Journal of the Mechanical Behavior of Biomedical Materials 82 (2018) 133–144

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

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

Mechanical characterization and numerical simulation of a subcutaneous implantable 3D printed cell encapsulation system

Federica Adamo[a,1], Marco Farina[b,c,1], Usha R. Thekkedath[b], Alessandro Grattoni[b,2,*], Raffaella Sesana[a,2]

[a] Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy
[b] Department of Nanomedicine, Houston Methodist Research Institute, 6670 Bertner Avenue, R8-216, Houston, TX 77030, USA
[c] Department of Electronics and Telecommunications, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy

ARTICLE INFO

Keywords:
Subcutaneous implant
Cell transplantation
Structural analysis
Additive manufacturing
Finite element analysis

ABSTRACT

Cell transplantation in bioengineered scaffolds and encapsulation systems has shown great promise in regenerative medicine. Depending on the site of implantation, type of cells and their expected function, these systems are designed to provide cells with a physiological-like environment while providing mechanical support and promoting long-term viability and function of the graft. A minimally invasive 3D printed system termed neovascularized implantable cell homing and encapsulation (NICHE) was developed in polylactic acid for subcutaneous transplantation of endocrine cells, including pancreatic islets. The suitability of the NICHE for long term in vivo deployment is investigated by assessing mechanical behavior of both fresh devices under simulated subcutaneous conditions and NICHE retrieved from subcutaneous implantation in pigs. Both experimental and numerical studies were performed with a focus on validating the constitutive material model used in the numerical analysis for accuracy and reliability. Notably, homogeneous isotropic constitutive material model calibrated by means of uniaxial testing well suited experimental results. The results highlight the long term durability for in vivo applications and the potential applicability of the model to predict the mechanical behavior of similar devices in various physiological settings.

1. Introduction

Cell therapy is emerging as an attractive treatment option for various chronic medical conditions such as diabetes, hypogonadism, hemophilia, and Parkinson's disease. However, clinical cell transplantation efforts are hindered by several challenges including loss of graft function due to graft dispersion, limited vascularization or innervation, the need for continuous immunosuppression, and the lack of adequate mechanical support to the graft (Farina et al., 2018). Current research is focused on the development of encapsulation materials and strategies for graft protection. Cell encapsulation consists on the inclusion of cells within a matrix or device to achieve ad hoc delivery of hormones or other molecules as an advanced strategy for various therapeutic applications. The encapsulating architecture must enable the transplanted cells to function as an artificial organ, while allowing for the bi-directional transport of oxygen, metabolic products and release of the therapeutic agents such as hormones and enzymes. Physical parameters of materials, such as porosity, rigidity, chemical composition, and surface functionalization play a dominant role in the success of the encapsulation. Further, the optimal implantation site must allow for minimally invasive surgical procedures, the ability to locate and retrieve the graft at need, and offer the potential for cell replacement or replenishment. As such, several studies have focused on the development of devices for subcutaneous placement. Because of the potential external stresses associated to this site of implantation, subcutaneous implants have to withstand mechanical forces, while conforming to the surrounding tissue (Schwab et al., 2008). Further, in the context of their long-term clinical adoption, safety and mechanical robustness must be assured for repeated mechanical solicitations and fatigue.

With the goal of addressing the current challenges in the field, we developed an innovative refillable macro-encapsulation system termed neovascularized implantable cell homing and encapsulation (NICHE) for the subcutaneous transplantation of pancreatic islets and Leydig cells (Sabek et al., 2016) In previous studies, the systems demonstrated to provide a suitable environment for the transplantation of cells in vivo and achieved neovascularization and long term viability and survival of

* Corresponding author.
E-mail address: agrattoni@houstonmethodist.org (A. Grattoni).
[1] equal contribution.
[2] co-senior authorship.

https://doi.org/10.1016/j.jmbbm.2018.03.023
Received 15 November 2017; Received in revised form 16 March 2018; Accepted 19 March 2018
Available online 21 March 2018
1751-6161/

transplanted cells in rodent models (Farina et al., 2017). The system was generated in implantable grade polylactic acid (PLA) by 3D printing adopting the fused deposition modeling (FDM) technology.

PLA was approved in 1970 by the US Food and Drug Administration (FDA) for use in the food and pharmaceutical industries. Due to its biocompatibility, non-toxic nature of degradation products, and mechanical properties, PLA is one of the most promising biopolymers used tissue engineering, drug delivery systems as well as sutures and clips. PLA is a thermoplastic and slowly biodegradable aliphatic polyester derived from naturally occurring organic acid (lactic acid). The melting point is in the range of 180–220 °C with glass transition temperature 60–65 °C. In physiological conditions PLA biodegrades by hydrolysis of the ester backbone resulting in the formation of non-harmful and non-toxic compounds (Weir et al., 2004). Their degradation products are easily excreted through kidneys or eliminated in the form of carbon dioxide and water through metabolic processes. Importantly, PLA is an ideal candidate for 3D printing manufacturing.

3D printing, also known as additive manufacturing (AM), has emerged in the biomedical field as a new tool for the custom-made fabrication of prosthesis, scaffolds, bone replacements with well-defined architectures based on patient-specific tissue defects (Liu et al., 2017; Sing et al., 2017). The potential of AM has been highlighted in recent pioneering developments of artificial organs and bio-artificial tissues for tissue regeneration (Murphy and Atala, 2014). Among them, notable examples include the regeneration of functional skin and cartilage in situ demonstrated by Skardal et al. (2012) and Cui et al. (2012), respectively. Also, various commercial companies are producing 3D bioengineered tissue for safety and efficacy testing of pharmaceutical products. Organovo (San Diego, CA) has pioneered 3D-printing for liver tissue, and is currently working on a kidney tissue product (Pondrom, 2016).

3D Printing is particularly advantageous not only for rapid design iterations in the development stage, but also to create one-of-a-kind, customized complex devices that are either impossible to fabricate or present prohibitive cost when using conventional manufacturing methods such as injection molding. Important recent examples of AM of polymeric systems for biomedical applications are customized implants (Chen et al., 2017), prosthetics (Ten Kate et al., 2017), pre-surgical medical models (Le Bras, 2018) and medical devices (Dodziuk, 2016; Cristofari et al., 2017). More specifically, PLA was used for cell encapsulation systems (Narayanan et al., 2016), bioactive porous scaffolds for tissue (Do et al., 2015) and dental regeneration (Liaw and Guvendiren, 2017). Together with massive potential, AM carries some challenges and limitations: The precision of material deposition and manufacturing tolerances are poor as compared to other conventional techniques. The accuracy of printing strongly depends on material, 3D technology, and processing parameters used, which have been investigated by various authors (Salmi et al., 2013). The geometry of the printed structures is approximated due to deposition procedure, the resolution of the printer and the 3D software adopted. As a result, irregularities, air gaps, stairs steps between layers and poor layer-to layer adhesion affect the mechanical and chemical properties of the printed part (Dizon et al., 2018) with notable effect on elastic modulus, yield strength, and fracture (Torrado Perez et al., 2014; Miller et al., 2017; Park and Rosen, 2016).

In this work we present a thorough investigation of the mechanical properties of our 3D printed NICHE system and developed a finite element model (FEM) of the structure with the objective of generating a simulation tool for the development of NICHE or other implantable 3D-printed PLA architectures.

FEM analysis is extremely useful for optimizing device design by estimating the mechanical response of materials and tissues (Huo et al., 2015; Mengoni et al., 2016), which is extremely challenging to be assessed in vivo. Intact NICHE capsules were examined under compressive, and four point bending stresses, in wet and dry conditions. Chicken dermal tissue was used to mimic the effect of the skin in subcutaneous applications. Further, NICHE retrieved post vascularization and tissue engraftment from subcutaneous implantation in Yucatan minipigs were tested under compressive stress to assess the effect of ingrown tissues and degradation on the mechanical performances of the capsule. Creep and fatigue testing were also executed on the NICHE to assess irreversible deformations as well as failure modes. Failure and crack position were examined focusing on the presence of fragments. To develop the FEM, experimental uniaxial monotonic testing was performed on 3D printed PLA dogbone specimens to determine the constitutive material properties for the material under dry and wet conditions. By using 3D printed “solid” specimens, we intrinsically took into account the potential presence of defects such as voids, steps, and geometrical errors due to the printing process in the determination of the constitutive material properties. This approach was successfully adopted by Zeltmann et al. (2016) and Beretta and Romano (2017) in the evaluation of printed structures in both metallic and polymeric systems. Compressive tests on the chicken dermal skin were also used to obtain the properties of tissues to be implemented in the FEM. Finally, FEM numerical results and experimental data were compared with the objective of evaluating if the linear elastic-plastic homogeneous constitutive material model was suited to simulate the experimental behavior of the macro encapsulation system.

2. Materials and methods

To characterize the mechanical properties of the thermoplastic polymer material (PLA) and of the encapsulation system under different loading conditions, a set of monotonic, creep and cyclic tests were performed in dry and wet conditions (samples previously immersed in a sodium chloride solution 0.9% w/v NaCl for 24 h and stored at 23 ± 2 °C). In addition, dermal tissue collected from chicken leg (after feather removal) was adopted to simulate the mechanical behavior in the subcutaneous environment.

The study was conducted as follows. Firstly, the analysis of the mechanical uniaxial monotonic behavior of the base material was performed through monotonic tensile tests (Krone et al., 2013; Li et al., 2014), run on 3D printed dogbone specimens, in dry and wet conditions. The results were used to calibrate the constitutive material models of the FE simulations.

In a second phase, testing of encapsulation system specimens was executed to evaluate the stiffness of the devices, failure loads, and failure locations in dry and wet conditions, by means of the application of monotonic, cyclic, and central compression test. To simulate the subcutaneous environment, devices enveloped in chicken dermal tissues (thickness 2 mm) were used. Compression tests were executed on NICHE with and without chicken dermal tissue interposed between the puncher and the capsule or wrapping the NICHE entirely. Additionally, compression testing was performed on NICHE retrieved from subcutaneous implantation in pigs. These capsules were completely filled by ingrown tissue during a 4 weeks vascularization and engraftment period (see details in Section 2.3). This better mimicked the actual NICHE operating conditions in the subcutaneous environment. Four-point monotonic bending tests were used to measure bending failure modes and loads. Mechanical characterization of the tissue alone was also performed via compression testing under monotonic conditions.

In a third phase, the FE numerical simulations of experimental analyses were performed. While simulation of dogbone monotonic tests aimed at validating the material constitutive model, simulations of device testing were executed to validate the numerical model, in complex loading, with and without subcutaneous tissue. The first objective of these simulations was to validate the numerical device model. In particular, we investigated if the constitutive, isotropic, and homogeneous material model calibrated by means of experimental uniaxial tests was suitable for simulating the experimental behavior of the device. The second objective was to compare the numerical stress distribution, and the value and location of the maximum stresses,