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Viscoelastic properties of rapid prototyped magnetic nanocomposite scaffolds for osteochondral tissue regeneration

Roberto De Santis[a,*], Antonio Gloria[a], Teresa Russo[a], Alfredo Ronca[a], Ugo D'Amora[a], Giacomo Negri[b], Dante Ronca[c], Luigi Ambrosio[d]

[a]*Institute of Polymers, Composites and Biomaterials, National Research Council of Italy, V. le J.F. Kennedy, 54, 80125 Naples, Italy.*
[b]*Villa Betania Evangelical Hospital, Via Argine,604, 80147 Naples, Italy.*
[c]*Institute of Orthopaedics and Traumathology, II University of Naples, Via L. De Crecchio, 2-4, 80138 Naples, Italy.*
[d]*Department of Chemical Science and Materials Technology, National Research Council of Italy, Piazzale Aldo Moro 7, 00185 Roma, Italy*

* Corresponding author. Tel.: +39-081-242536; fax: +39-081-2425932. *E-mail address:* rosantis@unina.it

Abstract

Poly(ε-caprolactone) and poly(ethylene glycol) based magnetic nanocomposite scaffolds were fabricated using fused deposition modeling and stereolithography approaches, and a hybrid scaffold was obtained by combining these additive manufacturing technologies. Viscoelastic properties in compression were investigated at 37°C, spanning a range frequency of four decades. Results suggest that poly(ε-caprolactone) and poly(ethylene glycol) based scaffolds adequately reproduce viscoelastic properties of subchondral bone and articular cartilage tissues, respectively. By combining fused deposition modeling and stereolithography it is possible to manufacture a hybrid scaffold suitable for osteochondral tissue regeneration.

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Keywords: Magnetic scaffolds; Fused deposition modeling; Stereolithography; Nanocomposite; Poly(ε-caprolactone); Poly(ethylene glycol); Magnetic nanoparticles; Viscoelastic properties.

1. Introduction

Osteoarthritis represents the most common joint disorder causing severe pain and disability [1,2]. This pathology damages subchondral bone and cartilage, and total joint arthroplasty (e.g., knee and hip prostheses) is still the choice for the treatment of the late stage osteoarthritis [3,4]. Metals, ceramics, polymers and composites are the materials used to fabricate these prostheses [4-7], and often acrylic cements [8,9] are used to fix the prosthesis to bone. However, due to several drawbacks of prosthetic devices, revision of an implanted prosthesis is required [10]. In this scenario, osteochondral bone regeneration represents a great challenge.

A scaffold for tissue engineering has to satisfy several requirements: an interconnected porous architecture able to promote cell-material interaction and extracellular matrix deposition; withstand the forces acting on the bone-joint segment and transfer the stress to the hosting tissue allowing the mechanical stimulation of tissue cells; tailored mechanical and degradation properties in order to gradually transfer the loading function to the newly forming tissue [11-17]. In particular, polymer-based composite materials can be designed to achieve enhanced functional and mechanical properties [7,15,16,18]. Benefiting from the large variety of biocompatible polymers, spanning from cross-linked networks to weak gels, scaffolds may be designed in the form of solid-like [15-22], strong gel-like [23-25] or injectable [26-30] formulations according to the specific application. Advanced scaffolds for tissue engineering able to release, *in situ*, drugs or cells could be obtained by integrating different technologies, also involving several strategies to improve cell attachment (i.e., surface functionalization) [30-35].

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Different approaches have been proposed to fabricate 3D porous scaffolds. Conventional methods (e.g., salt leaching, solvent casting, phase inversion, etc.) are not capable of precisely controlling pore geometry, spatial distribution and interconnectivity [16,36,37]. On the other hand, additive manufacturing, also known as 3D printing or solid freeform fabrication, represents the key to fabricate customized and reproducible scaffolds [38]. Among these techniques, Fused Deposition Modeling (FDM) offers the opportunity to process highly filled composites, thus obtaining 3D morphologically controlled micro/nano-composite scaffolds. This technique involves a moving nozzle to extrude a fiber of polymeric or composite material by which the physical model is built layer-by-layer [16,20,37-41]. Conversely, stereolithography relies on the UV polymerization process. As in the case of FDM, stereolithography allows to process composite materials, but the amount of fillers is highly limited by the viscosity of the reactive photo-curable solution [16,36,42,43].

Recently, novel routes in tissue engineering have involved the use of magnetic nanoparticles (MNPs) to induce tissue growth through magnetic fields. Iron doped hydroxyapatite nanoparticles and iron oxide have been included into a polymeric matrix in order to guide bone regeneration [44-46]. The superparamagnetic feature of magnetic nanocomposite materials has allowed to develop an advanced and efficient *in vitro* method for the seeding of magnetically labeled cells, and a remarkable bone regeneration has been observed *in vivo* [47]. Moreover, this approach allows also to benefit from the effects of induced hyperthermia [48, 49].

In a joint connective tissues can be considered as composite materials mainly composed of collagen, hydroxyapatite and water-gel containing proteoglycans [50-52], and the time-dependent properties (viscoelasticity) of bone and cartilage are strongly related to the age, organ and district site [50,53-55]. On the other hand, mimicking of the mechanical and viscoelastic properties has shown to be very effective for improving the design of prosthetic [56,57] and scaffold devices [58]. Dynamic Mechanical Analysis (DMA) is a powerful tool to study the viscoelastic behavior of materials [8,50,53-55,57]. It is recognized that mechanisms occurring during tissue adaptation rely on a cellular mechanotransduction process driven by dynamic rather than static loading [59,61]. Of course, viscoelastic properties of scaffolds are of paramount importance especially in relation to the remarkable effects of dynamic mechanical stimulation, *in vivo* and *in vitro* through bioreactors, on tissue adaptation/regeneration [59-62].

Accordingly, through the combination of additive manufacturing approaches, the aim of the current research was the design and preparation of scaffolds for bone and cartilage regeneration, as well as the assessment of the viscoelastic features through DMA.

2. Materials and Methods

Poly(ε-caprolactone) (PCL) and PCL/MNPs nanocomposites scaffolds were fabricated through FDM technique (Fig. 1a), while poly(ethylene glycol) diacrylate (PEGDA) and PEGDA/MNPs scaffolds were manufactured using stereolithography (Fig. 1b). 3D cylindrical hybrid scaffolds (Fig. 1c) were also obtained through a proper combination of these technologies.

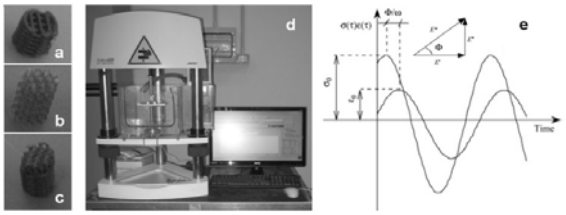

Fig. 1. (a) PCL/MNPs 80/20 scaffold obtained through FDM; (b) PEG/MNPs 95/5 scaffold obtained through stereolithography; (c) Multimaterial scaffold obtained combining stereolithography and FDM; (d) mechanical spectrometer equipped with a water bath at 37°C; (e) typical dynamic mechanical measurement and analysis for determining viscoelastic properties.

2.1. Scaffolds preparation: FDM

PCL pellets (M_w=65,000, Sigma-Aldrich, St. Louis, MO) and PCL/MNPs pellets were processed through FDM using a 3D plotter (Bioplotter, Envisiontec GmbH, Gladbeck, Germany) to manufacture 3D cylindrical scaffolds (6 mm in diameter, 8 mm in height) with a 0/0/90/90° lay-down pattern (Fig. 1a). PCL/MNPs pellets were obtained by dissolving PCL in tetrahydrofuran (THF, Sigma-Aldrich, St. Louis, MO) and adding MNPs and ethanol to the PCL/THF solution during stirring. Sonication through the ultrasonic bath (Branson 1510 MT, Danbury, CT) promoted the dispersion of the nanoparticles in the solution. The polymer/filler weight ratio (wt/wt) was 80/20.

The stainless steel injector of the FDM apparatus was pressurized to 8.5 bar and it was heated to a temperature of 90°C and 120°C for PCL and PCL/MNPs scaffolds, respectively. The material was deposited at a speed of 35 mm/min through a nozzle with an inner diameter of 600 µm.

2.2. Scaffolds preparation: stereolithography

PEGDA and PEGDA/MNPs photo-curable solutions were processed through stereolithography (Envisiontec Perfactory Mini Multilens SLA). Briefly, MNPs were dispersed in the monomer solution through sonication. PEG/MNPs 95/5 and PEG/MNPs 90/10 formulations were prepared, also including the photo-initiator Lucirin-TPO (4% wt).

Surfaces, which are periodic in three independent directions, were generated using K3DSurf v0.6.2 software, and diamond like architectures, according to the boundary conditions x^2+y^2:[-4π,4π] and z:[-8π,8π], were obtained using the following trigonometric functions:

$$\sin(x)\sin(y)\sin(z)+\sin(x)\cos(y)\cos(z)+\cos(x)\sin(y)\cos(z)+\cos(x)\cos(y)\sin(z)=C \quad (1)$$

where C is the offset value that regulates the porosity of the structure. This value was set at 0.4 to obtain a porosity of about 80%.