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Selective Immobilization of Biomolecules on PTMC Network Surfaces Using Micro Contact Printing

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Summary: Using poly(trimethylene carbonate) (PTMC) network surfaces containing unreacted macromer methacrylate groups, thiol-functionalized biotin (SH-undecyl-biotin) can be bound to the surface. With micro contact printing techniques and making use of the biotin-streptavidin (SAv) binding system patterned surfaces with biological functionality can be prepared in a very straightforward manner. This method has high potential in numerous biomedical applications.

Keywords: microcontact printing; PTMC networks

Introduction

In the past years, microcontact printing (μCP) has emerged as a simple method for the preparation of patterned surfaces. The process in which a textured elastomeric stamp is inked with a molecule of interest, which is then transferred to a substrate surface, provides a fast way of obtaining surfaces to which these molecules are coupled in a desired pattern.[1,2] These systems are very versatile, and a variety of molecules can be immobilized.[3,4] Currently, conventional glass is commonly employed as a substrate for immobilizing biomolecules following e.g. biotin-streptavidin coupling schemes.[2] PTMC networks are polymers with good mechanical properties (even at low molecular weights), easy to process and biocompatible, demonstrating high potential for implantable biological applications, unlike glass. Furthermore, by using PTMC surfaces with high density of free methacrylates, it is possible to successfully couple biotin and streptavidin by means of simple reactive micro contact printing techniques (Figure 1). With this simple, straightforward method, functional PTMC networks surfaces can be produced, allowing a subsequent successful biotin – streptavidin coupling to the surface. Additionally, this method allows the possibility of creating a ''chain'' of assembled molecules of interest, with high potential for biomedical applications.

Experimental Part

A liquid resin based on three-armed PTMC (Figure 2) macromers (MW = 8000 g/mol) was prepared as described elsewhere.[5] The resin (1% wt in $CHCl_3$) was spin coated on a piranha-cleaned glass microscope slide (4000 rpm, 30 s) and a homogeneous film with an approximate thickness of 70–80 nm was obtained. Next, the PTMC macromer film was then partially crosslinked by exposure to UV light (365 nm) for 5, 10, 15 and 30 minutes.

Afterwards, a featured PDMS stamp (with 100 μm diameter dots and 20 μm spacing) was inked for 1 minute with a 1 mM solution of SH-undecyl-biotin

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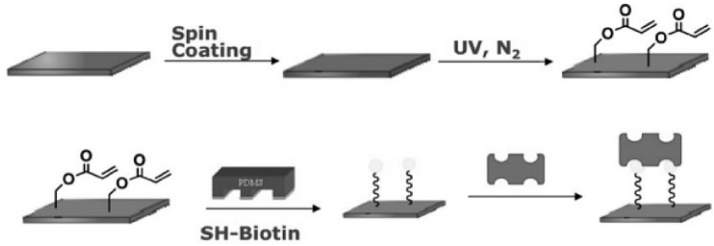

Figure 1.
Preparation of patterned structures by reactive micro contact printing of SH-undecyl-biotin onto unreacted methacrylate groups via Michael addition, followed by incubation with Cy2-streptavidin.

((3aS,4S,6aR)-11-Sulfanylundecyl 5-(Hexahydro-2-oxo-1H-thieno [3,4-d] imidazol-4t-yl) pentanoate) in DMF. After blowing the stamp dry with N_2, it was brought into conformal contact with the PTMC network surface. To obtain appropriate biotin immobilization conditions, several contact printing times were investigated. After stamp removal, the surface was carefully washed with PBS to remove excess of SH-undecyl-biotin and was immediately incubated for 1 hour with a fluorescently labelled streptavidin solution (SAv-Cy2) 0.1 μM in PBS. After several washing steps with PBS and water, the surfaces were gently dried with N_2 and the pattern was visualized by fluorescence microscopy. As a control, 11-Mercapto-1-undecanol was prepared and the same streptadivin incubation protocol was applied. On the control surface, this molecule (11-Mercapto-1-undecanol) has an SH group on one side that can react with the free methacrylates present on the polymer surface. However, on the other side, the reaction with streptavidin is not possible. Therefore, with this control one can prove that the appearance of the pattern is exclusively due to the streptavidin-biotin interaction.

Results

The ability to strongly immobilize proteins and other biomolecules onto substrates can present a useful advantage; in particular the functionalization of inorganic surfaces with organic molecules for various applications

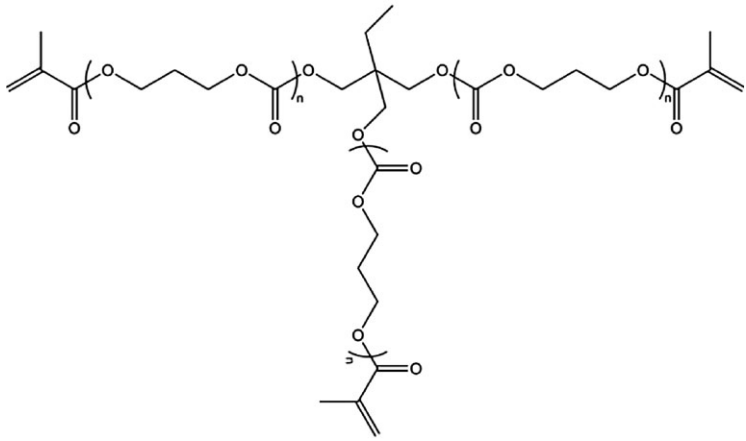

Figure 2.
Three-armed poly(trimethylene carbonate) (PTMC) macromer structure from which the PTMC networks were prepared.

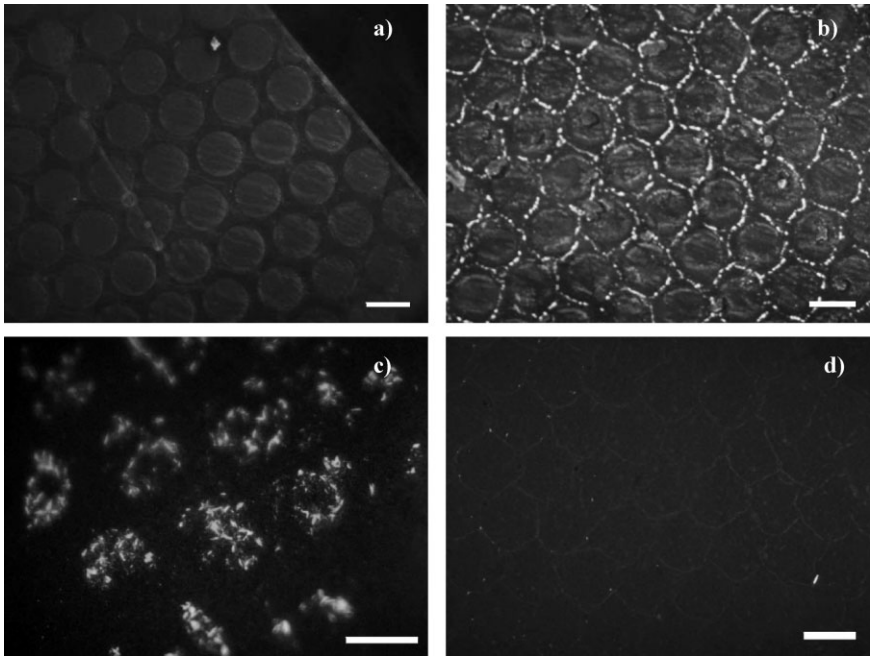

Figure 3.
Fluorescent images obtained upon a) 10 min UV exposure of PTMC macromer and 15 min biotin printing, b) 15 min UV exposure of PTMC macromer and 15 min biotin printing, c) 30 min UV exposure of PTMC macromer and 15 min biotin printing, d) Control PTMC surface, where 11-Mercapto-1-undecanol was printed and then followed by incubation with fluorescent streptavidin (SAv-Cys2). Scale bar = 100 μm.

such as sensors, electronics and biotechnology.[6]

Employing simple micro contact printing techniques, it was possible to successfully immobilize SH-undecyl-biotin to a PTMC networks surface (Figure 3a, b and c). First, the PTMC networks surfaces were partially exposed to UV and several crosslinking times were investigated to determine the best substrate conditions, ensuring the presence of free methacrylate groups. Next, the SH-undecyl-biotin printing conditions were explored by varying the printing time. All the results were compared by means of fluorescence imaging.

When the crosslinking time is extensive, less SH-undecyl-biotin can be coupled to the surface, leading to a decrease in streptavidin binding (Figure 3c), whereas with shorter crosslinking times (5 minutes or less) the surfaces were not suitable for manipulation. On the other hand, for the SH-undecyl-biotin printing, it was found that shorter printing times led to ineffective printing on the surface, whereas longer times exhibited some streptavidin smear on the surface. After selecting the best crosslinking/printing conditions, functional PTMC surfaces were prepared. It was found that the best conditions for biotin coupling and stamping were 15 minutes of biotin printing with PTMC crosslinking times of 10 (Figure 3a) and 15 minutes respectively (Figure 3b). The streptavidin was successfully coupled to biotin after 1 hour incubation period with the surface, where well defined structures were printed and can be observed in Figure 3.

Conclusion

A fast and flexible method for the selective immobilization of biomolecules by micro contact printing on PTMC networks surfaces was developed. By using simple

contact printing methods, the selective immobilization of biomolecules on partially crosslinked PTMC network surfaces was achieved. This method can be considered as a new strategy to pattern biomolecules on PTMC networks surfaces and presents significant advantages over the conventional spotting technologies. It is very easy to implement, it is cost effective, and allows a precise control over the surface's architecture. PTMC networks are polymers with good mechanical properties (even at low molecular weights), easy to process and biocompatible, demonstrating high potential for implantable biological applications.

This fact leads to the possibility of coupling several molecules of interest to a PTMC network surface for specific cell attachment or for drug delivery applications.

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