Acute 1L-1β Luciferase Signal and Immune Response to Surface Modified Polyurethane-Type Shape Memory Polymers: A Pilot Study

Xinying Cheng[1,4], Jian Fei[2,3], Lin Ye[4], Marcela MM Bilek[5] and Shisan Bao*[1,6]

[1]Discipline of Pathology and School of Medical Sciences, Bosch Institute, Australia

[2]School of Life Science and Technology, China

[3]Research Centre for Model Organisms, China

[4]Centre for Advanced Materials Technology, Australia

[5]Applied and Plasma Physics, Australia

[6]Tongren Hospital, China

*Corresponding author: Shisan Bao, Discipline of Pathology and School of Medical Sciences, Bosch Institute, Australia

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ABSTRACT

Polyurethane-type Shape Memory Polymers (SMPU) are promising biomaterials due to the ability to change shape by external stimulus. However, SMPU suffer from hydrophobicity and bio-inertness which restricts their possibilities as implantable biomedical applications. Plasma Immersion Ion Implantation (PIII) is a one-step covalent method to functionalise SMPU and to create a tuneable, biocompatible surface by attaching biomolecules. In this pilot study, collagen was used to immobilise on PIII treated SMPU surfaces and SMPU ± PIII and ± collagen were subcutaneously implanted in 1L-1β mice. Real-time 1L-1β luciferase signal was detected at day 1 and acute inflammatory responses at day 7 were determined using H&E staining histologically. The results indicate significantly less host immune responses to PIII treated SMPU with collagen coating. This pilot study provides a solid foundation for further acute/chronic study *in vivo*.

Keywords: SMPU; PIII; Collagen; Host Immune Response; Inflammation; Subcutaneous Implantation

Introduction

As one of smart materials, Shape Memory Polymers (SMP) have been investigated extensively for biomedical applications because of their shape memory properties [1], such as vascular stents, aneurysm occlusion devices, and clot extraction devices [2-5]. Polyurethane-type Shape Memory Polymers (SMPU), one of the most common SMP, have the advantage of a glass transition temperature closed to body temperature [6], facilitating the activation of shape memory recovery within the body. However, SMPU still suffer from inherent hydrophobicity and bio-inertness [7]. This may bring about immune reactions and poor tissue integration. Therefore, creating bioactive interfaces prior to implantation is of great importance [8].

Plasma surface treatments, such as Plasma Immersion Ion Implantation (PIII), are efficient methods to change surface properties without changing bulk properties, involving wettability, chemical structure, and hardness. These changes can contribute to the improvement of interactions between biomolecules and biomaterial surfaces [9]. Additionally, PIII enables one-step covalent attachment of biomolecules without the participation of any chemical reagents [10], facilitating the applications of SMPU in biomedical industry easily. It has been documented that protein can be covalently immobilised on PIII treated polymer surfaces, leading to the improvement of cell adhesion, proliferation, and viability on the interfaces [11-13]. In our previous study, covalent

immobilisation of collagen has been observed on PIII treated SMPU surfaces *in vitro* [14]. Therefore, we aim to conduct a pilot study to study biocompatibility of SMPU ± PIII and ± collagen *in vivo*. The acute 1L-1β luciferase signal was detected in real time and acute inflammatory response to the implanted SMPU was determined at the histopathological level.
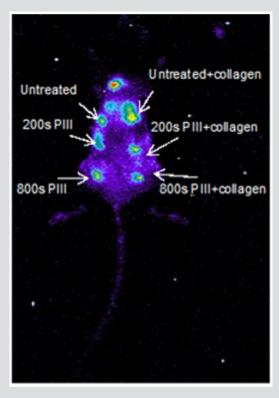
Materials and Methods

Polyurethane-type Shape Memory Polymers (SMPU) were purchased from DiAPLEX Co., Ltd (Tokyo, Japan). SMPU incorporates hard segments of 4, 4' Diphenylmethane Diisocyanate (MDI) and polyether type soft segments of polypropylene glycol and polyethylene glycol, as described [15].

Plasma Immersion Ion Implantation (PIII) was carried out at a pressure of 2×10^{-3} Torr with an RF nitrogen plasma powered at 100 W and accelerated by high voltage pulsed bias of 20 kV for pulse durations of 20 μs at a frequency of 50Hz, as described previously [16]. The SMPU was treated for durations of 200 s and 800 s, corresponding to ion implantation fluences of 2.5×10^{15} ions/cm^2 and 1×10^{16} ions/cm^2 respectively [14]. PIII treatment was performed on SMPU samples 10 days prior to implantation and stored at room temperature. Collagen type I from rat tail was purchased from Sigma-Aldrich (cat. No. C3867, Sydney, Australia) and diluted to 10 μg/ml with PBS. Six types of SMPU samples (1.5 cm × 2 cm) were prepared:

a)   untreated SMPU,

b)   200 s PIII treated SMPU,

c)   800 s PIII treated SMPU,

d)   collagen coated untreated SMPU,

e)   200 s PIII treated SMPU with collagen coating, and

f)   800 s PIII treated SMPU with collagen coating.

SMPU samples (diameter = 4 mm, thickness = 0.5 mm) were implanted in six subcutaneous pockets at the back of IL-1β luciferase mice (the Research Centre for Model Organisms, Shanghai, China), and all samples were sterilised using UV light in a biosafety cabinet for 15 min prior to implantation. Real time 1L-1β luciferase signal was detected using MAG Biosystems Lumazone imaging system. The harvested implants included surrounding tissues and capsules (1.5 cm × 1.5 cm) were collected at day 7 for histological analysis. Total cell number in the capsule surrounding SMPU implants was determined using H&E staining and analysed using one-way ANOVA in in GraphPad Prism Version 7.0 (GraphPad Software, CA, USA). This experiment has been approved by the University of Sydney Animal Ethics Committee (protocol number K20/12-2011/3/5634) and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purpose.
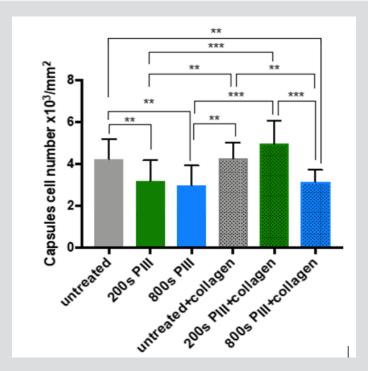

Figure 1: 1L-1β luciferase signal detected in SMPU at day 1 post implantation: untreated SMPU, 200s PIII treated SMPU, 800 s PIII treated SMPU, untreated SMPU with collagen coating, 200 s PIII treated SMPU with collagen coating, and 800 s PIII treated SMPU with collagen coating.

Results and Discussion

Figure 1 shows the real-time 1L-1β expression of SMPU implants in the back of mice at day 1. Compared to other samples, untreated SMPU with collagen coating had a substantially higher level of acute immune response. Smaller 1L-1β areas were observed in both SMPU with 200 s/ 800s PIII treatment and collagen coating than those with PIII treatment only, indicating less acute immune response to PIII treated SMPU with collagen coated. Figure 2 shows total cell numbers in capsules surrounding SMPU implants using H&E staining at day 7. Both 200 s PIII treated SMPU and 800 s PIII treated SMPU had significantly lower total cell numbers than untreated SMPU by 25.1% ($p < 0.01$) and 24.3% ($p < 0.01$),

respectively. These two PIII treated SMPU also had lower total cell numbers than untreated SMPU with collagen coating by 25.4% ($p < 0.01$) and 24.6% ($p < 0.01$), respectively. There was no significant difference between SMPU with 200 s PIII treatment and 400 s PIII treatment, while collagen coated SMPU with PIII treated for 200 s had a higher total cell number than collagen coated SMPU with 800 s PIII treatment ($p < 0.001$). This can be attributed to that more collagen were immobilised on the 800 s PIII treated SMPU than on the 200 s PIII treated SMPU. In addition, no significant difference was observed between untreated SMPU and collagen coated untreated SMPU, which might be due to collagen replaced by other proteins in extracellular matrix.

Figure 2: Total cell numbers quantified in capsules surrounding SMPU implants at day 7 post implantation, *p < 0.05, **p < 0.01, ***p < 0.001.

Conclusion

By comparing SMPU ± PIII and ± collagen implants in vivo, significantly lower acute inflammatory responses were observed in PIII treated SMPU with collagen coating than other untreated or single treated (either PIII treatment or collagen coating) at day 7. This pilot study provides a solid foundation for further acute/ chronic inflammation study of SMPU as implantable biomedical applications.

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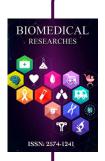
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