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Schwann-cell cylinders grown inside hyaluronic-acid tubular scaffolds with gradient porosity

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ABSTRACT

Cell transplantation therapies in the nervous system are frequently hampered by glial scarring and cell drain from the damaged site, among others. To improve this situation, new biomaterials may be of help. Here, novel single-channel tubular conduits based on hyaluronic acid (HA) with and without poly-L-lactide acid fibers in their lumen were fabricated. Rat Schwann cells were seeded within the conduits and cultured for 10 days. The conduits possessed a three-layered porous structure that impeded the leakage of the cells seeded in their interior and made them impervious to cell invasion from the exterior, while allowing free transport of nutrients and other molecules needed for cell survival. The channel's surface acted as a template for the formation of a cylindrical sheath-like tapestry of Schwann cells continuously spanning the whole length of the lumen. Schwann-cell tubes having a diameter of around 0.5 mm and variable lengths can thus be generated. This structure is not found in nature and represents a truly engineered tissue, the outcome of the specific cell–material interactions. The conduits might be useful to sustain and protect cells for transplantation, and the biohybrids here described, together with neuronal precursors, might be of help in building bridges across significant distances in the central and peripheral nervous system.

Statement of significance

The paper entitled "Schwann-cell cylinders grown inside hyaluronic-acid tubular scaffolds with gradient porosity" reports on the development of a novel tubular scaffold and on how this scaffold acts on Schwann cells seeded in its interior as a template to produce macroscopic hollow continuous cylinders of tightly joined Schwann cells. This cellular structure is not found in nature and represents a truly engineered novel tissue, which obtains as a consequence of the specific cell-material interactions within the scaffold.

1. Introduction

Ideas for neural cell therapies have flourished since it was discovered that tissue regeneration was viable [1] even in the central nervous system (CNS) [2,3]. However, the regeneration in CNS tracts has not proved to be satisfactory due to the poor axonal growth rate of adult neurons [4,5] and neurite retraction after a short time if no synaptic connection has been reached [6]. In addition, astrocyte activation after trauma leads to a glial scar which, though advantageous in preventing the lesion from spreading, is detrimental for the reconnection of neural populations [7].

Proposals to overcome these difficulties involve the use of systems composed of biocompatible structures supplying some of the functions of the lost extracellular matrix, exogenous or endogenous healthy cells transplanted within scaffolds, and different cytokines or molecular cues to provide a regenerative guide [8]. A number of studies aiming at the repair of nerve or brain tissue have been conducted with promising results, especially those which used cylindrical or tubular structures to mimic nervous tracts [9–11]. These anisotropic structures provide physical guidance for cell migration and axonal penetration and elongation

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and they permit the investigation of the effects of guidance stimuli to achieve efficient functional regeneration.

Schwann cells (SCs) are the main supportive cells of neurons in the peripheral nervous system (PNS), and play an important role in neurodegeneration and protection: they have an immunomodulatory effect and they wrap axons creating the myelin sheath around them [12,13]. Furthermore, they constitute a source of multiple growth factors which promote axon regeneration and angiogenesis [14,15]. SCs have been extensively employed in *in vitro* cultures [16,17] and *in vivo* models [18,19] as candidates for restoring defects in nerve bundles. Although they are cells of the PNS, Schwann cells are able to regenerate and myelinate axons also in the CNS when transplanted [15,20,21]; for these reasons, SCs have been proposed as cells for transplantation in therapies for spinal cord injury and other CNS disorders [22–27].

However, *in vivo* experiments with glia, neuronal precursors and stem cells [28,29] show a significant loss of transplanted cells at the site of grafting, leading to a poor restoration of functional properties [30]. In order to face this problem, porous scaffolds with tailored properties and structure can be used to provide cells with a friendly environment and to prevent them from leaking out of the lesion site [18,19,31].

Different types of hyaluronic acid (HA)-based scaffolds have been implanted into CNS lesions to evaluate their biocompatibility and potential for inducing neural regeneration [31–34]. HA is a glycosaminoglycan present naturally in the extracellular matrix of several tissues in the human body. As a biomaterial, it is biodegradable, biocompatible and resorbable. It is composed of hydrophilic polymeric chains and has numerous advantages compared with other hydrogels, since it can be easily processed, delivered in a minimally invasive way, and has mechanical properties similar to soft nervous tissues [35,36]. In addition, its degradation rate and the diffusion of active molecules through it can be modulated by selecting a specific range of molecular weights, or by using different crosslinking agents and fabrication procedures. Furthermore, HA induces a low inflammatory response and may contribute to neovascularization through its low-molecular weight oligomers [31,37,38]; in addition, it can be chemically functionalized in different ways to improve cell adhesion, proliferation, migration and differentiation [39,40]. For all these reasons, HA seems a promising material for nerve and brain tissue repair [31,38–41].

The stabilization of the HA hydrogel through crosslinking [42,43] is mandatory if one wishes to obtain structures that are insoluble and capable of retaining their shape. Here we employ divinyl sulfone (DVS) as a cross-linking agent. DVS is known to be toxic because of the reactivity of its vinyl groups [44,45], so its use implies a risk of toxicity if remnants of this molecule are not completely washed from the material. However, once reacted with the OH groups of the HA molecule these double bonds disappear, and, if non-reacted molecules are thoroughly removed, the resulting HA-DVS networks are devoid of inflammatory, pyrogenic or cytotoxic effects [46–49]. HA-based materials employed in clinic for several uses in humans contain HA-DVS networks have been approved by the US Food and Drug Administration (FDA) [50,51].

In the present work, a concept is developed which might find application in the regeneration of neural tracts in the CNS and of nerves in the PNS. A HA conduit with a cylindrical inner channel and aligned poly-L-lactic acid (PLLA) microfibers located in its lumen has been produced and characterized, and tested for cultures of Schwann cells. The concept rests on the hypothesis that the regeneration of neural tracts will require a niche of cells supportive for axon growth (such as Schwann cells and maybe other neuroglia) and a structured, directional material substrate, onto which neurons may project their axons. In our concept this directional substrate is provided by the PLLA microfibers, while the HA conduit, thanks to the peculiar pore morphology of its walls, should be able to isolate and protect the transplanted supportive cells seeded in its interior from the external hostile microenvironment, permitting oxygen and nutrients exchange and removal of waste products. The Schwann cells in the interior of the conduit, besides being a pump for neurotrophic and angiogenic factors, should act as natural scaffolding for axon outgrowth, and the microfibers should guide axonal growth. Our study represents, thus, a first step in this wider concept; here solely the interaction of Schwann cells with the materials of the conduit has been investigated and characterized.

2. Materials and methods

2.1. Materials preparation

A polytetrafluoroethylene (PTFE) thin block with 1.5 mm-wide grooves of square section was used as a mold for the conduits (sketch in Fig. 1A). In each groove, a single poly-ε-caprolactone (PCL; PolySciences) fiber of 400-450 μm diameter was located using PTFE washers having external diameters of 1.5 mm every 3 cm of fiber in order to keep it centered. These fibers acted as a template for the lumen of the conduits. Solutions of HA (1.5–1.8 MDa, from Streptococcus equi; Sigma–Aldrich) 1%, 3% and 5% wt/wt in sodium hydroxide 0.2 M (NaOH; Scharlab) were prepared by gently stirring for 24 h. Conduits made with such solutions are named onwards HA1, HA3 and HA5, respectively. Divinyl sulfone (DVS; Sigma–Aldrich) was then added as a crosslinker (via a Michael 1,4-addition reaction) in a 9:10 DVS:HA monomeric units molar ratio. Following its addition, the solutions were stirred for 10 s more and injected in the mold grooves.

After 10 min the mold was set in a closed Petri dish to avoid evaporation and chilled to −20 °C for a minimum of 5 h. After fully frozen, the solution in the mold was lyophilized (Lyoquest-85, Telstar) for 24 h at 20 Pa and −80 °C to generate HA microporous matrices due to the sublimation of water crystals. Next, the channel-generating PCL fibers were carefully removed, resulting in the final conduits. One of the exterior surfaces of the conduit, thus, remains in contact with air during the fabrication process. This upper side of the conduit will be referred to as its *top face*. Finally, the conduits were hydrated in distilled water for 2 h, cut 6 mm length, and stored at 4 °C in sterile water until use (up to 4 weeks).

HA porous films (hereinafter HAf) were also prepared by injecting the 5% wt HA solution with DVS in the same proportion as above in a glass *Petri* dish and drying them for 24 h under a ventilation hood at RT, in order to compare them with the conduits and study the effect of the 3D architecture.

In some of the HA5 conduits thus prepared 20 PLLA fibers of 30 μm diameter (Natureworks 6251D, Ingeo) were located inside their channel, as a loose bundle of individual fibers; the conduits-with-fibers thus prepared are henceforth referred to as HA5-PLLA conduits. Also, PLLA films (hereinafter PLLAf) were obtained by solvent-casting from a solution of PLLA pellets (PURASORB PL 18 with inherent viscosity 1.8 dl/g, Corbion Purac) in chloroform at a 10% w/v concentration and air-dried for 24 h at RT. The HAf and PLLAf membranes obtained were die-cut into 7 mm-diameter disks, to obtain a seeding area in the planar substrates comparable to glass coverslips (same size), used as positive controls in cell culture experiments.

2.2. Morphological analysis

The conduits' structure and microporosity was observed in a JSM 6300 scanning electron microscope (SEM; JEOL) at 10 kV after

ID	Title	Pages
186	Schwann-cell cylinders grown inside hyaluronic-acid tubular scaffolds with gradient porosity	13