Biomaterials 39 (2015) 155–163

Contents lists available at ScienceDirect

Biomaterials

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

USPIO-labeled textile materials for non-invasive MR imaging of tissue-engineered vascular grafts

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ARTICLE INFO

Article history:
Received 8 September 2014
Accepted 23 October 2014
Available online 21 November 2014

Keywords:
Tissue engineering
Vascular graft
Textile material
MRI
USPIO

ABSTRACT

Non-invasive imaging might assist in the clinical translation of tissue-engineered vascular grafts (TEVG). It can e.g. be used to facilitate the implantation of TEVG, to longitudinally monitor their localization and function, and to provide non-invasive and quantitative feedback on their remodeling and resorption. We here incorporated ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles into polyvinylidene fluoride (PVDF)-based textile fibers, and used them to prepare imageable tissue-engineered vascular grafts (iTEVG). The USPIO-labeled scaffold materials were molded with a mixture of fibrin, fibroblasts and smooth muscle cells, and then endothelialized in a bioreactor under physiological flow conditions. The resulting grafts could be sensitively detected using T1-, T2- and T2*-weighted MRI, both during bioreactor cultivation and upon surgical implantation into sheep, in which they were used as an arteriovenous shunt between the carotid artery and the jugular vein. In vivo, the iTEVG were shown to be biocompatible and functional. Post-mortem ex vivo analyses provided evidence for efficient endothelialization and for endogenous neo-vascularization within the biohybrid vessel wall. These findings show that labeling polymer-based textile materials with MR contrast agents is straightforward and safe, and they indicate that such theranostic tissue engineering approaches might be highly useful for improving the production, performance, personalization and translation of biohybrid vascular grafts.

1. Introduction

In recent years, significant progress has been made in the development of tissue-engineered materials for cardiovascular purposes, including e.g. blood vessels, heart valves and myocardium [1–8]. However, in spite of the fact that tissue engineering strategies have demonstrated clear potential for improving the biocompatibility and the performance of cardiovascular grafts, the translation of such biohybrid materials into clinically useful products, suitable for implantation into patients, has been slow [9–11]. Among the reasons for this are practical issues, such as difficulties in automated and GMP-based production [12], as well as clinical limitations, related e.g. to the inability to monitor the position and performance of tissue-engineered grafts upon implantation [13].

Materials and methods for non-invasive imaging therefore hold great potential for implementation in tissue engineering, enabling the longitudinal assessment of implant localization, function, maturation, acceptance and remodeling [14]. Consequently, integrating non-invasive imaging in tissue engineering might foster the development of individualized and improved cardiovascular implants, and it might facilitate their clinical translation. Much current interest in this regard is directed toward the use of

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http://dx.doi.org/10.1016/j.biomaterials.2014.10.076
0142-9612/

magnetic resonance imaging (MRI), as it does not involve ionizing radiation, has the capability of imaging tissues at any depth with excellent soft tissue contrast, and has a resolution close to the cellular level. The application of MRI to cardiovascular tissue engineering is of particular interest, since it can provide valuable information on critical parameters such as: i) the morphology and the composition of regenerated vessels; ii) the patency of stent and graft materials; iii) the localization, function and remodeling of the implant material over time; iv) the fate of in vitro colonized cells upon implantation; and v) important (patho-) physiological processes, such as perfusion, inflammation and thrombosis [15–18].

Whereas tissue morphology and vascularization can be assessed without the application of contrast agents, e.g. via relaxometry and time-of-flight (perfusion) measurements, scaffold materials and cells cannot be depicted using conventional MR techniques. The incorporation of MR contrast agents into the cells embedded in tissue-engineered vascular grafts (TEVG) has been shown to be an efficient method for 'indirectly' visualizing the localization and function of TEVG. Ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles are routinely used MR contrast agents [19,20], and have already been employed to label aortic smooth muscle cells (SMC) and endothelial cells (EC) in vascular grafts [21,22]. From a clinical point of view, EC would arguably be the most relevant cellular target to image, in particular if eventually intending to use such constructs in combination with ^{18}F-FDG-based PET imaging, to more easily identify and more accurately quantify vascular inflammation.

Here, to 'directly' visualize vascular grafts in vivo, to monitor their remodeling and resorption, and to facilitate imaging also in case of non-cellularized implants, we developed materials and methods to visualize the scaffold materials themselves. To this end, as exemplified by Fig. 1, USPIO nanoparticles were incorporated into polyvinylidene fluoride (PVDF)-based textile materials, which were knitted into vascular scaffolds. The scaffolds were then molded with a composite of smooth muscle cells (SMC), fibroblasts (FB) and fibrin, and the inner lumen was colonized with endothelial cells (EC) in a bioreactor, to avoid inflammation and thrombus formation. During bioreactor cultivation, the image-guided and tissue-engineered vascular grafts (iTEVG) were monitored using MRI at three different time points. As proof-of-principle for in vivo visualization, they were finally also implanted into sheep, as an arteriovenous shunt between the carotid artery and the jugular vein. USPIO labeling allowed a clear delineation of the textile-based scaffold materials using MRI, and it did not negatively affect the stability, biocompatibility and functionality of the iTEVG. Such theranostic tissue engineering approaches are considered to be relevant both at the preclinical level and in patients, enabling individualized and improved vessel replacement treatments.

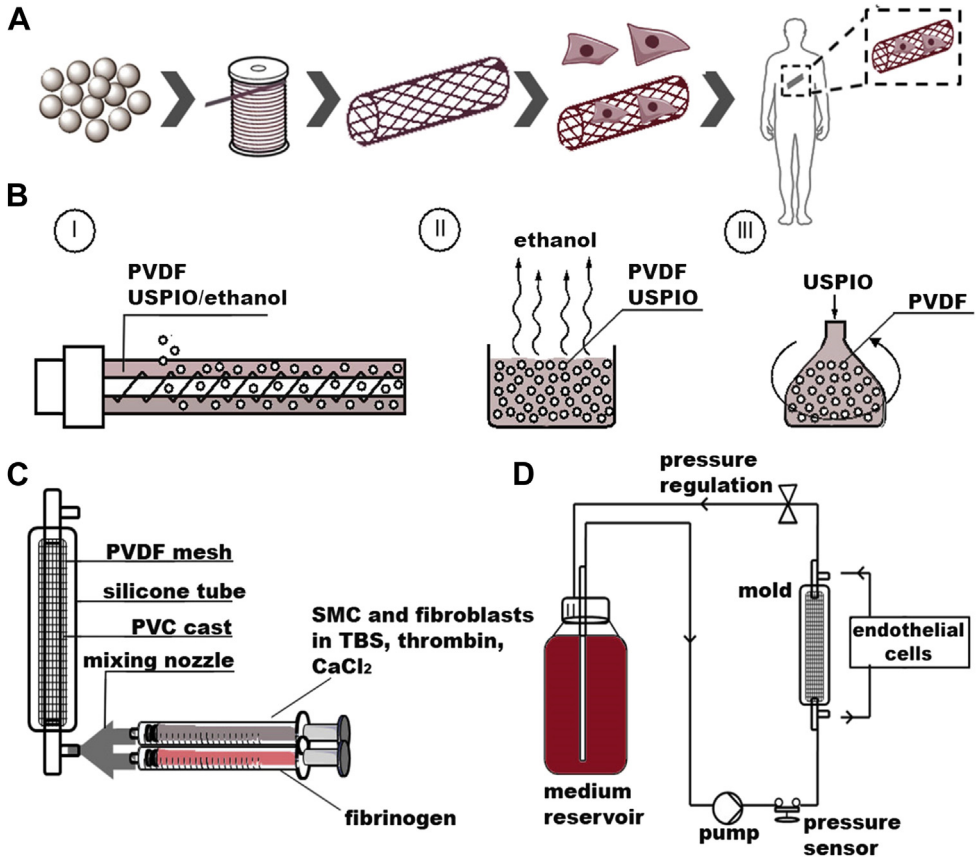

Fig. 1. Production process for USPIO-labeled tissue-engineered vascular grafts. A) PVDF-based polymeric pellets were processed into fibers via melt-spinning and the fibers were further processed into knitted tubular meshes. The meshes were used as scaffolds for the colonization with cells, to produce a biohybrid tissue-engineered vascular graft. B) The incorporation of USPIO nanoparticles into PVDF was carried out in three different ways, in order to find the most suitable procedure for achieving a uniform particle distribution within the matrix. C) An injection molding technique was employed to colonize the PVDF meshes with cells. PVDF meshes were positioned between two cylinders and then covered with the smooth muscle cell/fibroblast/fibrinogen suspension. The polymerization of fibrinogen to fibrin was achieved by using thrombin and calcium chloride. D) Schematic depiction of the bioreactor circuit with pulsatile luminal flow for the mechanical conditioning of the graft. After 7 days, endothelial cells were seeded onto the luminal side of the graft.