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Inflammatory response to a novel series of siloxane-crosslinked polyurethane elastomers having controlled biodegradation

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A series of polyurethane polymers was synthesized with increasing proportions of silicone in the form of polydimethylsiloxane (PDMS) utilised as a cross-linking agent, based on an aromatic, non-biostable polyetherurethane (PEtU). Eight formulations ranging from 0–50% PDMS were constructed into porous and non-porous films. These were implanted subcutaneously in rats, both unstrained and 100% strained, for 3 and 6 months. Degradation was determined by FTIR-ATR. Porous films were implanted for 6 and 12 months intramuscularly in both rats and rabbits. These were explanted and examined for inflammatory cell markers by immunohistochemistry. Both low and high percentages of siloxane gave rise to increased degradation, with 20–40% PDMS resulting in the least degradation. Infrared spectral changes correlated well with both visual examination and observation by SEM. Changes to the concentration of siloxane gave rise to differences in the thickness of fibroblastic capsule and infiltration of inflammatory cells in both films & scaffolds. Cellular infiltration was greatest in the films with lower siloxane concentrations. Macrophage activation (MHC-I & MHC-II expression) was least in the higher siloxane variants. It is concluded that by varying the siloxane content in the PEtU matrix we can obtain an acceptable inflammatory response with a relatively short degradation time.

1. Introduction

Polyurethane elastomers are widely used in medical devices because they have good mechanical properties whilst having generally acceptable biocompatibility [1]. Their use in many applications is limited due to their inherent degradability [2–5], which is often accompanied by high levels of inflammatory reaction to the degradation products. Many strategies have been utilised to reduce this bioinstability, including the inclusion of carbonate end caps and silicone moieties within the backbone of the polymer. Nevertheless, elevated levels of inflammation are often observed at the site of implantation of polymer biomaterials [6, 7] and this has potentially dramatic consequences for the fate of implanted cells in tissue engineering applications.

Many tissue engineering scaffolds are constructed from degradable materials that have rigid and inelastic polymer strands [8], many for the sole reason that they already have FDA approval for clinical use. It is generally recognized that the tissue development around and within a construct crucially depends on the initial mechanical properties. There are, therefore, many applications in the field of tissue engineering in which it would be desirable to have a material with the elastomeric properties of a polyurethane accompanied by controlled degradation without an unacceptable inflammatory response.

In this study, we created a series of polymers with the same basic chemistry but with increasing proportions of silicone in the form of polydimethylsiloxane (PDMS) utilised as a cross-linking agent. Ultimately, we intend to determine the best compromise in the proportion of silicone giving rise to a desirable level of biostability and enhanced levels of biocompatibility. In the current study, we present the profile of degradation and the nature of the host response in two different animal models observed at time periods up to 12 months implantation *in vivo* of 8 different formulations of the

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polymer fashioned into porous sheets suitable for tissue engineering applications.

2. Materials and methods

A medical-grade, aromatic, non-biostable polyetherurethane (PEtU) was used as the base material for the polymer series. The PEtU was purified using a soxhlet apparatus in a 1:1 (v/v) methanol-acetone mixture. The final reaction solvent was a mixture of 1:1 (v/v) tetrahydrofurane (THF) and 1,4-dioxane (DX) and was purified in a rotating evaporator. The cross-linking reaction was performed with PDMS in a three neck reactor flask equipped with a water condenser at 82 °C for 6 h under stirring conditions. PDMS was included in the non urethane component of the reaction mixture at concentrations of 0% (pure PEtU), 10%, 20%, 30%, 40%, 50%, 60%, 80% and 100%, termed S0, S10, S20, S30, S40, S50, S60, S80 and S100 respectively, with a 1:1 ratio of urethane and non-urethane components. This was stored as a solution of 3% (w/v) in THF-DX 1:1 (v/v), protected from light until used.

Flat sheets of the polymers were created by solvent casting onto glass at room temperature in an atmosphere of nitrogen (Fig. 1). Porous scaffolds (Fig. 2) were made by bringing the PEtU-PDMS solution to the point of precipitation by the addition of 17% (v/v) distilled water then processing them using phase inversion in a bespoke "spray machine" that has been described in detail elsewhere [9, 10]. Briefly, polymer solution was sprayed into a fine spray of water, and the resultant precipitate deposited onto a spinning mandrel.

An *in vivo* model which enhances degradation of films by exposing a free edge to a degrading environment by implantation of thin sheets of polymer under strain was utilized. Pieces of each polymer formulation 100–200 μm thick were implanted subcutaneously into the dorso-lumbar region of adult Wister rats for either 3 or 6 months and were either unstrained or strained to 100% of their original length by clamping dumbell-shaped polymer pieces over Delrin formers using silicone sleeves. Explants were rigorously washed with detergent and carefully trypsinised to remove any adherent protein. Degradation was determined using FTIR using a Germanium ATR crystal by comparison of the ratios of the signal of various hard and soft segment polyurethane groups (e.g. urethane carbonyl at 1703–1730 cm^{-1}) to Si—CH_3 bonds at 805 cm^{-1} in implanted polymers to non-implanted controls.

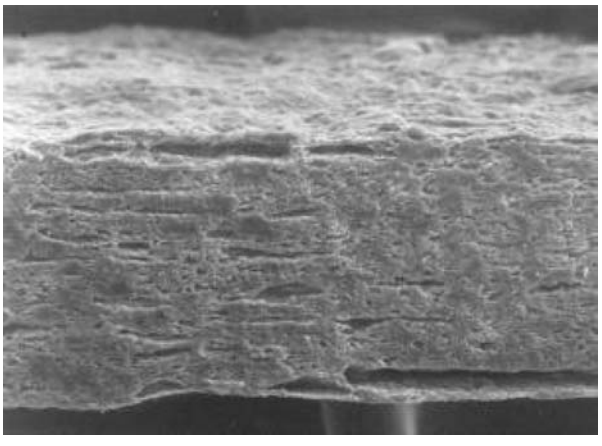

Figure 1 Unimplanted, non-porous S30 film.

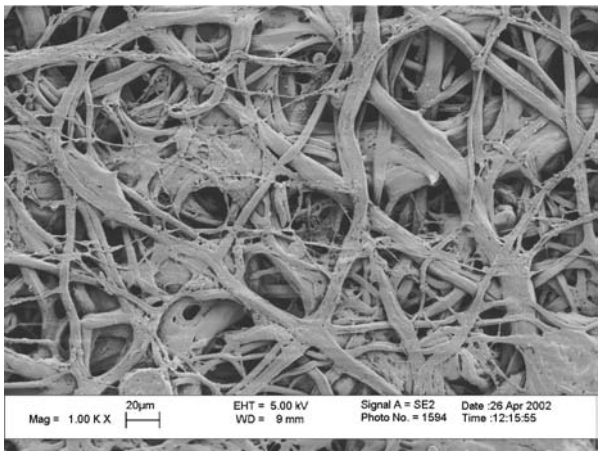

Figure 2 Unimplanted S20 scaffold.

Additionally, both porous scaffolds and polymer films were implanted intramuscularly in adult Wister rats and New Zealand white rabbits for 6 and 12 months respectively. Explants were fixed in formalin and embedded in methacrylate resin, sectioned to 3 μm and analysed by conventional histology (haematoxylin and eosin) and immunohistochemistry using inflammatory cell markers. An avidin-biotin staining protocol was used with fast red as the final enzyme substrate. A panel of mouse anti-rat monoclonal antibodies was used to stain serial sections of the samples targeting the following cell types and subsets, expression of receptors and specific molecules: immature macrophages and monocytes (ED1), differentiating (mature) macrophages (CD163) (ED2), α-β receptor on all T-lymphocytes, CD2 (mature T-lymphocytes), CD4 (helper/inducer T-lymphocytes), CD54 (ICAM-1), MHC (major histocompatibility complex) class I, MHC class II, TGFβ (Transforming growth factor β), γ-δ receptor on T-lymphocyte subset and natural killer cells. All monoclonal antibodies were purchased from Serotec (Oxford, UK). The secondary antibody was biotinylated rabbit anti-mouse IgG from DakoCytomation (Cambridge, UK). PBS was used as the process negative control and the panel of antibodies was run together on two samples per full staining protocol to act as internal reference and isotype controls for the staining procedure.

3. Results

Polymers containing both low and high percentages of siloxane demonstrated significant degradation (Figs. 3 and 4) after 6 months for flat sheets and 3 months for porous scaffolds in the thin edge degradation model in the rat when strained at 100% of their original length. Degradation was much reduced in the unstrained samples. The materials containing percentages of siloxane of 20–40% resulted in the least degradation. Infrared spectral changes correlated well with both visual examination and observation by SEM.

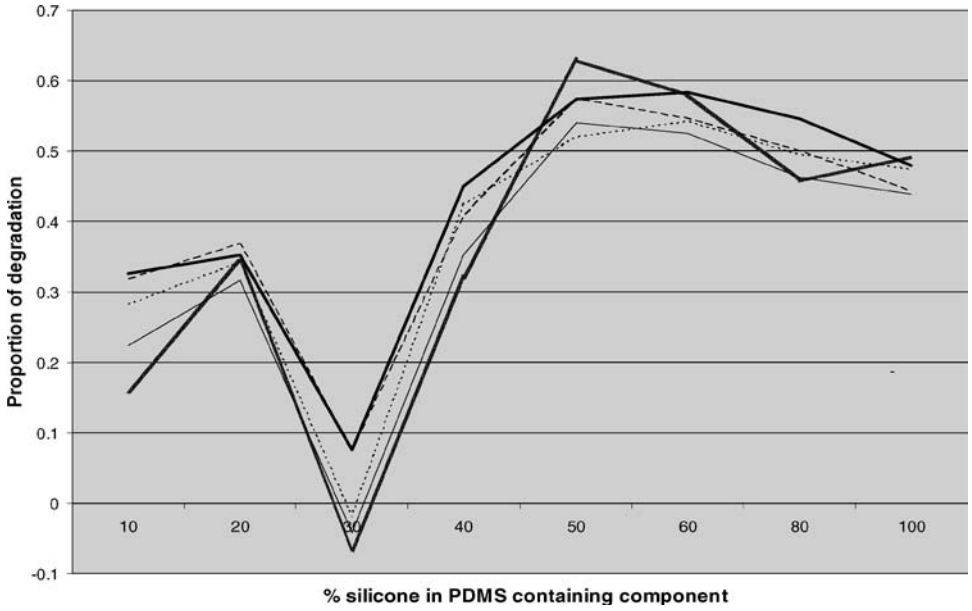

Figure 3 Degradation of polymer series hard segment components as measured by FTIR (..... Putative MDI (1597 cm^{-1})), (– – – C—N & Amide III (1220 cm^{-1})), (— Urethane carbonyl (1703 cm^{-1})), (▬ bonded & unbonded carbonyls), (▬ Urethane N—H & C—N (1530 cm^{-1})).

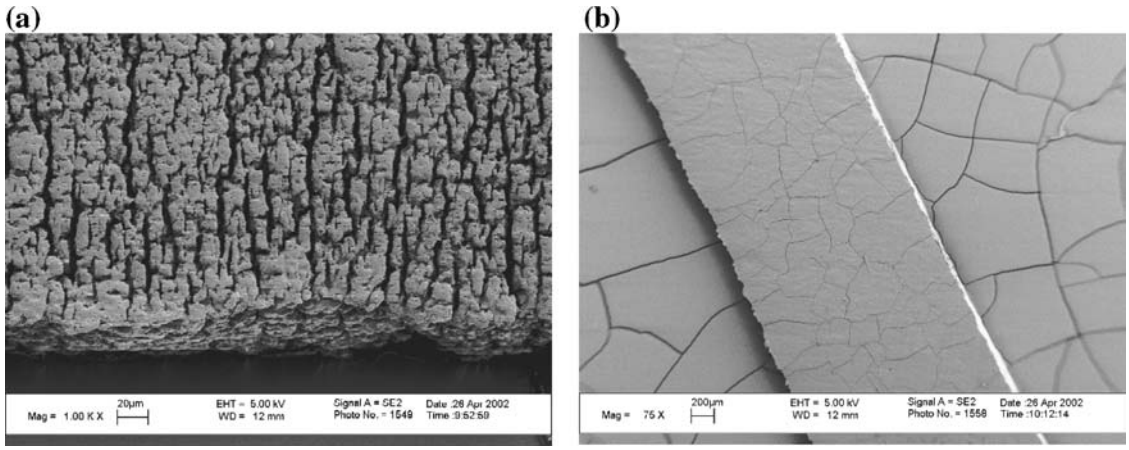

Figure 4 Scanning electron micrographs of (a) S10 and (b) S30 implanted subcutaneously in Wistar rats at 100% strain for 6 months.

Changes in the concentration of siloxane within the polymer formulation gave rise to differences in the thickness of the resultant fibroblastic capsule and the infiltration and identity of inflammatory cells (Fig. 5) both in scaffolds and surrounding the thin films. The nature of the capsule in the rabbit model mirrored those performed in the rat model (Fig. 6). Cellular infiltration was generally greatest in the films with lower siloxane

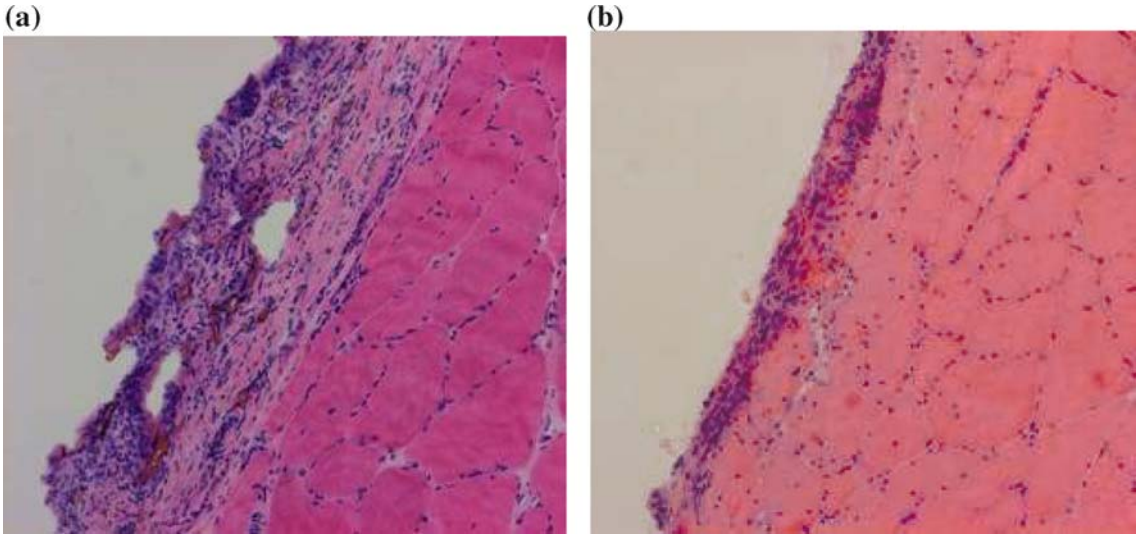

Figure 5 Histology of porous scaffolds, after 6 months intramuscular implantation in the rat: (a) S10 and (b) S80.

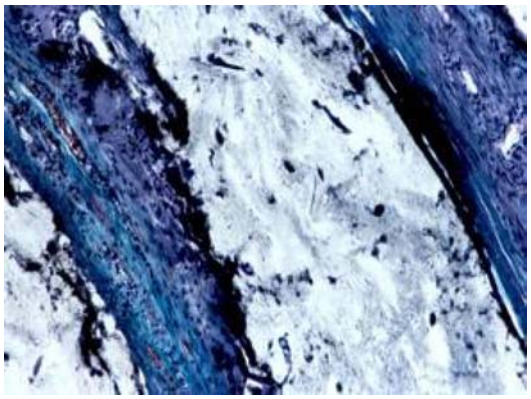

Figure 6 Non-porous S30 film after 12 month intramuscular implantation in a rabbit model.

concentrations. However there was no linear relationship, so that the cell number and fibrous layer increased over the range S0 to S30, then declined steadily over the range S40 to S100, such that the apparent response was much smaller in the high siloxane containing polymers than pure PEtU. The cellular recruitment was predominantly directed towards macrophages. At all time periods studied, both ED1 (immature cells and monocytes) and ED2 (CD163 positive, differentiating cells) positive macrophages were observed at high intensities, and in all formulations, although more intensely at low siloxane concentrations. Macrophage activation (MHC-I & MHC-II expression) was least in the higher siloxane variants (Fig. 7), suggesting that for these polymers, at least, the cells present were in a benign state, with

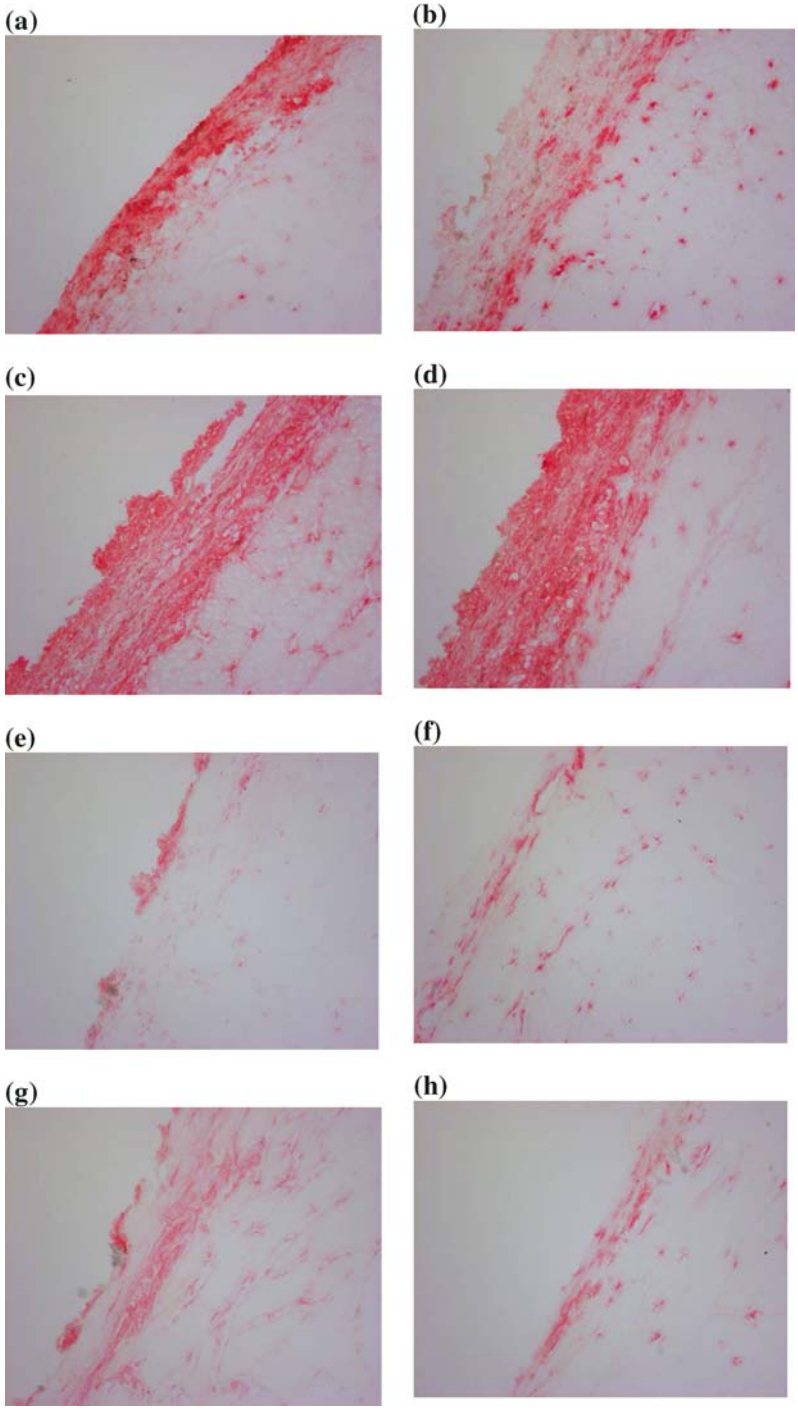

Figure 7 Response of S10 (a)–(d) and S80 (e)–(h) to intramuscular implantation in a rat model for 6 months: (a) and (e) ED1: immature macrophages and monocytes; (b) and (f) ED2: CD163 positive differentiating macrophages; (c) and (g) MHC-I positive; (d) and (h) MHC-II positive.

the PEtU—PDMS degradation products inducing little local stimulation of either the inflammatory or immune systems.

4. Discussion

Many tissue engineering scaffolds are chosen for their physical and processing properties and their degradation profile and often the mechanical properties are not fully considered, despite the importance of biomechanical signalling to the nature of the extracellular matrix that develops, and in turn its resultant mechanical properties. This has led to the common exploitation of fast degrading polymers such as PLLA, PLGA and P4HB, with the expectation that degradation could occur totally *ex vivo* prior to implantation. The hypothesis obviates the need to consider the host response to the scaffold completely. However, the nature of the extracellular matrix is likely to be compromised over such a time scale.

Modified polyurethanes are often chosen for construction of a medical device due to their durability. It is generally expected that the degradation products will produce at least some form of adverse host response. For this reason, we wanted to establish the link between degradation profile and inflammatory response *in vivo* to a series of polyurethane-derived polymers with similar chemistry and controlled biostability.

Whilst not explicitly stated, it generally perceived that softer implants are likely to induce smaller fibrous capsules due to the reduction in mechanical abrasion that results. Although this is generally true within this series, S30 produced a thicker fibrous capsule than did S0, S10 or S20. Interestingly, the capsule was not formed as a result of the degradation products either, since S30 was the most biostable formulation of the polymer series. Additionally, S80 and S100 degraded to a significantly greater degree than did S30, yet produced the weakest inflammatory response, when judged against the markers studied, suggesting that the degradation products of the interpenetrating PEtU—PDMS network were generally benign.

This points to the role of the adsorbed protein layer as the driver for the host response to this series of implants. The hydrophobicity of the material increased as siloxane was added to the material formulation and this is likely to have had a dramatic effect on the conformation of the adsorbed layers of protein, and also on the composition of the protein layers at the surface of the implant.

Whilst it is well known that the host response to biomaterials depends almost completely on the adsorbed protein layer for non-degradable implants, the more complex interplay between degradation products and adsorbed proteins is not at all established. The perceived situation would be that for urethane-containing materials, the more stable the composition of polymer, the less the impact on the inflammatory system, with degradation products being responsible for much of the observed reactions (e.g. phagocytic cell infiltration). This study completely negates that view, and for this series of polymers, certain variants (e.g. S80) of the faster degrading formulations result in a smaller inflammatory impact.

Whilst it is necessary to study the detailed inflammatory profile of this series of polymers in more depth, it is important to note that this may be the first of a generic series of tissue engineering scaffolds which can be regarded as successfully achieving the required specifications for strength and elasticity that are required for many cardiovascular applications.

5. Conclusions

It has been demonstrated that the degradation rate can be modulated in these polymers, materials containing 30% siloxane providing the longest degradation times. However, material with 80% siloxane showed the lower inflammatory response. It can be concluded that by varying the siloxane content in the PEtU matrix we can obtain an acceptable inflammatory response with a relatively short degradation time, suggesting that a standard medical-grade polyurethane can be chemically modified such that it is an excellent candidate scaffold for tissue engineering in mechanically challenging applications.

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