Chitosan Micro-Grooved Membranes with Increased Asymmetry for the Improvement of the Schwann Cell Response in Nerve Regeneration

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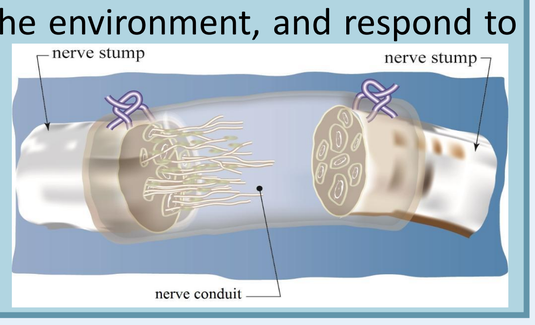
1. Introduction

- **Peripheral nerve injuries** (PNIs), are a common type of injury around the world, affecting more than one million people every year [1]. There are still no efficient therapeutic treatments for PNIs.
- **Nerve guidance conduits (NGCs)**, artificial scaffolds for nerve regeneration, represents a new strategy in the treatment of PNIs [2].
- **Chitosan** is a promising natural polymer, obtained via the controlled deacetylation of chitin [3]. This biomaterial is completely biocompatible and non-immunogenic. Plain **chitosan nerve conduits** are already in use in Europe [4].
- **Cells can perceive** physico-mechanical stimuli from the environment, and respond to them, in a process called **mechanotranducsction** [5].
- New evidence shows that **cells can respond also to the symmetry of patterns** [6].

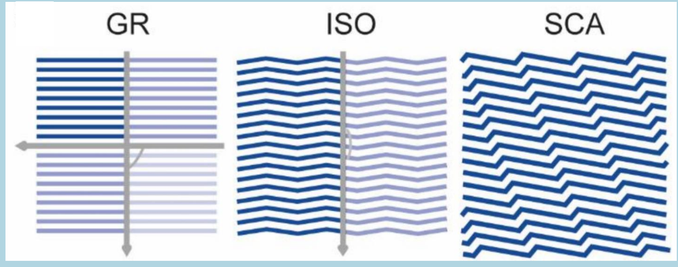


Schematic representation of the implantation and functioning of a polymeric **nerve conduit**

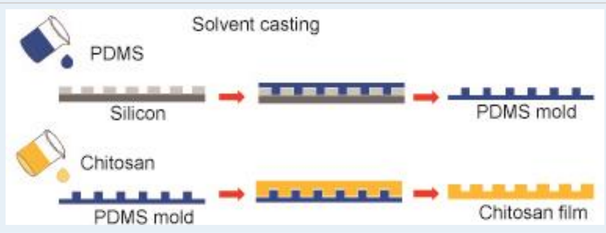
2. Methods

- Here, we **develop and test in vitro chitosan-based microstructured substrates**, aiming to introduce new strategies to improve the scaffolds for peripheral nerve regeneration.
- The patterns on the chitosan membranes present **various levels of asymmetry**.
- **Schwann cell (RT4-SC)-material interaction** is studied via immunocytochemistry and optical and confocal microscopy.

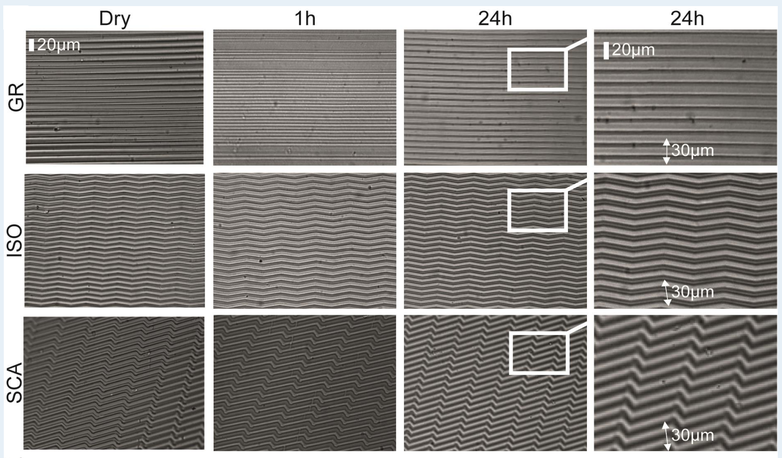
CAD designs of the three patterns: gratings (GR), isosceles triangles (ISO), and scalene triangles (SCA).



3. Chitosan Micropatterned Membranes

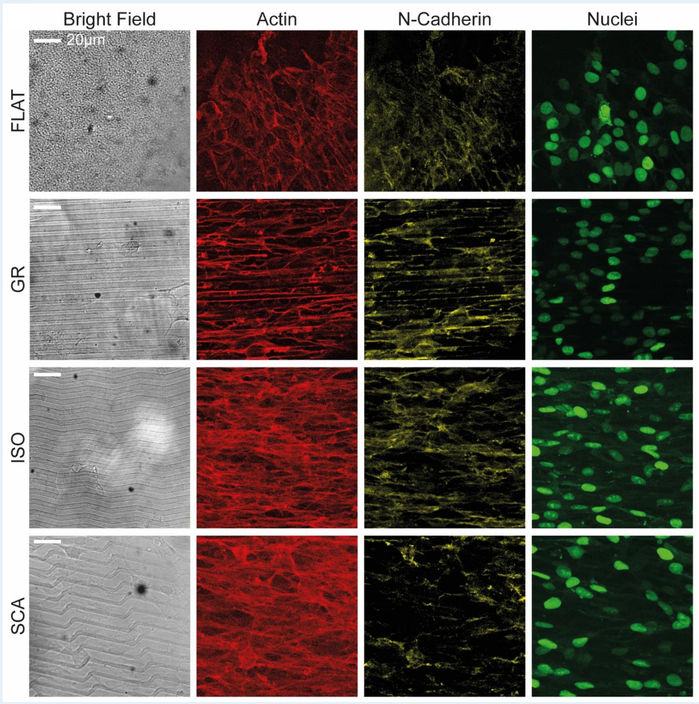


The **microtextured chitosan membrane fabrication** process with **two molds** by replica molding and solvent casting.

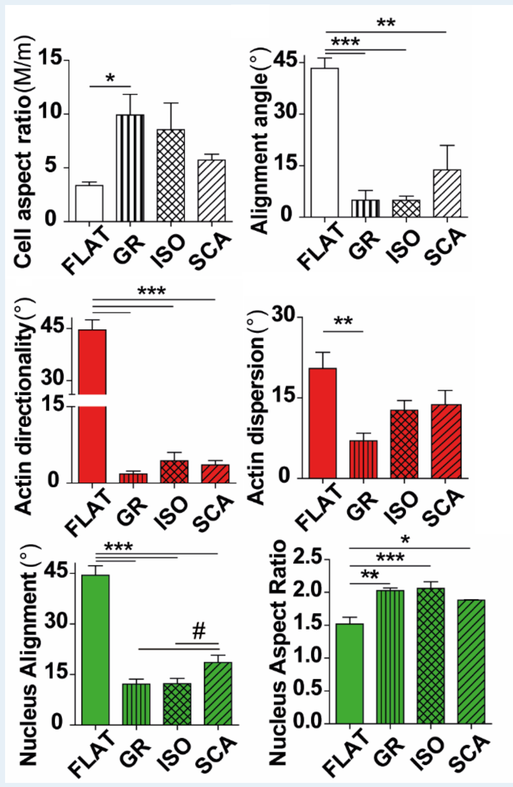


Optical microscope images of chitosan micropatterned membranes for the three patterns: GR, ISO, and SCA.

4. Cell growth and morphology

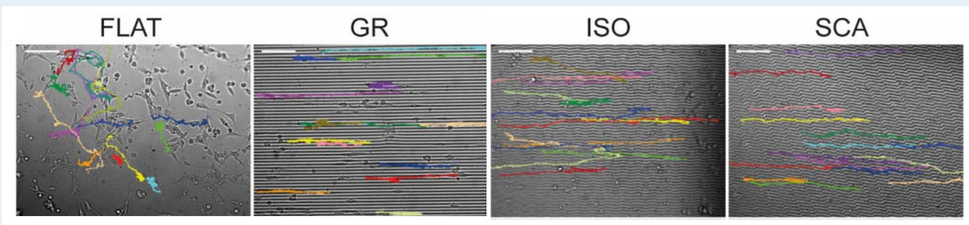


Confocal images of RT4-Schwann cells cultured on GR, ISO, SCA and FLAT chitosan membranes.

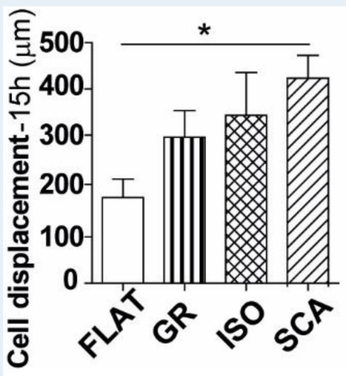


RT4-Schwann cell morphological analysis.

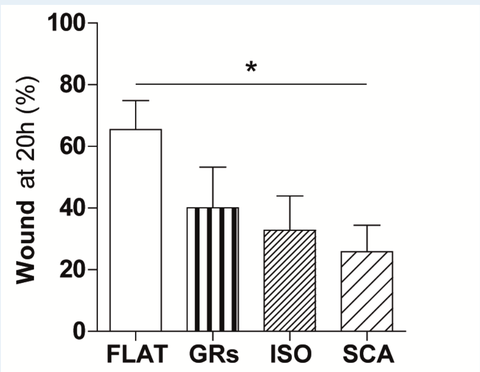
5. Cell migration



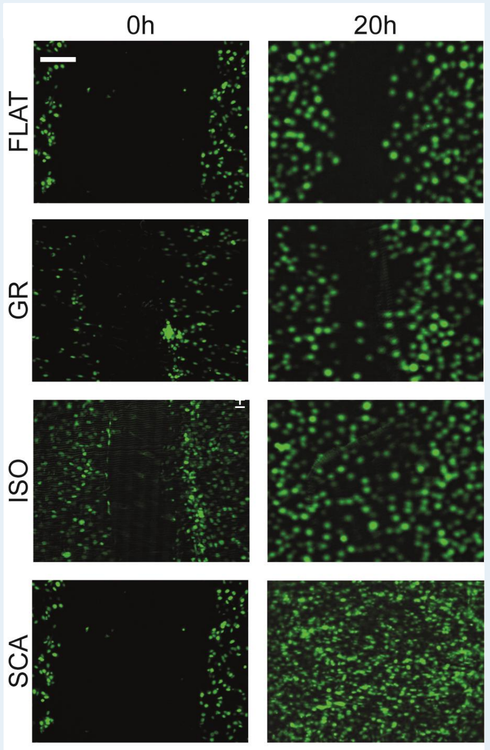
Single cell migration.



Single cell migration analysis: final cell displacement (at t=15 h)

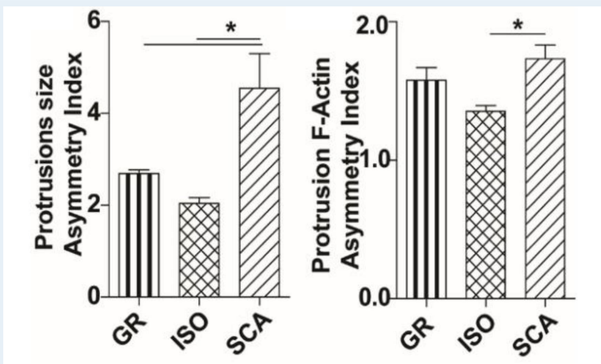


Wound-healing quantification

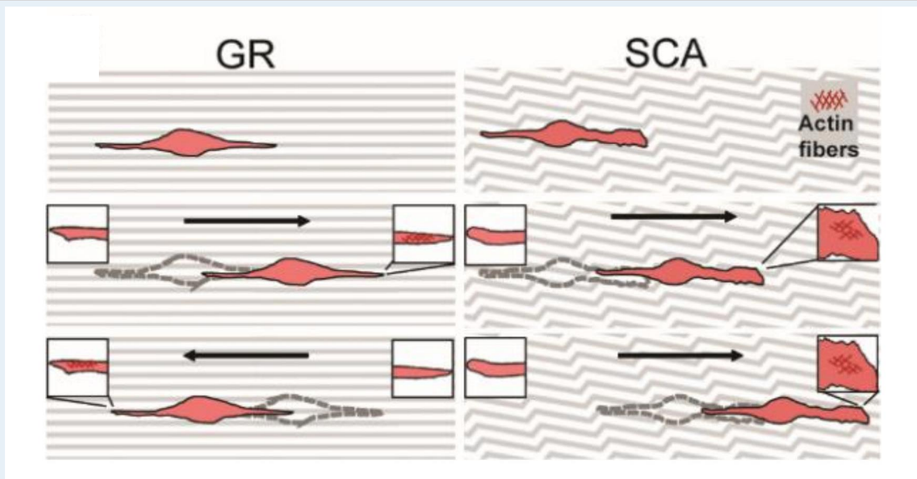


Collective cell migration.

6. Proposed mechanism



Cell protrusion size asymmetry index



Model of the asymmetric development and directional migration of RT4-SCs on SCA chitosan substrates. The symmetry of the pattern influences these processes, promoting or demoting differently the spreading dynamics at the two cell edges on GR or SCA. The black arrows indicate the cell migration direction.

7. Conclusions

- The films presented **precise and stable directional and asymmetric micro-topographies.**
- Asymmetric directional topographies (ISO and SCA) orientated Schwann cells leaving a **higher degree of freedom to cell shaping**.
- The **asymmetry of SCA reflected on the cell shape**, leading to cells with one end bigger than the other.
- SCA promoted a **farther displacement of the cells** and an increased persistence on one direction of migration.
- Overall, the **SCA chitosan membranes showed a promising topography** for the improvement of Schwann cells migration performance, and therefore of nerve regeneration scaffolds.

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