues. hMSCs are "trophic" and highly regenerative [7,9]. They can adapt to the microenvironment *in vivo*, and be directed by the growth factors released by platelets and vascular cells after vessel injury (PDGF, TGF-β1, and bFGF) toward a vessel reparative function [7,8]. Most notably, the *in vivo* experiments demonstrated that aligned hMSC constructs facilitate endothelial cell (EC) and smooth muscle cell (SMC) recruitment and organization in addition to providing excellent long-term graft patency, implying that local cues within injured vessels *in vivo* may direct hMSCs toward a vessel repairing function [1]. The unique properties of hMSCs offer us opportunities to use this single cell type to engineer an off-the-shelf tissue engineered blood vessel (TEBV) that can be readily used as allografts by any patient without time concerns.

Cell sheet engineering enables nondestructive cell harvest from thermosensitive polymer-coated surface by controlling the conformational change of the polymer coating [12,13]. The thermosensitive polymers change their hydrophobicity to hydrophilicity when the environmental temperature decreased below the lower critical solution temperature (LCST) of the polymers. This technique avoids the use of proteolytic enzyme to digest the ECM structure and intracellular junction, thus conserves the cell sheet completeness to the maximum extent [13]. Hydroxybutyl chitosan (HBC) is a thermosensitive polymer derived from the biopolymer chitosan, a polysaccharide with similar structure to glycosaminoglycans (GAG) [14,15]. When blended with collagen, the obtained polymer complex coating favors the hMSC attachment, proliferation and phenotypic expression, in addition to the easy removal of hMSC cell sheets from the coated substrate surfaces upon exposure to a temperature lower than the LCST of HBC. In our previous study, we have successfully used the HBC to coat the nanogratings for the ease of harvesting aligned hMSC cell sheets. In the next step, we will use the prealigned hMSC cell sheets to produce a TEBV with well-defined 3D cellular organization similar to that of the SMC organization in natural vessels.

The optimal functionality of a tissue depends on its appropriate histological organization. In natural blood vessels, the SMCs and reinforcing extracellular matrix (ECM) fibers form an elastomotor helix inclined to the vessel centerline [16-18]. The angle between the elastomotor helix winding and the longitudinal axis of the vessel is 30-50° in large arteries, and gradually increases as the vessel diameter decreases [17,18]. The alignment of cells also plays an important role in providing tissues with stronger mechanical properties. Inspired by the structure of natural blood vessels, we are trying to mimic the 3D spiral and interwoven organization of SMCs in real blood vessels with hMSCs. Towards this goal, we firstly fabricate hMSCs into cell sheets that have a high degree of alignment and confluency. We then engineer them into a scaffold-free tissue engineered vascular graft, with one cell sheet layer inclined 30−50° to the centerline, and the second layer perpendicularly to the first. We anticipate that the endurance of TEBVs to pressure and stretch stress of blood flow will be significantly improved by the 3D cellular organization that highly mimics the orientation of SMCs.

To mimic the 3D spiral and interwoven organization of SMCs in real blood vessels, it is crucial to fabricate an hMSC cell sheet that has a high degree of alignment and confluency. A grated substrate can effectively orient cells [19,20]. Other than the width, the depth of the grating is also an important parameter. At the microscale, deep gratings appear to produce a non-uniform cell sheet [21]. The portion of the cell layer grown on the ridges tends to be thinner, rendering the cell sheet more prone to tearing during handling and processing. Furthermore, deep grooves would likely lead to an increase in the time required for an intact sheet to form [21]. We have previously studied hMSC alignment on nanogratings, and established that nanopatterns with a grating depth of 250 nm exert a more pronounced effect than micropatterns in aligning

cells [22,23]. However, although the nanogratings align hMSCs, the cells have a great tendency to grow into an uneven patchy layer [24]. A desirable cell sheet should comprise cells forming tight junctions with each other and secrete plenty of ECM proteins to hold the cell sheet together [25-27]. A non-uniform or patchy structure could make the cell sheet vulnerable to tearing during handling, in addition to compromising the quality of the engineered tissue. Another complication of culturing hMSCs on nanopatterns is the differentiation driven by nanotopographical cues. Nanostructures stimulate hMSCs to differentiate along the neuronal, myogenic, and osteogenic lineages in a proliferative, non-differentiation medium, while decreasing their proliferation [23,28,29].

Low O_2 is a native physiological condition of the hMSC niche, which effectively supports hMSC survival, maintains their primitive status, increases the ECM secretion, and considerably improves the uniformity of cell layers [24,30-32]. We have previously demonstrated that hMSCs grown under 2% O_2 conditions secret abundant ECM proteins [24,31,32], facilitating the cells to grow into even layers with highly aligned morphology on nanogratings [24]. Moreover, the cells maintained elevated self-renewal ability and preserved higher multi-lineage differentiation ability of the hMSCs than their counterpart cultured under conventional 20% O_2 [24,32]. Thus, for engineering vascular grafts using hMSCs, it is necessary to maintain their undifferentiated status of hMSCs during the process of fabricating hMSC vascular grafts. Our previous studies have shown that physiologically low O_2 conditions favor the *in vitro* expansion of hMSCs, prevent their differentiation, stimulate the secretion of ECM proteins, and considerably improve the uniformity of cell layers [24,31,32]. It is also an important environmental parameter that regulates the developmental process and metabolic behavior of blood vessels [33,34]. Therefore, we are incorporating physiologically low O_2 conditions in the fabrication process, ensuring a high quality hMSC cell sheet and a subsequent mechanically strong TEBV while maintaining the "trophic" and regenerative properties of hMSCs.

Once wrapped around the temporary mandrel, the 3D tubular cellular assemblies need to be further matured to fuse all the cell layers together. Static culture impairs diffusion of nutrients and O_2 [35] to 3D tissue constructs. In addition, blood vessels reside in a dynamic environment *in vivo*. During the cardiac cycle, the arteries are exposed to significant mechanical strains and variable O_2 levels. The shear stress in the human arteries is in the range of 15~30 dyn/cm^2 [36], whereas the O_2 level is about 12% (90 mmHg) on average [34,37]. Dynamic culture systems have been widely utilized [38-41] to mimic physical effects of blood flow and pressure in engineering vascular grafts [42-45]. To provide sufficient nutrients and also mature the TEBVs in the natural environment of coronary arteries, it is necessary to develop a bioreactor system that can replicate the physiologically relevant O_2 and flow conditions of coronary arteries in one single unit. Therefore, we are currently developing a novel bioreactor system that has the capacity to stabilize the TEBVs in a hydrodynamic microenvironment by providing controllable O_2 tension and physiological pulsatile force to the 3D tubular cellular constructs.

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