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Response of mesenchymal stem cells to the biomechanical environment of the endothelium on a flexible tubular silicone substrate

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Abstract

Understanding the response of mesenchymal stem cells (MSCs) to forces in the vasculature is very important in the field of cardiovascular intervention for a number of reasons. These include the development of MSC seeded tissue engineered vascular grafts, targeted or systemic delivery of MSCs in the dynamic environment of the coronary artery and understanding the potential pathological calcifying role of mechanically conditioned multipotent cells already present in the vessel wall. *In vivo*, cells present in the coronary artery are exposed to the primary biomechanical forces of shear stress, radial stress and hoop stress. To date, many studies have examined the effect of these stresses in isolation, thereby not presenting the complete picture. Therefore, the main aim of this study is to examine the combined role of these stresses on MSC behaviour. To this end, a bioreactor was configured to expose MSCs seeded on flexible silicone substrates to physiological forces – namely, a pulsatile pressure between 40 and 120 mmHg ($5.33-1.6 \times 10^4$ Pa), radial distention of 5% and a shear stress of 10 dyn/cm^2 (1 Pa) at frequency of 1 Hz for up to 24 h. Thereafter, the 'pseudovessel' was assessed for changes in morphology, orientation and expression of endothelial and smooth muscle cell (SMC) specific markers. Hematoxylin and eosin (H&E) staining revealed that MSCs exhibit a similar mechanosensitive response to that of endothelial cells (ECs); they reorientate parallel with direction of flow and have adapted their morphology to be similar to that of ECs. However, gene expression results show the cells exhibit greater levels of SMC-associated markers α-smooth muscle actin and calponin ($p < 0.05$).

Keywords: Mesenchymal stem cell; Silicone; Bioreactor; Endothelial cell; Smooth muscle cell; Soft tissue biomechanics

1. Introduction

The studies of vascular disease, intervention and post-operative care are evolving as the role of undifferentiated cells in the vasculature is being investigated more intensely. The multipotentiality of these cells can be harnessed if properly directed and understood. However, their plasticity must be consistently controlled, while guarding against differentiation into inappropriate cell types. The study of the effect of vascular physiological forces on mesenchymal stem cells (MSCs) is of particular interest. MSCs are well characterised and can be successfully propagated in large numbers making them suitable for clinical applications [1]. While the multipotentiality of these cells has been widely explored under biochemical stimulation [2], the role of biomechanical stimulation is less well understood.

Several aspects of cardiovascular research would benefit from a greater understanding of cell response to a vascular mechanical environment. The potential of being able to provide autologous terminally differentiated vascular phenotype cell populations would be a major clinical breakthrough in vascular tissue engineering [3,4].

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Similarly, many novel therapies currently being researched to treat a broad range of diseases (including but not exclusive to vascular diseases) involve the localised or systemic delivery of MSCs as a direct therapeutic tool or with/on some form of carrier [5–8]. In many of these pilot studies, MSCs tagged with a fluorescent protein have been seen in other non-targeted organs [9–11]. If these cells are circulating, there is a chance they would adhere to vessel walls and differentiate under biomechanical stimulation and lead to ectopic tissue formation.

Furthermore, subpopulations of cells found in the vessel medial layer have been found to exhibit characteristics similar to those of MSCs isolated from the bone marrow. These cells have shown the capacity to undergo osteogenesis and chondrogenesis *in vitro* [12–14]. In addition to lipid accumulation at sites of vascular disease, more unusually, cartilage and bone-like tissues are also sometimes found at sites of vascular disease [12–17]. This leads to the inference that these undifferentiated cells in the vessel wall under unfavourable mechanical conditioning could lead to ectopic tissue formation. Therefore, a system which can apply a range of loading conditions on similarly undifferentiated cells could lead to a greater understanding of the influence of mechanical factors on pathology of certain types of arteriosclerosis [18,19].

In embryonic stem cells, FLK-1$^+$ cells have shown the capacity to differentiate into endothelial cells (ECs) through biomechanical [20] and biochemical signalling [21] in isolation and in combination [22]. In adult cells, attempts have been made to differentiate MSCs derived from bone marrow [23] and from the umbilical cord [24] into ECs, however, the effect of mechanical signalling along this differentiation pathway is largely unstudied. Cyclic tensile strain has been shown to promote differentiation towards a smooth muscle cell (SMC) phenotype [8,25]. Shear stress has been shown to promote EC differentiation from endothelial progenitor cells [26], while also playing a crucial role in vasculogenesis and re-endothelialisation [27].

Combinations of flow and pressure stimulation have been shown to enhance expression of SMC markers in rat stromal cells [28]. The combined effects of laminar flow and cyclic stretching have recently been shown to enhance tissue growth in tissue engineered heart valves [29], however, the magnitude of these forces is not as great as those found along the vessels endothelium. It is clear from these studies that mechanical signalling plays a crucial role in vascular cell development and differentiation.

It is the objective of this study to examine the combinatorial effect of the three major forces of pulsatile flow, cyclic pressure and stretch experienced by cells lining the endothelium. The capacity of MSCs to differentiate into vascular phenotypes by these biomechanical stimuli is investigated. MSCs and human umbilical vein endothelial cells (HUVECs) are seeded onto silicone 'pseudovessels' and subjected to mechanical conditioning for 24 h. Cellular responses are characterised by changes in orientation, morphology and gene expression.

2. Materials and methods

2.1. Cell source

Bone marrow aspirates were obtained from the iliac crest of normal donors. All procedures were performed with informed consent and approved by the Clinical Research Ethical Committee at University College Hospital, Galway. Donors are selected from what are generally considered ethically approved guidelines: healthy males and non-pregnant females between the ages of 18 and 45 years old, tested negative for HIV, hepatitis B and hepatitis C. MSCs were isolated and expanded in culture as described previously by direct plating [30]. Briefly, aspirates were washed with medium (DMEM–low glucose containing penicillin–streptomycin solution (both Sigma–Aldrich, Ireland) at 100 U penicillin-G and 0.1 mg streptomycin/ml) and centrifuged; the precipitated cells were suspended in medium with 10% selected foetal bovine serum and plated at a final density of approximately 3.0×10^5 cells/cm^2. Serum was selected based on maintenance of MSC proliferation and multipotency in culture. Cells were seeded on T-175 flasks (Sarstedt, Ireland) and maintained at 37 °C with 95% humidity and 5% CO_2 in the same medium. After 5 days, red blood cells were washed off with phosphate-buffered saline and fresh medium added. Colonies of adherent cells formed within 9 days. At the end of primary culture, adherent colonies were detached by treatment with 0.25% trypsin and 0.53 mM EDTA (Sigma–Aldrich, Ireland). Cells were plated in hMSC medium (DMEM–LG; 10% FBS; 1% antibiotic) at 5.7×10^3 cells/cm^2. All MSC preparations were characterised for surface expression of CD14, CD34, CD45, CD73 (BD Pharmingen, UK) and CD105 (Serotec, UK) using a FACs ARIA sorter (Becton Dickinson, UK). FACS analysis indicated the presence of a uniform population of cells negative for CD14, CD34, CD45 (<2%) and positive for CD73 and CD105 (>95%) [31]. Cell multipotency was confirmed through adipogenic, osteogenic and chondrogenic assays. Cultures were passaged at 4–6 day intervals and expanded to passage 4 for experimentation.

2.2. Bioreactor design

This system is an enhanced version of that described in a previous study [32] which now allows the incorporation of radial distention of tubular constructs under pressurisation. It is designed to fit inside a standard incubator in an environment of 100% humidity, 5% CO_2 at 37 °C. The bioreactor system consists of a peristaltic pump (520U, Watson Marlow, UK) which operates at a speed of 30 rpm. The pump generates a cyclic flow pattern of culture medium through the system and the average flow rate is controlled by varying the tubing diameter through which the medium is pulsed. The basal pressure in the system is controlled by varying the height of the reservoir. Both the mean and amplitude levels of the cyclic pressure waveform in the pseudovessel are controlled by pinching the flow loop distal of the pseudovessel chamber. Flow rate is monitored independently by a non-contact ultrasonic flowmeter and recorded by a Labview® (National Instruments, TX, USA) program [33]. Mean wall shear stress, τ_{mean}, is calculated by the Hagen–Poiseuille equation [34]:

$$\tau_{mean} = \frac{4\mu Q}{\pi R^3};$$

where μ is the dynamic viscosity of the medium, Q is the total flow volume and R is the vessel's internal radius. Similarly, pressure is monitored by a pressure probe and recorded in Labview®. Tube distention is examined by observing the expansion of the pseudovessel using a video-extensometer. Typical waveforms for flow rate, pressure and radial distention are shown in Fig. 1. Radial distention is quantified as the ratio of the instantaneous outer diameter to the original unpressurised outer diameter of the pseudovessel.

2.3. Pseudovessel formation

Medical-grade silicone (4600, Elastosil, Wacker) was used to produce pseudovessels. It exhibits similar levels of radial distention to those of a native vessel when subjected to physiological levels of pressure [35]. When cured this material has a tensile strength of 6 MPa and an elongation at break of