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Vascular Prostheses: Performance Related to Cell-Shear Responses

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Background. **This work concerned the endothelialization of vascular prostheses and subsequent improvement of functionality with respect to tissue engineering. The aim of the study was to investigate the initial, pre-shear stress cellular behavior with respect to three vascular biomaterials to explain subsequent cellular responses to physiological shear stresses.**

Materials and methods. **Expanded polytetrafluoroethylene (ePTFE), polyethyleneterephthalate (polyester; Dacron; PET), and electrostatically spun polyurethane (PU) (all pre-impregnated with collagen I/III) were cell-seeded with L929 immortalized murine fibroblasts or human umbilical vein endothelial cells (HUVECs). Cytoskeletal involvement, cell height profiles, and immunohistochemistry were examined after 7 d static culture.**

Results. **All three vascular biomaterials demonstrated different structures. Cell behavior varied both between the materials and the two cell types: cytoskeletal involvement was greater for the HUVECs and the more fibrous surfaces; height profiles were greater for the L929 and PET, and lowest on PU. Immunohistochemistry of HUVEC samples also showed differences: PU revealed the greatest expression of intercellular adhesion molecule-1 and E-selectin (PET and ePTFE the lowest, respectively); ePTFE produced the greatest for vascular cell adhesion molecule-1 (PET the lowest).**

Conclusions. **Material substrate influenced the cellular response. Cells demonstrating firm adhesion increased their cytoskeletal processes and expression of cell-substratum and inter-cellular adhesion markers, which may explain their ability to adapt more readily to shear stress. The fibrous PU structure appeared to be most suited to further shear stress exposure. This study demonstrated the potential of the underlying vascular material to affect the long-term cellular functionality of the prosthesis.**

Key Words: **tissue engineering; blood vessel prosthesis; cell responses; electrospinning; endothelial cells; regional blood flow; static culture; shear stress.**

INTRODUCTION

An ideal solution to blood vessel replacement, particularly those of small diameter (<6 mm), remains an important objective, both for tissue engineering research and clinically; seeding of vascular endothelial cells onto the luminal surface of the prostheses (endothelialization) has been used as an associated technique since the 1980s, producing varying degrees of success [1–7]. In general, though, this technique is still of extreme research interest, particularly with respect to the improvement of long-term results, e.g., an increase in graft thromboresistivity [1–11]. To develop cell retention, many groups are also now examining the effect of pre-impregnating the material surface with extracellular matrix (ECM) proteins, such as fibronectin and collagen [12]; reports have stated that the addition of these proteins increases cell adhesion and spreading [4–7, 9, 13–15].

Once endothelial cells are seeded onto the prosthesis, their adhesion has to be maintained, particularly on exposure to shear stresses resulting from blood flow. There is evidence that shear stress can alter cell retention (both increase and decrease) and change cell morphology and gene expression [2, 3, 6, 8, 9, 11, 16–22]. It is also well known that cells react accordingly to varying underlying substrate topography, producing different cell coverage and responses [3, 6, 7, 14, 15, 23–29].

In previous work, three different vascular prosthesis materials were examined to quantify and compare cell adhesion [6]. The effect of collagen-impregnating the grafts was investigated and the results of exposure to physiologically relevant shear stresses representative

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of femoro-popliteal bypass determined. L929 murine fibroblasts and human umbilical vein endothelial cells (HUVECs) were used to cell-seed expanded polytetrafluoroethylene (ePTFE), polyethyleneterephthalate (PET), and electrostatically spun polyurethane (PU). The HUVECs demonstrated a greater response to shear stress than fibroblasts, with the HUVECs spreading to a greater degree. Impregnation of the three materials was seen to improve initial cell attachment, with initial cell coverage and resistance to fluid shear stress being greatest on collagen-impregnated ePTFE and comparable on PET and PU [6]. However, cell loss from all three materials when exposed to shear stress was significant [6].

These experiments indicated the range of cellular responses produced from the application of shear to the three different biomaterials [6]; however, the reasoning behind these differing responses was not fully understood, and has not been explored in the literature. As previously discussed, the cellular responses, particularly those of adhesion and retention, are affected not only by the shear stresses but also by the underlying substrate (coating and topography of graft) [2–9, 11–29]. Hence, for the clinical performance and outcome of the vascular graft to be optimized, an explanation for this range of responses is required. The aim of this paper was to study the initial cellular behavior with respect to the three materials before the onset of shear stresses (static conditions), in order to understand the cell responses later seen when exposed to the shear stresses. The same two cell types as previously investigated were used [6]. Previous research has examined the effect of the vascular prostheses on the endothelialization [1, 29], and the results of shear on the attached cells on different materials [2, 3]; however, the novelty of this study lies in the correlation of the initial cellular results (examined here) to the subsequent behavior on application of initial shear stress [6], all with respect to the underlying substrate.

MATERIALS AND METHODS

Vascular Prosthesis Materials

Three biomaterials were used for the study: ePTFE (IMPRA Graft; C. R. Bard Inc., Murray Hill, NJ), Dacron (Laboratoire Pérouse Implant, Ivry Le Temple, France) (PET), and electrostatically spun PU scaffolds (Tecoflex SG-80A, Thermedics Inc., Woburn, CA; fabricated in Division of Clinical Engineering). These were characterized using a field emission scanning electron microscope (SEM) (Leo, Welwyn Garden City, United Kingdom) and its associated annotation measurement software with regard to their pore size and microstructure. Reflective light microscope images were also taken of the structure (Zeiss, Welwyn Garden City, United Kingdom). Pore/inter-fiber sizes were measured, using SEM, at the widest points of the spaces ($n = 20$ per sample with 4 repeats). Sixteen 2 cm $\times$ 1 cm samples were cut of each material.

Collagen Impregnation of Materials

Samples were prepared according to the protocol previously described [6]. Briefly, samples were ethylene oxide sterilized and allowed to degas for 2 wk, before being impregnated with bovine Type I/III purified and dehydrated collagen (Symatese Biomatériaux, Chaponost, France). Samples were covered in the collagen solution and placed in a vacuum oven for 10 min. Excess solution was then rinsed off with phosphate-buffered saline (PBS) and aspirated off and the samples incubated (37°C, 5% CO_2, humidified) for 30 min.

Cell Culture

Primary derived HUVECs and L929 immortalized murine cell-line fibroblasts (ECACC, Salisbury, United Kingdom) were cultured. L929 cells used 199 medium containing modified Earle's salts (Gibco, Invitrogen Corporation, Paisley, United Kingdom), 1.25 GM/L $NaHCO_3$, L-glutamine, L-amino acids, 1% streptomycin and penicillin, and 5% bovine fetal calf serum (Cambrex, Nottingham, United Kingdom). HUVECs were grown in 40% DMEM (Dulbecco's Modified Eagle's Medium), 40% 199 (containing modified Earle's salts) (Gibco, Invitrogen Corporation), 20% bovine fetal calf serum (Cambrex), 1% nonessential amino acids, 1% sodium pyruvate, and 1% streptomycin and penicillin.

Tissue culture flasks were precoated with 0.1% gelatin. L929 cells were subcultured on a weekly basis with corresponding medium changes. HUVECs were subcultured once approximately 60% to 80% confluent with contacting adjacent cells; medium was changed every 3 d. HUVECs were seeded from the fourth passage.

Use of the HUVECs had approval granted from the Local Research Ethic Committee; this facilitated the collection of umbilical cord tissue and the harvesting of the cells by named individuals, with informed consent from the patients. The issue of cell and tissue storage was managed through the University of Liverpool Human Material Inventory.

Cell-Seeding of Vascular Materials

All three collagen-impregnated materials were seeded separately with L929 and HUVEC cells; 2.1×10^5 cells/mL were suspended with 1 mL of cell suspension added to each sample. This seeding density was calculated to be subconfluent at the total culture period, to accurately examine the material-cell interactions. Medium was changed every 3 d; the total culture period was 7 d for all samples. Four repeats of each culture condition (i.e., material substrate, and cell type) were performed.

Cytoskeletal Staining of Samples

All of the cell-seeded samples were fixed (after rinsing with PBS) using 4% formaldehyde, 2% sucrose fixative solution at 37°C, 5% CO_2, humidified for 10 min, then rinsed with PBS. Samples were permeabilized using 0.5% Triton X100 solution at 4°C for 5 min, then rinsed again with PBS. Primary antibodies were added and incubated for 1 h at 37°C: L929 cells with 0.22 mg/mL anti-vinculin (V4139; Sigma, Gillingham, United Kingdom); HUVECs with 0.22 mg/mL monoclonal mouse anti-human vinculin (Serotec, Oxford, United Kingdom). This was followed by a trio of wash steps performed in the dark at room temperature for 3 min each, using 0.1% Tween 20 solution (ICN Biomedicals Inc., Aurora, OH). Secondary antibodies were added for 1 h at 37°C: L929 cells with 0.1 mg/mL Alexa Fluor 568 goat anti-rabbit IgG (H + L) (Molecular Probes, Invitrogen, Eugene, OR); HUVECs with 0.1 mg/mL rhodamine (rhodamine-conjugated goat IgG fraction to mouse IgG whole molecule) (Sigma, Gillingham, United Kingdom). The Tween 20 wash step was then repeated. Oregon-green phalloidin (Molecular Probes, Leiden, The Netherlands) was added at a concentration of 5 μg/mL for 30 min at 4°C. Samples were washed with PBS before mounting with fluorescence stabilizing mountant containing DAPI stain (Vectashield with DAPI H-1200) (Vector Laboratories Inc., Burlingame, CA). Samples were kept in the dark, at 4°C, until analysis.