

Material performance

Poly(vinylidene fluoride) and copolymers as porous membranes for tissue engineering applications



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ABSTRACT

Poly(vinylidene fluoride) (PVDF) and its main copolymers - poly(vinylidene fluoride-co-hexafluoropropene), P(VDF-HFP), and poly(vinylidene fluoride-co-trifluoroethylene), P(VDF-TrFE) - were processed by solvent casting at room temperature in the form of porous membranes. Copolymer membranes showed higher degree of porosity than PVDF, the average pore size being larger for P(VDF-TrFE) than for P(VDF-HFP) and PVDF. All membranes show high hydrophobicity with water contact angles in the range 94° to 115°, and electroactive beta phase contents above 90%. The adhesion and proliferation of both C2C12 myoblast and MC3T3-E1 pre-osteoblast cells on the membranes were investigated. It is demonstrated that PVDF membranes promote higher cell proliferation while P(VDF-HFP) membranes show the lowest proliferation for both kinds of cell. The proliferation on P(VDF-TrFE) membranes is cell dependent, higher for MC3T3-E1 cells but lower for C2C12 cells, related to the effect of the highly porous structure on the preferred morphology of each cell type, as the higher pore size and porosity of the P(VDF-TrFE) membrane induce cell elongation, which is preferred just by the C2C12 muscle cells.

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1. Introduction

Tissue engineering (TE) is continuously evolving as an exciting and multidisciplinary field aiming to develop biological substitutes to restore, replace or regenerate defective tissues [1]. Successful tissue engineering generally relies on two essential elements: cells and scaffolds, scaffold design being, therefore, a key aspect of TE.

Scaffolds are primarily designed to support cell colonization and formation of tissue, mimicking the tissue extracellular matrix [2]. Scaffolds for TE should meet several design criteria with respect to geometry, microstructure and physicochemical properties. The scaffold should be designed according to the structure of the tissue into which it is to be implanted [3]. An ideal scaffold should be: (i) biocompatible; (ii) bear the proper shape; (iii) appropriate mechanical strength depending on the desired application; and (iv) an extensive network of interconnected pores for cell grow and proliferation [4]. Synthetic polymers are more frequently considered in the preparation of polymer scaffolds, as their properties can

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be finely tuned for the desired application [4,5]. These scaffolds are typically required to be biodegradable for medical implants or related applications [6] but not necessarily so for cell culture studies, expansion or differentiation prior to medical use of the cells [7,8].

Within this approach, quasi-two dimensional membranes can also play an important role in manipulating cell functions and, depending on the type of cells and the specific application, quasi-two dimensional membranes are even required [9,10]. Pore size, pore interconnectivity and total porosity are essential features of these membranes, pore size influencing cell adhesion *in vitro* and ability to colonize the scaffold [11], also affecting cell morphology and phenotypic expression [12]. In this way, biocompatible but not necessarily biodegradable quasi-two dimensional membranes are used for specific cell proliferation and differentiation studies or before medical implantation [8].

Biomedical applications based on fluorinated polymers have recently attracted much interest due to their unique properties, including high dielectric constant, chemical resistance, lubricity, sizing tolerance for device fabrication and biocompatibility. Poly(vinylidene fluoride) (PVDF) and its copolymers have been further recognized as important and unique materials for biomedical applications due to their high electroactive response, showing the highest piezo, pyro and ferroelectric responses among polymeric materials. In particular, piezoelectricity, the ability to convert mechanical signals into electrical ones and *vice-versa*, allows the development of smart scaffolds to stimulate cell growth and differentiation of specific tissues undergoing electro-mechanical stimuli in their function, such as bone and muscle tissues, as well as the fabrication of devices such as biosensors and mechanical sensors and actuators [13,14].

PVDF is a semi-crystalline polymer and exhibits five distinct crystalline phases: α , β , δ , γ and ϵ . The β -phase (Fig. 1) is the one with the largest interest for technological applications due to its electroactive properties: piezoelectric, pyroelectric and ferroelectric. This phase is obtained with a porous microstructure directly by solution casting with solvent evaporation and crystallization temperatures below 70 °C. Two of the most relevant co-polymers of PVDF are poly(vinylidene fluoride-co-trifluoroethylene) (P(VDF-TrFE)) and poly(vinylidene fluoride-co-hexafluoropropene) (P(VDF-HFP)) (Fig. 1) [14]. P(VDF-TrFE) shows a Curie temperature (T_c) below melting temperature (T_m) and crystallizes in the ferroelectric β -phase regardless of

processing technique, melt or solution casting, for VDF contents between 50 and 80%. P(VDF-HFP) (Fig. 1) is also a semi-crystalline polymer with lower degree of crystallinity than PVDF and with strong ferroelectric properties when samples are prepared by solvent casting [14].

The main differences between the copolymers with respect to their piezoelectric properties are the following [14]: d_{31} (pC/N) is between 8 and 22 for PVDF, 12 for P(VDF-TrFE) and 30 for P(VDF-HFP) and d_{33} (pC/N) is between –24 and –34 for PVDF, –38 for P(VDF-TrFE) and –24 for P(VDF-HFP).

PVDF d_{33} and d_{31} values are overall comparable with the ones observed for the copolymers, however PVDF presents generally higher degree of crystallinity, which results in higher electroactive β -phase content in the sample. Further, the surface morphology of polymer and copolymers are generally different in terms of spherulite size and organization [15].

As previously mentioned, cell-material interactions play critical roles in the success of scaffolds for tissue engineering, since chemical and physical cues of biomaterials regulate cell adhesion, migration, proliferation and differentiation [16]. Several tissues and cells are responsive to electrical fields and stimuli [17], such as bone [18], nerves [19] and cardiac and skeletal muscle [20,21].

Some studies have demonstrated the influence of pore size and degree of porosity of some membranes on cell proliferation for tissue engineering applications [22–24]. The optimal pore size of porous membranes for MC3T3 cell proliferation is still controversial [10,22,25]. To our knowledge there are no studies using PVDF and copolymers porous membranes for C2C12 and MC3T3 cell proliferation studies despite the fact that the electroactive properties of these membranes are interesting for muscle and bone regeneration [26,27]. Due to the similar electroactive properties of the materials, the use of different copolymer will allow study of the influence of the different morphologies and surface energy of the material on cell response.

Therefore, this study is devoted to evaluate comparatively the suitability of PVDF, P(VDF-TrFE) and P(VDF-HFP) membranes for tissue engineering applications by studying the adhesion and proliferation of both C2C12 and MC3T3-E1 cells. This study is relevant due to the aforementioned interest of piezoelectric polymer for tissue engineering applications and due to the differences of polymer and copolymers in terms of electroactivity, degree of crystallinity and microstructure, which certainly affect cell-material interactions.

2. Experimental details

2.1. Materials

Poly(vinylidene fluoride) (PVDF) Solef[®] 6020 ($M_w = 670–700$ kDa), poly(vinylidene fluoride trifluoroethylene) (P(VDF-TrFE)) (70/30) and poly(vinylidene fluoride-co-hexafluoropropylene) P(VDF-HFP) Solef[®] 21216 ($M_w = 570–600$ kDa) were acquired from Solvay; N,N-dimethylformamide (DMF) was obtained from Merck.

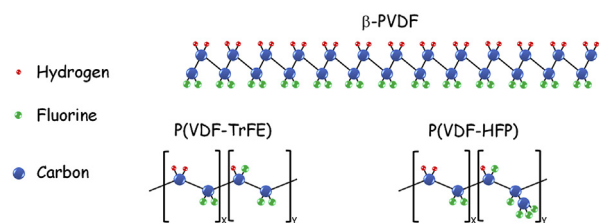


Fig. 1. Schematic representation of the β -PVDF chain conformation and the P(VDF-TrFE) and P(VDF-HFP) repeat units.

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