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Integration of Membranes in 3-D Biodegradable and Biocompatible Microfluidic Bioreactors

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Abstract- In the last two decades, a multidisciplinary approach on the delivery of cells to the body and the development of neo-tissue has advanced. On the one hand, the science of polymers focuses on the development of biocompatible materials for cell culturing and tissue engineering applications, while on the other hand (micro-) engineering has an important part in providing a specific topography that directs the cells and provides a more similar architecture to that of tissues. Soft and flexible elastomers have gained more and more interest, especially due to their biodegradability and tunable mechanical properties that can mimic the natural tissue. The class of aliphatic polyester elastomers, amongst which poly (glycerol sebacate) is a very representative material, has grown considerably. Herein, we report the design of novel polyester elastomers that are synthesized from monomers found within the human metabolism. The thermoset properties of these polymers as well as their optical transparency make them ideal materials for microsystems technology. Therefore, microfluidic devices were developed out of these polymers in order to facilitate the design of a tissue-engineered organ. A porous membrane was inserted in the microfluidic device to enable co-culture and distribution of nutrients to the cells. Furthermore, the reported polymers and microfluidic structure can serve a multitude of applications, from tissue engineering to point-of-care diagnostics or compound screening.

***Keywords*:** aliphatic polyesters, elastomers, three-dimensional, membrane, microfluidics, cell culture

1. Introduction

The field of tissue engineering, pioneered by Robert Langer and his co-workers, combines mainly 3 different disciplines that have the same goal: to regenerate or repair specific body tissue. Amongst these disciplines, material science or polymer science deals with the synthesis and characterization of materials that are biocompatible with the human body and even biodegradable if required by the specific application. Engineering, on the other hand, has the purpose of designing the topography of the biomaterial such that it provides a good environment that resembles the internal structure of the respective organ. And last but not least, biology and medicine provide knowledge on how to grow certain cells into a specific tissue. Synthetic thermoset elastomers such as poly (glycerol sebacate) and other poly (polyol sebacates) (Bruggeman *et al.*, 2008) have become one of the most favored materials for tissue engineering

due to their biodegradability and wide range of mechanical properties that are similar to that of the native tissue (Rai *et al.*, 2012).

In the present paper we report the synthesis of new poly (polyol sebacate)-derived elastomers, where the polyols are glycerol and erythritol. The mentioned monomers are compounds also found in the human metabolism, therefore they are considered biocompatible and biodegradable. Based on all these characteristics, the obtained polymers are good candidates for tissue engineering applications. Microfabrication technology is an interesting tool used to impart a specific topography to these materials (Sackmann *et al.*, 2014). Microfluidic bioreactors have been mostly developed by bonding a patterned layer on top of a flat one (Fidkowski *et al.*, 2005) or by stacking and bonding single-layer microfluidic networks (Bettinger *et al.*, 2006). Nevertheless, microfabrication is fundamentally a 2-dimensional (2-D) technique as it creates patterns on 2-D surfaces (Ryu *et al.*, 2006; Cheng *et al.*, 2015). The present work proposes the integration of a porous membrane inside a microfluidic device, in between 2 micro-aligned patterned layers. From the best of our knowledge, this is a unique approach that is intended to mimic the extracellular matrix (ECM) required to have proliferating cells. The membrane will impart the 3-dimensionality that cells need to grow, while the microchannels will guide the cells and align them in a specific way as to better mimic the native tissue.

2. Methods

2. 1. Synthesis of Elastomers

All reagents were purchased from Sigma-Aldrich and used as received, unless stated otherwise. The monomers used were sebacic acid, sugar alcohols and small or long chain diols. The molar ratio was varied in order to tune the mechanical properties of these materials. The reagents were mixed in a reactor at 130 °C for different reaction times. The pre-polymer was crosslinked on alginate coated molds after the synthesis in order to obtain films. The films were characterized from a physico-chemical and mechanical perspective.

2. 2. Silicon Master Fabrication

Patterned silicon wafers were processed by standard lithographic processes (Borenstein *et al.*, 2002). Briefly, clean 4-inch silicon wafers were spin coated with SU-8 100 (Microchem Co.) and exposed to UV light in an aligner through a mask. The mask was designed using AutoCAD 2008 and printed on a transparency. The exposed photoresist was then developed and baked for 1.5 hours at 120 °C.

2. 3. Bioreactor Development

The micro-pattern of the silicon master was transferred to the synthesized materials. The patterned polymer layers were aligned and bonded. In between these patterned layers, porous membranes were inserted in order to create proper 3-D conditions for cell culturing. The devices were cut using a proprietary designed vertical cutter and the cross section was evaluated using scanning electron microscope (SEM).

3. Results and Discussion

3. 1. Synthesis Of Elastomers

Poly (polyol sebacates) are thermoset materials and possess several characteristics that make them a good choice for developing microfluidic bioreactors. Amongst these features, we mention optical transparency that is especially important during the alignment of two patterned microfluidic layers. The tunability of the mechanical properties, making these materials range from very elastic to very rigid, together with their tunable degradation time are important characteristics that were considered when choosing to synthesize these materials (Figure 1).

The monomers used in the synthesis, namely sebacic acid, glycerol, and erythritol are intrinsic to the human body. Furthermore, sebacate polymers have already been approved by the U.S. Food and Drug

Administration (FDA) for use in tissue engineering and implant applications in general. After synthesis all pre-polymers (oligomers) obtained have a low molecular weight between 3000 g/mol and 18000 g/mol and, at this point, they could still be melted and cured into a desired shape. After the curing process, the polymers ranged from flexible to more rigid elasticity which is verifiable through the Young's modulus that varies between 0.6±0.14 MPa and 4.3±0.06 MPa. Therefore, the mechanical testing demonstrated the tunability of this type of elastomers. The advantages of these materials include the fact that they are biodegradable, pliable, they can be easily processed through different scaffolding techniques, and the synthesis is fast, straightforward and can be transferred cheaply to industrial production.

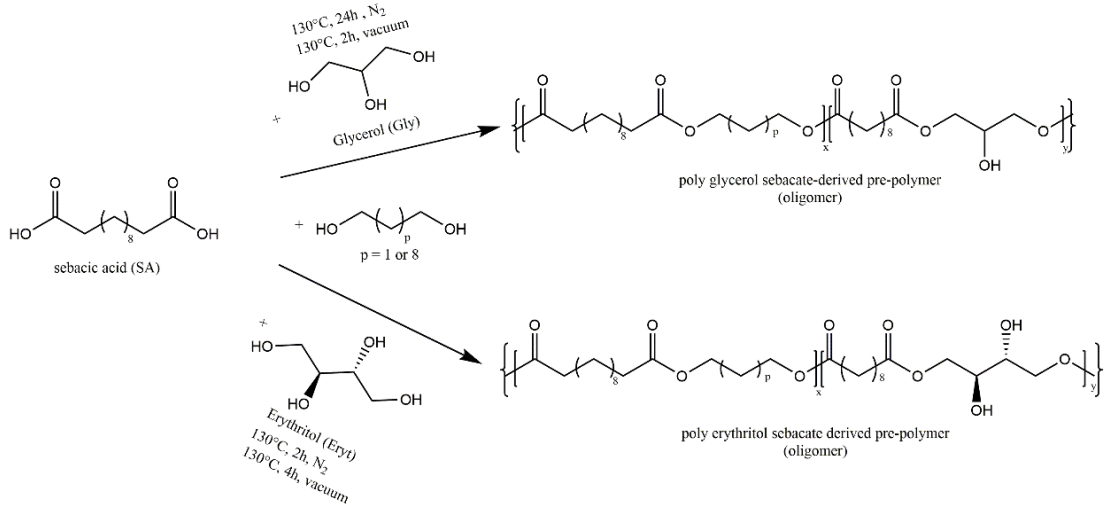

Fig. 1. Schematic representation of the material synthesis

3. 2. Bioreactor Development

Up to now only single-layer microfluidic systems were stacked together in order to create microfluidic networks out of biodegradable polymers such as poly(glycerol sebacate) (PGS). Although multiple layers are present, the stacking does not offer a real 3-D environment as the cells will still grow in 2-D channels. In the current work we have aligned and bonded two patterned microfluidic layers in between which a porous membrane is inserted (Figure 2A, B). This could impart the 3-D functionality that cells need in order to grow and proliferate into a more complex tissue.

The patency of the microfluidic network was evaluated by injecting a solution of blue ink with water through the microchannels. Teflon tubes go through the inlet and outlet chambers, and are sealed by using medical grade silicone glue. The perfused system (Figure 2C) presented no leaks and the bonding between the microfluidic layers was sufficiently strong to allow perfusion flow rates ranging from 0.1 µl/min to 200 µl/min. Higher flow rates were not used, as cells would be subjected to shear stresses that might be harmful for their development and proliferation. *In vitro* compatibility tests are currently ongoing to determine the (non-)cytotoxicity of these materials. Tests with different cell lines will also be considered for the obtained bioreactors.

4. Conclusion

We have successfully synthesized novel biodegradable elastomers with tunable mechanical properties and applied them in the development of microfluidic devices. Up to now, most cell and tissue culturing is done at best using flat uniform biodegradable layers, or 2-dimensional (planar) microsystems. As cells tend to flatten or change their morphology on 2D surfaces, and as complex tissues contain different cell types that have to interact, we have inserted a porous membrane in the elastomeric microfluidic devices. Therefore, the key feature of the presented microsystems is that they contain a 3-dimensional environment

(i.e. given by the porous membrane), which can be suitable for cell culturing and complex tissue engineering applications. Via this way, cells of different types can be cultured together and aligned according to their *in vivo* model. Future work will include *in vitro* characterization studies using the obtained devices.

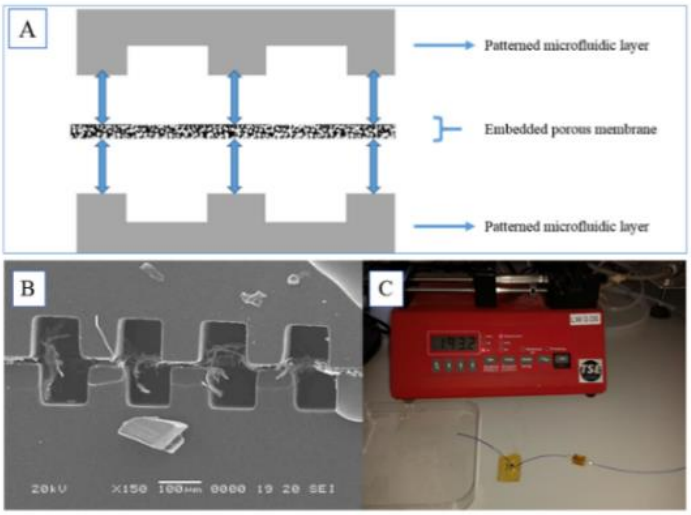

Fig. 2: Three-dimensional environment in a microsystem; A – schematic representation of the microfluidic design; B – cross section of a microsystem with multiple channels in a poly (erythritol sebacate)-derived polymer; C – perfusion system used for the bioreactor.

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