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Electrospun degradable polyesterurethane membranes: potential scaffolds for skeletal muscle tissue engineering

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Abstract

Skeletal muscle tissue engineering represents an attractive approach to overcome problems associated with autologous transfer of muscle tissue and provides a valid alternative in muscle regeneration enhancement. The aim of this study was to investigate the suitability, as scaffold for skeletal muscle tissue engineering, of a known biodegradable block copolymer (DegraPol®) processed by electrospinning in the novel form of microfibrous membranes. Scaffolds were characterized with reference to their morphological, degradative and mechanical properties. Subsequently, cell viability, adhesion and differentiation on coated and uncoated DegraPol® slides were investigated using line cells (C2C12 and L6) and primary human satellite cells (HSCs). The membranes exhibited absence of toxic residuals and satisfactory mechanical properties (linear elastic behavior up to 10% deformation, E modulus in the order of magnitude of MPa). A promising cellular response was also found in preliminary experiments: both line cells and HSCs adhered, proliferated and fused on differently coated electrospun membranes. Positive staining for myosin heavy chain expression indicated that differentiation of C2C12 multinucleated cells occurred within the porous elastomeric substrate. Together the results of this study provide significant evidence of the suitability of electrospun DegraPol® membranes as scaffolds for skeletal muscle tissue engineering and that they represent a promising alternative to scaffolds currently used in this field.

Keywords: Block polyesterurethane; Electrospinning; Skeletal muscle tissue engineering; Satellite cell; Myoblast; Myotube

1. Introduction

Skeletal muscle, which is responsible for control of voluntary movement and maintenance of structural contours of the body, may be injured by exposure to myotoxic agents, such as bupivacaine or lidocaine; sharp or blunt trauma, such as punctures or contusions; ischemia, occurring with transplantation; exposure to excessively hot or cold temperatures [1]. Skeletal muscle tissue function is also affected by primary myopathies, which are characterized by a progressive wasting of skeletal muscle tissue that leads to deterioration of movements and, in the most severe cases, such as in Duchenne's Muscular Dystrophy (DMD), to complete paralysis and death [2,3].

Mature skeletal muscle is predominantly comprised of multinucleated, post-mitotic fibers that do not regenerate when damaged. Nevertheless, a population of quiescent myogenic progenitors, satellite cells, are capable of regeneration and compose 1–5% of the total nuclei of a mature muscle [4]. Satellite cells, sequestered between the sarcolemma and the individual mature muscle fibers, normally do not divide but can be induced to proliferate in response to specific local factors; they can migrate through the basal lamina sheets to enter the

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injured area, where they fuse with pre-existing, damaged fibers or fuse to form new myotubes [5]. Once the proliferation potential of satellite cells is exhausted, there is no further regeneration and skeletal muscle is replaced by connective tissue. When muscle structure is irreversibly compromised or individual muscles (or part of them) have been ablated by surgical procedures or major injuries, the perspective of engineering new muscle fibers via satellite cells becomes an attractive though difficult goal.

Engineering muscle constructs in vitro would overcome problems associated with autologous transfer of muscle tissue and provide a valid alternative for tissue replacement in the enhancement of muscle regeneration. In addition, advantages of a readily available, non-immunogenic muscle for reconstruction include elimination of donor site morbidity and reduction of operative time and length of rehabilitation [5].

Many attempts have been made so far to reconstruct skeletal muscle tissue in vitro [6–15]; together the results of these studies suggest that is indeed feasible to engineer bioartificial muscles; nonetheless, with current technology, tissue-engineered skeletal muscle analogues are far from being a clinical reality. Morphologically, they fall short of actual skeletal muscle in many respects, including small-diameter myofibers, low myofiber organization and excessive extracellular matrix content. Since myofibers need to be packed parallel to each other to generate sufficient force for contraction [10,16], the lack of structural organization in engineered constructs results in too little active force to make them useful in clinical applications.

A key factor to overcome these problems is the design of appropriate scaffolds able to support cell fusion and the formation of long, continuous muscle fibers, which are essential to the adult phenotype of muscle. In accordance with the established "contact guidance" theory, in fact, cell proliferation and differentiation can be directed in preferential directions associated with chemical, structural and/or mechanical properties of the substratum [17,18].

In this context, the recent tendency to develop microfibrous polymeric scaffolds, in most cases made of classic degradable polyesters (PLA, PGA and copolymers), revealed promising results and succeeded in driving myofiber development and orientation along the preferential direction of the scaffold fibers [17,19-20]; nevertheless, the majority of these scaffolds suffer from some disadvantages, mainly with respect to their high tensile modulus (in the order of magnitude of GPa) and low-yield elongation (3–4%) [21–24]. The relative inflexibility and the limited possibility to modulate the mechanical properties of the cited polymers make them hardly compatible with the widespread "dynamic culture approach" in bioreactors, based on the hypothesis that contractile functional skeletal muscle tissue will fully develop in vitro only if subjected to mechanical strains that prevail during normal in vivo organogenesis and growth [6–10].

In order to overcome the above cited problems, we propose the use of DegraPol®, a degradable block polyesterurethane, consisting of crystallizable blocks of poly((R)-3-hydroxybutyric acid)-diol and blocks of poly(ε-caprolactone-*co*-glycolide)-diol linked with a diisocyanate. The use of DegraPol® as scaffold for tissue engineering has been investigated for a long time: now there is significant evidence of its in vitro [25–27] and in vivo [27,28] biocompatibility properties, as well as of its elastomeric behavior [29]. The results of these studies, together with the possibility to process the polymer by electrospinning in the form of microfibrous membranes, suggest that DegraPol® hold promises to be used also as scaffold for skeletal muscle tissue engineering.

Hence, in this study electrospun DegraPol® membranes were characterized with reference to their morphological, degradative and mechanical properties. Subsequently, we investigated the behavior of different myogenic cell types (C2C12, L6 and primary human satellite cells (HSCs)) in response to the scaffolds, either uncoated or coated with different proteins (Matrigel®, fibronectin and collagen).

2. Materials and methods

2.1. DegraPol® electrospun membranes

DegraPol® scaffolds were manufactured by electrospinning after solving the block copolymer with chloroform (30 wt%): electrospinning voltage (18 kV) was applied with a high-voltage supply between a needle and a rotating cylindrical collector. As-spun membranes were dried under vacuum at room temperature. The membranes, about 100 μm thick, can be roughly described as reticulated meshes of randomly or partially ordered fibers (about 10 μm in diameter) fixed at intersecting points. Before cell culture, 13 mm diameter DegraPol® slides were cut and sterilized by immersion in 70% (v/v) ethanol overnight and allowed to dry at room temperature in a sterile hood.

2.2. Scaffold characterization

The morphology of DegraPol® scaffolds was examined by means of a scanning electron microscope (SEM): samples were sputter coated with gold (Emitech K550, 4 min, 20 mA, 10^{-1} mbar) before examination under a Stereoscan S360 microscope (Cambridge Instruments) at an accelerating voltage of 15 keV.

As to the polymer degradation profile, in vitro degradation experiments were performed as follows: