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Analysis of the biocompatibility of perfluoropolyether dimethacrylate network using an organotypic method

Rachid Jellali [a,*], Jean-Luc Duval [a], Eric Leclerc [a,b,*]

[a] CNRS UMR 7338, Laboratoire de Biomécanique et Bioingénierie, Sorbonne Universités, Université de Technologie de Compiègne, France
[b] CNRS UMI 2820, Laboratory for Integrated Micro Mechatronic System, Institute of Industrial Science, University of Tokyo, 4-6-1 Komaba, Meguro ku, Tokyo 153 8505, Japan

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A B S T R A C T

In this work, we have investigated the potential of perfluoropolyether (PFPE) polymers for use in biomaterial applications, especially in cell culture and tissue engineering. PFPE substrates were synthesized by the photocuring of liquid PFPE urethane dimethacrylate. These surfaces were then modified by ECM protein coatings and microstructuration, to promote cell adhesion and migration. The surface properties of PFPE and PDMS (used as a reference) samples were studied by static contact angle measurements and AFM imaging. Both polymer surfaces were hydrophobic, having sessile air–water contact angles superior to 100°. Collagen and fibronectin coatings were found to change the wettability of PFPE and PDMS samples. The biological testing of substrates was done using a liver organotypic culture to evaluate the migration and density of liver cells. The results over seven days of culture demonstrated that the migration and density of cells cultured under untreated PFPE were higher than the migration and density of cells cultured under PDMS. ECM protein coatings enhanced cell migration from liver explants cultured on PFPE or PDMS. Furthermore, these coatings were more efficient in the case of a PFPE sample. From a second series of tests, in which the PFPE was microstructured, it was found that microstructures promoted the formation of a 3D cell layer. These results indicate that PFPE polymers have a potential for use in the development of biomaterials for tissue engineering and cell culture.

1. Introduction

Biomaterials, which are used for tissue replacement or as substrate in contact with living tissue and/or cells, must satisfy criteria such as biocompatibility, mechanical compliance and chemical stability. They are classified into four groups: polymers, metals, ceramics and composites [1]. During the last decades, the development of polymeric biomaterials has continuously grown. Today, because of their easy manufacturability, low cost and adequate mechanical and physical properties, polymers are widely used in tissue engineering, proteomics, prosthetics, regenerative medicine, microfluidic biochips and drug delivery systems [1–4]. Polymeric biomaterials have the advantage of being easily modified to meet the desired biomedical application. Due to the progresses in material science and bioengineering, the panel of the type of surface modifications is more and more wider, including photopatterning, surface grafting of functional groups, plasma treatment or coating with extracellular matrix proteins such as fibronectin, vitronectin, collagen or laminin [5–10].

Fluoropolymers are particularly interesting materials because of their versatility and their unique properties originating from the strong C—F bonds [11,12]. These materials exhibit high thermal stability, chemical resistance to acids, bases, oxidizing and reducing agents, and most solvents, resistance to ageing and weather, low inflammability and low surface energy [11,12]. Some fluoropolymers such as polyvinylidene fluoride (PVDF) and polytetrafluoroethylene (PTFE) are biocompatible and used in medical applications [13]. However, the high inertness of fluoropolymers makes it difficult to fabricate microstructures and 3D scaffolds from these materials and limits their use in tissue engineering applications [11].

Perfluoropolyethers (PFPEs) constitute another class of fluoropolymers interesting for a variety of applications because of their diverse characteristics. Thus, in addition to the common properties of fluoropolymers (cited above), PFPEs present diverse interesting characteristics such as very low glass transition temperatures, low modulus, optical transparency, gas permeability, biocompatibility and long-term biostability [13–20]. Contrary to other fluorinated polymers, PFPE materials are easily patternable by direct photolithography or by a replica molding process and offer the opportunity to build regular and precise structures [14,17,18,21,22]. These good properties suggest that PFPEs would be advantageous as biomaterials. Nevertheless, due to its hydrophobicity, PFPEs have been mostly used in ophthalmic

* Corresponding author at: CNRS UMR 7338, Laboratoire de Biomécanique et Bioingénierie, Sorbonne Universités, Université de Technologie de Compiègne, France.
E-mail addresses: rachid.jellali@utc.fr (R. Jellali), eric.leclerc@utc.fr (E. Leclerc).

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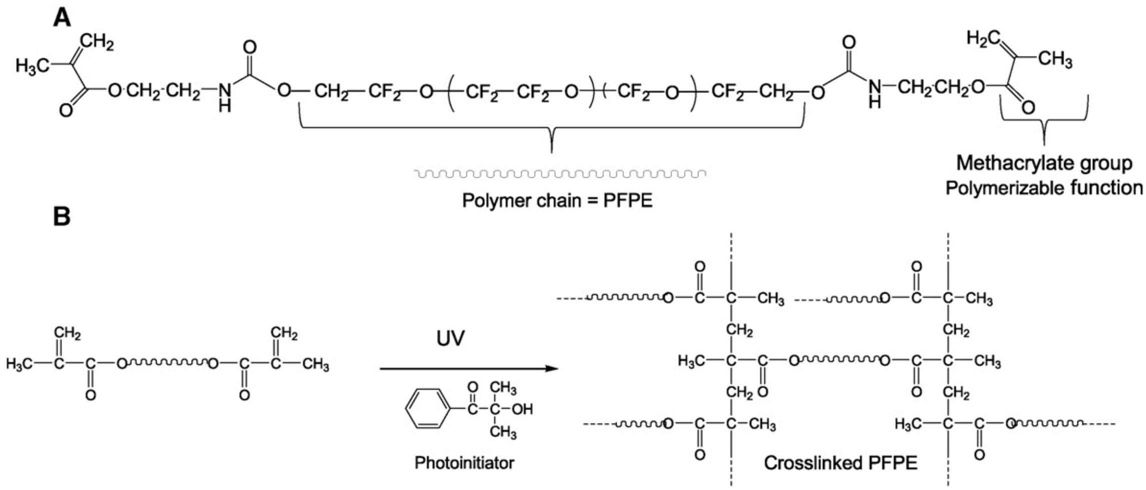

Fig. 1. (A) Chemical structure of perfluoropolyether (PFPE) urethane dimethacrylate (Fomblin MD40) and (B) reaction scheme of PFPE dimethacrylate crosslinking.

applications for corneal implant or contact lens [20,23–26], and their use in cell culture and tissue engineering remains very limited.

In this work, we studied the potential to extend the use of perfluoropolyether (PFPE) polymers as biomaterials for cell culture and tissue engineering. In fact, PFPE properties such as optical transparency, gas permeability, biocompatibility and long-term biostability, make this polymer a good candidate to build a cell bioreactor. To validate this hypothesis, we have compared the PFPE with a material widely used for cell culture, the Thermanox®, and a biomaterial used to build biochips, the polydimethylsiloxane (PDMS). First, the surface properties of PFPE and PDMS, with and without protein coatings, were investigated by contact angle measurement and AFM imaging. Second, the cell proliferation and migration from liver explants cultivated onto the pristine substrates and the substrates coated with fibronectin and collagen were studied. We have chosen to work with excised liver tissue samples from embryonic chick explants. The technique is based on chick embryo organotypic culture [6,27–31]. Organotypic culture represents an *in vitro* model that maintains the *in vivo* cellular interactions and allows tissues to keep a 3D multicellular organization. This method allows a rapid estimation of biocompatibility parameters such as adhesion, growth, proliferation and morphology using primary cells of targeted organs for each specific application (corneal tissues for eye implantation devices and titanium for bone repair devices for instance) [32]. Finally, the effect of PFPE surface topography on the behavior of embryonic liver was evaluated in this study as our final target is regeneration of liver.

2. Materials and methods

2.1. Preparation of polymer samples

PFPE samples were synthesized by UV photopolymerization of perfluoropolyether (PFPE) urethane dimethacrylate (Fomblin MD40, Solvey Solexis, Milan/Italy) (Fig. 1). Fomblin MD40 was mixed with 1 wt.% of a photoinitiator (2-hydroxy-2-methyl-1-phenyl-propan-1-one: Darocur 1173, Sigma-Aldrich) and deposited in a mold. The reactive UV-curable mixture was dried in a vacuum and cured for 10 min by means of a UV-KUB lamp (LED UV 365 nm, Kloe SA/France). The PDMS substrates were obtained by thermal curing of polydimethylsiloxane (Sylgard 184, Dow Corning, Midland/USA). Sylgard 184 contains two parts, a base and curing agent that are mixed in a 10:1 w:w ratio and cured for 2 h at 75 °C.

Structured PFPE was fabricated by a replica molding process using a PDMS master containing the positive pattern. The positive PDMS master was obtained from an SU-8 mold fabricated with standard photolithography [33]. Firstly, the Sylgard 184 mixed at a 10:1 ratio with the curing agent, was poured onto the SU-8 mold, degassed in a vacuum and cured at 75 °C for 2 h. The peeled negative PDMS stamp was then treated by air plasma (Harrick plasma cleaner) and silanized with the vapor of trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma-Aldrich) for 2 h in a desiccator [34]. Silanization of a positive PDMS stamp is necessary to prevent PDMS-to-PDMS irreversible bonding. Secondly, Sylgard 184 was poured onto the silanized PDMS stamp, degassed in a vacuum

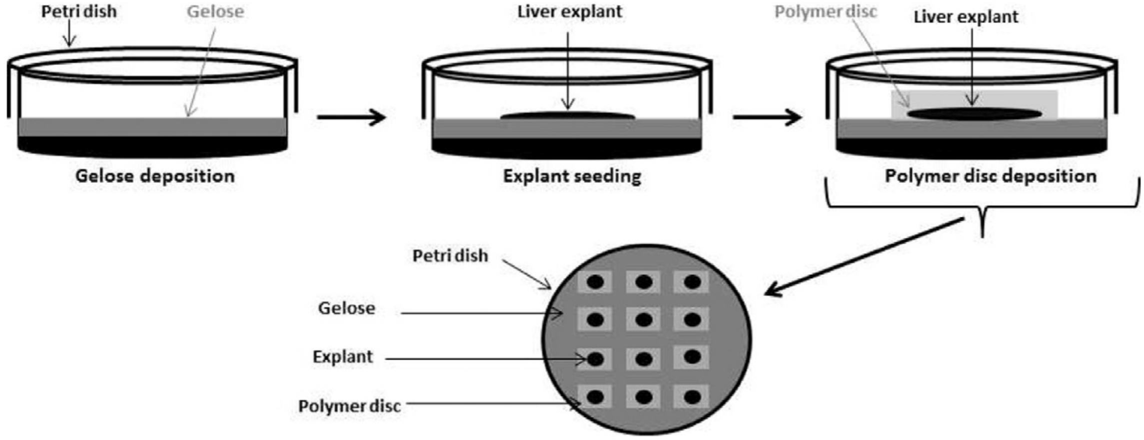

Fig. 2. Principle of the organotypic culture technique.