Materials Science and Engineering C 36 (2014) 226–236

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Biomaterials from blends of fluoropolymers and corn starch—implant and structural aspects

João D.A.S. Pereira [a,b], Regina C.T. Camargo [a], José C.S.C. Filho [a], Neri Alves [a], Miguel A. Rodriguez-Perez [b], Carlos J.L. Constantino [a,*]

[a] Faculdade de Ciências e Tecnologia, UNESP Univ Estadual Paulista, 19060-900 Presidente Prudente, SP, Brazil.
[b] Condensed Matter Physics Department, CellMat Laboratory, Faculty of Science, University of Valladolid, Valladolid Spain

ARTICLE INFO

Article history:
Received 29 May 2013
Received in revised form 7 October 2013
Accepted 6 December 2013
Available online 14 December 2013

Keywords:
Poly(vinylidene fluoride)
Poly(vinylidene fluoride-trifluoroethylene)
Corn starch
Bone healing

ABSTRACT

The development of polymeric blends to be used as matrices for bone regeneration is a hot topic nowadays. In this article we report on the blends composed by corn starch and poly(vinylidene fluoride), PVDF, or poly(vinylidene fluoride-trifluoroethylene), P(VDF-TrFE), to obtain biocompatible materials. Blends were produced by compressing/annealing and chemically/structurally characterized by micro-Raman scattering and Fourier transform infrared (FTIR) absorption spectroscopies, dynamic mechanical analysis (DMA) and scanning electron microscopy (SEM), besides *in vivo* study to evaluate the tissue response. Vibrational spectroscopy reveals no chemical interaction between the polymers and starch, absence of material degradation due to compressing/annealing process or organism implantation, and maintenance of α and ferroelectric crystalline phases of PVDF and P(VDF-TrFE), respectively. As a consequence of absence of interaction between polymers and starch, it was possible to identify by SEM each material, with starch acting as filler. Elastic modulus (E') obtained from DMA measurement, independent of the material proportion used in blends, reaches values close to those of cancellous bone. Finally, the *in vivo* study in animals shows that the blends, regardless of the composition, were tolerated by cancellous bone.

1. Introduction

Biomaterial must possess particular and well-controlled properties suited to each individual application. In load-bearing situations, emphasis may be placed on the material strength and its ability to withstand repeated loading and unloading cycles. In repairing small bone defects, the focus may lie on the material chemical composition and whether it is able to bond with surrounding bone tissue or trigger new growth [1]. The material porosity contributes greatly to both its mechanical properties and the way in which it behaves in vivo. Pores in a material act as stress concentrators, decreasing mechanical properties. On the other hand, they increase surface area, providing greater means for environmental interaction. For scaffolds (made intentionally with porous), interconnected macropores of 200–900 μm in diameter are induced within the material to allow bone growth. The increased surface area may then allow the degradable scaffold slowly to dissolve away as bone replaces it [2]. Besides pores, the piezoelectric effect in bone, which is known since 1950 and is related to collagen, plays an important physiological role in bone growth, remodeling and fracture healings [3,4].

Organic ferroelectric materials have attracted continuing attention for several decades, since the discovery of ferroelectricity on fluoropolymers, as in polyvinylidene fluoride (PVDF) and its copolymers, for instance, with trifluoroethylene P(VDF-TrFE). Fig. 1(a) and (b) shows the chemical structures of PVDF and P(VDF-TrFE) mers, respectively. The PVDF is a semicrystalline thermoplastic polymer with mechanical and chemical stability (high stability to acids and bases). Physical and electrical properties of PVDF depend on its molecular weight and distribution, chain conformation, crystalline form and chain defects [5]. Much of the interest in PVDF comes from its potential applications as material with piezoelectric, pyroelectric and ferroelectric properties, especially in the field of sensors and actuators [6–8]. Recently, Hong et al. [9] investigated nanograss structures of P(VDF-TrFE) and observed an enhancement of piezoelectricity, which was associated with nanopillars of P(VDF-TrFE). Poled vertically oriented P(VDF-TrFE) nanopillars were obtained using the flip-stacking poling method, leading to the increase of surrounding air between the electrode and polymer substrate, changing the surface capacitances and structural flexibility. Although PVDF and copolymers are studied as a biomaterial for a long time, for example in a study about the production of PVDF monofilament for suture [10], the interest due to their piezoelectric property is still great, and even recent studies seek to strengthen these properties [11]. The piezoelectricity can be associated with bone growth induction, since bones are piezoelectric compounds [12–14].

Among the various applications in which PVDF and its copolymers may be used, one has been studied for decades and is still studied: the application as biomaterial. The latter involves, for instance, the development of membranes and scaffolds for wound healing the culture of

* Corresponding author.
E-mail address: case@fct.unesp.br (C.J.L. Constantino).

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http://dx.doi.org/10.1016/j.msec.2013.12.008

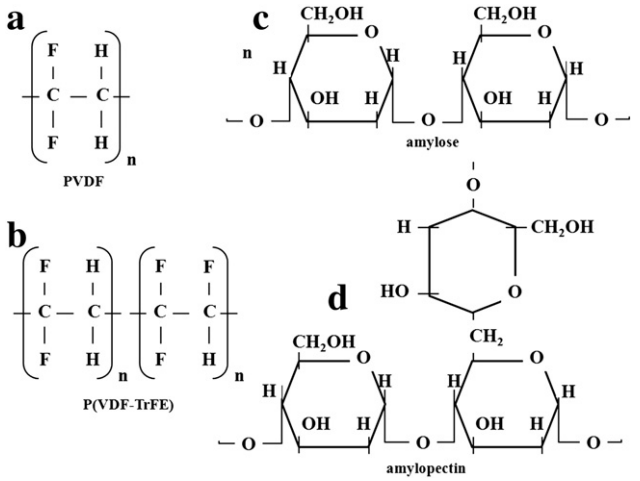

Fig. 1. Chemical structure of PVDF (a) and P(VDF-TrFE) (b) mers, amylose (c) and amylopectin (d).

neural cells, replacement and repair of neural tissue due to trauma or diseases, applications related to the repair of bone tissue, for favoring the adhesion, spreading and proliferation of mesenchymal cells (fibroblasts, fibronectin and osteoblasts) [15–21]. Callegari and Belangero [22] evaluated the interface formed between P(VDF-TrFE) and PVDF tubes (piezoelectric and non-piezoelectric) in rat bone tissue (implanted at the left femur in the intercondilian notch). Results of conventional optical microscopy and backscattered electronic scanning microscopy (SEM) indicate that the piezoelectric effect has an important role, actuating like a transducer, converting mechanical stimulus into electric stimulus favoring the bone tissue formation inside the polymeric tubes.

Starch is the carbohydrate reserve in plants, being usually isolated from corn, wheat, potato, tapioca and rice. Structurally, it is a heterogeneous combination of linear (amylose, 20–30%) and branched (amylopectin, 70–80%) polymers. Amylose has a linear structure 200 to 20,000 repeated glucose units α-1,4 connected by glycosidic links α-D-(1 → 4), forming a helical structure due to the bond angles between the glucose units. Amylopectin is a strong branched structure of short chains α-1,4 (30 glucose units) connected by links α-1,6, approximately every 20 or 30 glucose units along the chain. Amylopectin molecules are formed by approximately two million units of glucose [23]. The starch undergoes enzymatic degradation with the connections α-D-(1 → 4) being attacked by the enzyme α-amylase and the connections α-1,6 by glucosidases [24]. Fig. 1(c) and (d) shows the chemical structures of amylose and amylopectin, respectively. Starch-based blends have shown a great versatility of processing, being used in different application fields [25]. The use of starch as a biomaterial finds application as biodegradable polymeric systems for controlled drug release [26–28] and in tissue engineering scaffolds for bone regeneration and cartilage [29–32], and as bone replacement implants and bone cements [33]. Specifically in the orthopedic field, it is proposed to use blends based on starch as a material similar to bone for temporary fixation of fractures [34].

In our research, PVDF and P(VDF-TrFE) were chosen aiming their application as biocompatible materials, especially with respect to induction of bone growth. Complementarily, the corn starch was chosen due to its possibility of being absorbed by the body, creating a porous matrix for growing tissue. Three specific goals investigated here are related to (i) optimize the blends manufacture of PVDF or P(VDF-TrFE) with corn starch in cylindrical form; (ii) determine structural and mechanical properties of the blends; and (iii) implant such blends (cylinders) in the femur bone of mice to evaluate the effects on tissues after the implantation period. It is important mentioning that the PVDF or its copolymer will act as a mechanical support structure for bone regeneration process, with the tissue growing within the porous left by starch absorption, filling this space (bone integration). The bone integration is a desired property for applications involving bone failure or fixation of small bone fractures. Therefore, removing the PVDF or its copolymer is not a necessary condition. We performed the implantation of PVDF/starch and P(VDF-TrFE)/starch blends with a different approach from that used in literature to date, in which the implants are performed in soft tissues [16], or bone cells are deposited on membranes of PVDF [17], or even with implantation of PVDF or P(VDF-TrFE) polymers in bone tissue, without blending with other polymers [22].

2. Materials and methods

2.1. Production and characterization of samples

The PVDF Florafon F4000 HD was purchased from the Atochem Company as small slugs. PVDF underwent a grinding process in a cryogenic mill. Copolymer P(VDF-TrFE) 72/28 wt% was obtained in the Piezotech in powder form. Native corn starch was Amidex 3001 (Corn Products) provided by Dr. A.J.F. Carvalho (Universidade de São Paulo, campus São Carlos, Brazil), 28% amylose and average particle size of 25 μm.

The preparation of PVDF and P(VDF-TrFE) blends with corn starch was performed by compressing under annealing. This type of processing was chosen because it avoids the use of organic solvent, usually toxic to living organism. The materials for manufacturing the cylinders PVDF/starch and P(VDF-TrFE)/starch were mixed manually in a mortar and then sifted in a sieve with opening of 0.21 mm. Mixed contents were then distributed over holes in the mold, and this was placed between two sheets of Kapton superposed and pressed. The composition of polymer/starch is shown in Table 1, which proportions are based on a previous study [35]. The mold is a rectangular plate of aluminum 3.00 mm thick; 100.00 mm long and 96.00 mm wide, with 21 holes of 3.14 mm^2. The cylindrical samples have the following dimensions: 3.00 mm high and 2.00 mm in diameter, corresponding to a volume of 9.42×10^{-6} mm^3. These dimensions were chosen to allow for subsequent implantation in mice *in vivo*. PVDF or P(VDF-TrFE) cylinders were manufactured by melting the powder of these materials in a hydraulic press Forte Charlott, monitored by a temperature controller Contempcom model PXV. To produce the cylinders of PVDF/starch ($T = 190$ °C) and P(VDF-TrFE)/starch ($T = 155$ °C), 6 pressings of 120 s were performed and 2.04×10^6 Pa of pressure was used. During press, a relief of approximately 5 s between each press is made to release air bubbles that show up during the process.

SEM images were recorded using a Zeiss equipment model EVO LS15 (20 KV). The samples were metallized with 20 nm of gold in a Quorum equipment model Q 150R ES. Raman measurements were obtained in a Renishaw micro-Raman spectrograph, model in-Via, with the excitation laser line at 785 nm, power of microwatt (μW) at the sample, diffraction grating 1200 lines/mm, exposure time of 10 s and 5 accumulations for each spectrum. The micro-Raman spectrograph is equipped with a Peltier CCD detector (cooled to −70 °C) and XYZ motorized platform (step motor—0.1 μm) where samples are positioned, and coupled to spectrograph there is a Leica microscope, which 50× objective lens leads to a magnification of 500×. FTIR measurements were carried out in a Bruker spectrometer model Tensor 27 using the ATR mode (not polarized), 128 scans, and 4 cm^{-1} spectral resolution. Dynamical

Table 1
Composition of polymer/starch blends.

Sample composition	% in mass of each material
0.45 g PVDF/0.05 g starch	90.0% PVDF/10.0% starch
0.40 g PVDF/0.10 g starch	80.0% PVDF/20.0% starch
0.33 g PVDF/0.17 g starch	66.66% PVDF/33.33% starch
0.33 g P(VDF-TrFE)/0.17 g starch	66.66% P(VDF-TrFE)/33.33% starch