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Interactions between endothelial cells and a poly(carbonate-silsesquioxane-bridge-urea)urethane

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Abstract

We have recently developed a polymer which contains silsesquioxane in the form of nano-bridges poly(carbonate-silsesquioxane-bridge-urea)urethane (PCBSU) for cardiovascular device applications. The polymer has been characterised and the durability has been confirmed with long-term in vivo tests. The aim of this study was to test the cytocompatibility of the new polymer and to investigate any potential cytotoxic effects. To assess the effect of direct contact with PCBSU sections of polymer material were cut and placed into a 24-well plate. Six discs were seeded with 2×10^5 human umbilical vein cells (HUVEC). As a positive control, six wells were seeded with the same number of HUVEC. In a further experiment to assess indirect contact with PCBSU a sample of the polymer was powdered using a Micro-Dismembrator. Cell culture medium was exposed to powdered polymer (1–100 mg/ml) for a period of 7 days. HUVEC seeded as above were then exposed to the treated cell culture medium for 24 and 96 h. Finally, cell proliferation was studied over 16 days by seeding 2×10^5 HUVEC on films of PCBSU cast in glass Petri dishes. Cell viability and growth were assessed using Alamar blue™, lactate dehydrogenase and Pico green assays and morphology was studied by Toluidine blue staining and scanning electron microscopy. Viable cells were demonstrated to be present after 16 days seeded on PCBSU. Exposing cells to PCBSU-treated cell culture medium resulted in no apparent damage to the cells at concentrations of 1 or 10 mg/ml, and only a slight reduction at 100 mg/ml after 96 h exposure. This study demonstrates that PCBSU can support the growth of endothelial cells for a prolonged period and does not demonstrate any significant toxic effects to cells. Thus it has the potential to be used both as a medical device and as scaffolding in tissue engineering applications.

Keywords: Cell viability; Copolymer; Cytotoxicity; Bypass graft; Stent; Polymer; Silsesquioxane; Silsesquioxane nano-bridges; Medical device

1. Introduction

Current bio-materials available for clinical application such as bypass grafts and stents are predominantly made from two polymers, poly(ethylene terephthalate) (PET or Dacron) and polytetrafluoroethylene (PTFE) [1,2]. Dacron, especially in cardiovascular applications, is highly reactive towards blood and the surrounding tissue resulting in inflammation and neo-intimal proliferation with both grafts [3,4] and stents [5,6]. PTFE is widely used due to its high bio-stability and reasonable tolerance by the body but as with Dacron there is a high adherence of platelets and blood proteins to the surface [7].

Polyurethanes have begun to be widely investigated as an alternative in cardiovascular bio-medical devices due

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to their durability and flexibility in extrusion. Their use as a bypass graft to simulate the viscoelastic properties inherent in native arterial wall, which has been found to be of importance in improving the patency of cardiovascular devices [8], has been suggested. Polyurethanes demonstrate a reduction in surface thrombogenicity as compared to both Dacron and PTFE, which is due to the fact that the surface of polyurethanes can be easily modified with smart bio-molecules such as arginine–glycine–aspartate (RGD) or heparin [9].

Previously we have developed a bypass graft based on poly(carbonate-urea)urethane (PCU) which has been extensively assessed and is currently marketed as an access graft [10,11], where it is required for short periods until a suitable kidney has been found for transplantation. This material was still thrombogenic, but lesser than other polyurethanes due to its soft segment being based on carbonate linkages rather than ester or ether, as in the majority of conventional polyurethanes. It was felt that a need still remained for a polymer not requiring surface modification which employed a carbonate amorphous segment to improve its surface thrombogenicity. The properties engendered in the polymer were to achieve both anti-platelet and protein inhibitory qualities coupled with the ability to demonstrate cytocompatibility allowing cells to grow on its surface. In order to achieve this aim a polymer based on polyurethane and bridged monomers of silsesquioxane (pendant nano-bridge); poly(carbonate-silsesquioxane-bridge-urea)urethane (PCBSU) was developed [12]. Our original hypothesis was that by the incorporation of these into polyurethanes the resultant polymer would have anti-thrombogenic qualities. Being inorganic in nature silsesquioxanes negate the problems and complexities associated with the incorporation of drugs and bio-molecules such as heparin into the matrix of the material. The silesquioxane being bridged and pendant on the polymer surface permits the anti-platelet and anti-coagulant function but prevents the cytotoxic effects associated with materials containing silicon. This allows endothelial cells (EC) to grow on its surface as required in tissue-engineered cardiovascular medical devices.

Previous studies into the cytocompatibility of similar materials have investigated the effect on cells of either direct contact with the material or indirect contact by utilising cell culture medium exposed to material for a time prior to use. Techniques used to evaluate cytocompatibility include examining cell morphology by light microscopy and scanning electron microscopy (SEM), [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) metabolic activity assays, neutral red viability staining and cell counting by using Tryphan blue [13–16]. In this study, both the direct and indirect effects of PCBSU on human umbilical vein endothelial cells (HUVEC) have been explored by looking at the total amount of DNA present in the cells using a Pico green (PG) assay system, examining cell metabolism using an Alamar blue^{TM} (AB) metabolic assay and looking at cell damage by measuring lactate dehydrogenase (LDH) release. Cell morphology was studied by staining with Toluidine blue in the case of indirect contact and carrying out SEM studies in the case of direct contact.

This study was carried out to assess the cytocompatibility of PCBSU and determine if it could provide a suitable base on which to seed EC.

2. Materials and methods

2.1. Polymer Production

The synthesis of poly(carbonate-bridged silsesquioxane-urea)urethane has been described in detail previously [12] but in brief the inorganic urethane is made from 4,4′-methylenebis(phenyl isocyanate) (MDI), poly(hexamethylene carbonate)diol, and silsesquioxane dissolved in tetrahydrofuran here bis[3-(trimethoxysilyl)propyl]amine and chain extended with ethylene diamine in *N*,*N*′-dimethylacetamide (DMAC).

PCBSU graft was cast by pouring 3 ml of polymer (diluted 1:1 in DMAC) solution into a 10 cm diameter glass dish and was left for 18 h in a circulating air oven at 55–65 °C. Following casting, the graft material was thoroughly washed with phosphate-buffered saline (PBS) prior to use.

2.2. Endothelial cell culture

HUVEC were isolated from human umbilical cord vein following a previously described method [17]. In brief, cell numbers were amplified by tissue culture in cell culture medium (CCM) which was prepared as follows: 157 ml M199 medium, 4.5 ml sodium bicarbonate (7.5%), 1.5 ml penicillin/streptomycin (10,000 U/ml and 10 mg/ml, respectively), 40 ml fetal bovine serum and 3.6 ml 200 mM L-glutamine (Invitrogen Ltd, Paisley, UK). At confluence, cells were removed using 0.25% trypsin-EDTA (Sigma-Aldrich Company Ltd., Poole, UK) and split in a 1:2 ratio. Confluent cultures at passage three were used in all experiments.

2.3. Assessment of cytocompatibility

2.3.1. Indirect effect of PCBSU on HUVEC

A sample of the graft was powdered using a Mikro Dismembartor U (B. Braun Biotech International, Melsungen, Germany). Following powdering, graft samples were sterilised by autoclaving. Powdered graft was then added at concentrations of 1, 10 and 100 mg/ml to CCM and shaken for seven days at 37 °C in a