European Journal of Pharmaceutics and Biopharmaceutics 85 (2013) 78–86

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Plasma treatment for improving cell biocompatibility of a biodegradable polymer scaffold for vascular graft applications

Sarra de Valence [a], Jean-Christophe Tille [b], Chiraz Chaabane [c], Robert Gurny [a], Marie-Luce Bochaton-Piallat [c], Beat H. Walpoth [d], Michael Möller [a,*]

[a] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland
[b] Division of Clinical Pathology, University Hospital of Geneva, Geneva, Switzerland
[c] Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland
[d] Service of Cardiovascular Surgery, University Hospital of Geneva, Geneva, Switzerland

ARTICLE INFO

Dedicated to Hans Peter Merkle on the occasion of his 70th birthday

Keywords:
Tissue regeneration
Vascular graft
Polycaprolactone
Plasma
In vivo
Smooth muscle cells
Biocompatibility

ABSTRACT

Biodegradable synthetic scaffolds are being evaluated by many groups for the application of vascular tissue engineering. In addition to the choice of the material and the structure of the scaffold, tailoring the surface properties can have an important effect on promoting adequate tissue regeneration. The objective of this study was to evaluate the effect of an increased hydrophilicity of a polycaprolactone vascular graft by treatment with a cold air plasma. To this end, treated and untreated scaffolds were characterized, evaluated *in vitro* with smooth muscle cells, and implanted *in vivo* in the rat model for 3 weeks, both in the subcutaneous location and as an aortic replacement. The plasma treatment significantly increased the hydrophilicity of the scaffold, with complete wetting after a treatment of 60 sec, but did not change fiber morphology or mechanical properties. Smooth muscle cells cultured on plasma treated patches adopt a spread out morphology compared to a small, rounded morphology on untreated patches. Subcutaneous implantation revealed a low foreign body reaction for both types of scaffolds and a more extended and dense cellular infiltrate in the plasma treated scaffolds. In the vascular position, the plasma treatment induced a better cellularization of the graft wall, while it did not affect endothelialization rate or intimal hyperplasia. Plasma treatment is therefore an accessible tool to easily increase the biocompatibility of a scaffold and accelerate tissue regeneration without compromising mechanical strength, which are valuable advantages for vascular tissue engineering.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

For certain clinical applications such as the coronary artery bypasses, there is a need for synthetic alternatives to the current gold standard of autologous vascular material [1]. To achieve comparable long-term results to natural tissues, the ideal synthetic graft should degrade and remodel into a natural structure. This is the object of much research in the field of vascular tissue engineering where scaffolds and cells are being combined to promote the regeneration of an artery [2]. To not impede the natural healing process, the scaffold must have an excellent biocompatibility and allow a good cell infiltration.

Tissue regeneration in a biodegradable scaffold is dependent on several factors. Micro-architecture is important for guiding neo-tissue formation since pore size must be sufficient to allow cell migration and infiltration into the scaffold [3,4]. Surface properties of a scaffold are also very important to provoke specific cellular responses and direct tissue regeneration [5]. Poor hydrophilicity and a lack of natural recognition sites on the surface of a material will hinder cell-scaffold interactions and alter the tissue regeneration process. Surface properties depend on the biomaterial, but can be adjusted with different techniques such as surface hydrolysis [6], chemical grafting [7], self-assembly [8], or plasma treatment [9], which was investigated further in this study.

A gas plasma, which can be considered as the fourth state of matter, is a partially ionized gas consisting of electrons, ions, and neutral atoms or molecules. A plasma can be formed by radiofrequency oscillations, microwaves, or electrons from a hot filament discharge that will energize the electrons in excess of the ionization threshold. The energy of plasma electrons is sufficient to break molecular bonds and change the surface properties of a normally inert material [10]. For medical applications, plasma treatment has been used to improve the biocompatibility of surfaces [9,11] and to create functional groups for the attachment of other

* Corresponding author. Department of Pharmaceutical Sciences, University of Geneva, Quai Ernest Ansermet, 30, CH-1211 Geneva 4, Switzerland. Tel.: +41 (0)22 37 93 132; fax: +41 (0)22 37 96 567.
E-mail address: michael.moeller@unige.ch (M. Möller).

0939-6411/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejpb.2013.06.012

molecules such as drugs [12,13], peptides [14], or natural polymers [15–17].

In this study, the effect of increased surface hydrophilicity of a scaffold for vascular tissue engineering was explored. A micro- and nano-fibrous polycaprolactone scaffold was treated with an air plasma and evaluated both *in vitro* with smooth muscle cells and *in vivo* in subcutaneous and vascular locations.

2. Materials and methods

2.1. Scaffold preparation

Polycaprolactone micro- and nano-fiber scaffolds were produced by solution electrospinning. An 80'000 Da poly(ε-caprolactone) (PCL) (Sigma-Aldrich, Germany) was dissolved at 15% w:v in a chloroform/ethanol mixture (7:3 v:v). The solution was charged at 20 kV and ejected through an 18 G needle at a constant rate of 12 mL/h, and the fibers were collected 20 cm away on a rotating and translating cylindrical mandrel. The collection was done over 6 min on a 1 cm diameter mandrel to make the patches for cell culture and over 6 min on a 2 mm diameter mandrel for the subcutaneous and vascular implants. The scaffolds were placed under vacuum (0.2 mbar) for 18 h to evaporate any residual solvents.

The air plasma treatment was performed by placing the scaffolds in the center of a radiofrequency plasma chamber (PDC-002, Harrick Plasma, USA). Vacuum was applied to the chamber for 2 min, then the plasma was ignited at 30 W, and the treatment was done under a low inflow of air. Several treatment times from 5 sec to 5 min were done on patches for the *in vitro* investigations, and 60 sec treatments were done on the grafts meant for subcutaneous and vascular implantation. After treatment, the scaffolds were degassed for 1 h under vacuum to remove possible reactive oxygen species. The patches for cell culture were used within 1 week of plasma treatment, and the grafts were implanted within 2 weeks.

The patches for cell culture were sterilized by immersion in 70% ethanol for 2 h and rinsed at least three times 5 min in sterile phosphate buffered saline (PBS). The grafts for *in vivo* evaluation were sterilized by gamma irradiation at 25 kGy (Leoni Studer AG, Däniken, Switzerland). The sterilization of the scaffolds was performed after plasma treatment.

2.2. Scaffold characterization

The morphology of the fibers was evaluated by scanning electron microscopy (JEOL JSM-6510LV, Japan). Magnifications up to 5000× were used to observe potential differences between the untreated and plasma treated scaffolds.

The elemental composition of the polymer surface of untreated and 60 sec plasma treated patches, sterilized by gamma irradiation, was analyzed by X-ray photoelectron spectroscopy (XPS). Analysis was performed using an Axis Ultra instrument (Kratos, Shimadzu Corporation of Kyoto, Japan). The charge referencing was done by setting the C1s hydrocarbon peak at 285.00 eV. The elemental composition of the surface was determined with the standard Scofield photoemission cross-section.

The longitudinal mechanical properties were measured on 2 mm inner diameter and 2 cm long grafts, both untreated and plasma treated for 60 sec then sterilized by gamma irradiation. The grafts were clamped at a 1 cm inter-clamp distance and pulled at a rate of 10 mm/min until rupture (M1600, Schenk AG, Germany). The maximum stress, the strain at rupture, and the young's modulus were recorded. These measurements were done in triplicates.

The water contact angle was measured by placing a 20 μL droplet of bi-distilled water on an electrospun patch treated by plasma for 0, 5, 10, 20, 30, 45, 60, 90, or 300 sec (Type 28, Lorentzen & Wettre, Sweden) then sterilized by gamma irradiation. On random patches, water contact angles were measured on both the top side and the bottom side during plasma treatment. At least three droplets were analyzed on each patch and three patches for each treatment time were tested.

2.3. Smooth muscle cell culture

Primary porcine smooth muscle cells (SMC) with a rhomboid phenotype [18,19] were used to evaluate the biocompatibility of the plasma treated scaffolds *in vitro*. These cells were obtained from porcine carotid arteries by tissue explantation [19]. Air plasma treated patches for 10, 30, 60, and 90 sec as well as untreated patches were evaluated. The patches were cut into disks of the size of the wells of a 24 well plate (15 mm diameter). To prevent the patches from floating, fitted stainless steel rings were placed over each patch. Cells between passages P8 and P14 were trypsinized then counted and resuspended in supplemented culture media (Dublecco's Modified Eagle Medium (DMEM, Gibco-Invitrogen, Switzerland) with 10% fetal calf serum (FCS, Amimed, Bioconcept, Switzerland), 1% glutamine, and 1% penicillin). In each well, cells were seeded as a density of 280 cells/mm^2, and the plates were incubated at 37° and for 1, 3, and 7 days, with media changes every 48 h. Each experimental condition was performed in triplicates.

At each time point, a proliferation test was performed by incubating the cells with an XTT solution (Cell Proliferation Kit II (XTT), Roche Applied Science, Germany) for 6 h, then measuring the absorbance of 150 μL of the supernatant at a 480 nm, and subtracting the absorbance of the reference wavelength of 650 nm. Before performing the XTT tests, the patches were transferred to a new well plate to avoid measuring the metabolic activity from cells that could have gone off the patch and onto the tissue culture plastic.

To fix the cells, the patches were rinsed with DMEM + 2% HEPES (Gibco-Invitrogen, Switzerland) then fixed in a 1% solution of paraformaldehyde (Fluka, Switzerland) in DMEM for 30 min. After several rinses with PBS, the cells were permeabilized with methanol at −20° for 5 min then rinsed again with PBS. A double immunofluorescence was performed on the cells to label α-smooth muscle actin (αSMA) with FITC (green, primary mouse monoclonal IgG2a antibody specific for αSMA [20] at 1:50 in PBS, secondary FITC-conjugated goat anti-mouse IgG2a at 1:50 in PBS, Southern Lab, Birmingham, USA), S100A4 with Rhodamine (red, primary monoclonal IgM antibody specific for S100A4 at 1:5 [19], secondary rhodamine-conjugated goat anti-mouse IgM at 1:50, Southern Lab), and nuclear DNA with dapi (blue, 1:500 dilution, Sigma, Buchs, Switzerland). Primary antibodies were incubated for 90 min and secondary antibodies for 60 min.

Confocal microscopy (510 Meta, Carl Zeiss, Germany) was used to observe the morphologies of the cells. ImageJ software was used to measure the maximum diameter of the cells from 40× images. At least 20 cells were measured for each condition.

2.4. In vivo evaluation

2.4.1. Subcutaneous implantation

The scaffolds, implanted for subcutaneous evaluation, were in a tubular form with a 2 mm inner diameter and a 1 cm length and were either treated by plasma for 60 sec or untreated. Three Sprague–Dawley male rats were anesthetized with a 5% isoflurane induction then operated under 2% isoflurane. Small incisions were made in the skin, which was locally detached from the muscle to make space for the subcutaneous implant. The incisions were closed with two sutures, disinfected, and covered with an

Download English Version:

https://daneshyari.com/en/article/2083857

Download Persian Version:

https://daneshyari.com/article/2083857

Daneshyari.com