Chitosan blended micro-grooved membranes for nerve regenerative applications

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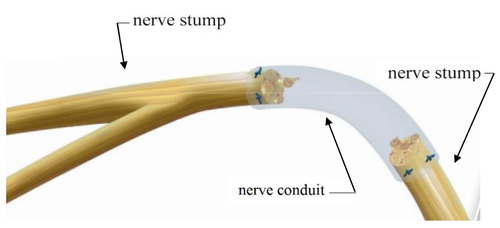
1. Introduction

- **Peripheral nerve injuries (PNIs)**, are a critical problem 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 emerging as a promising FDA-approved biopolymer for tissue engineering thanks to its properties of biocompatibility and biodegradability [3]. 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 **mechanotranducstion** [5]**.**
- We already demonstrated that **chitosan micro-grooved membranes** orient Schwann cells, with various degrees depending on the asymmetry of the pattern [6].

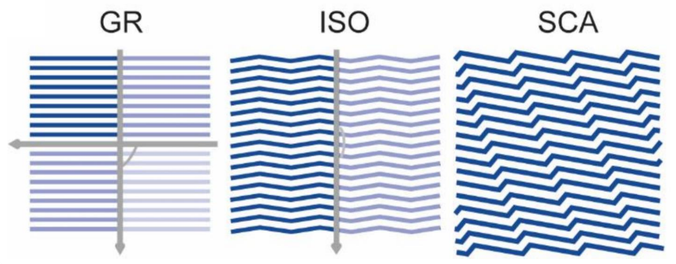
Schematic representation of the implantation and functioning of a polymeric **nerve conduit**



2. Materials and Methods

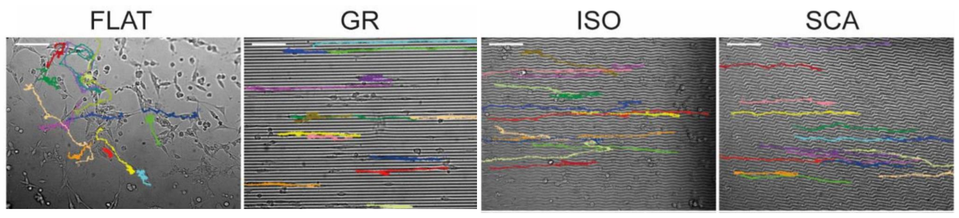
In this work, we **develop and test in vitro chitosan-glycerol blended microstructured substrates**, aiming to improve the effectiveness of our micro-grooved chitosan membranes.

- **Glycerol** is mixed in the chitosan solution at a 10% w/w concentration.
- **The mechanical properties of the membranes** are studied via stretching tests and differential scanning calorimetry (DSC).

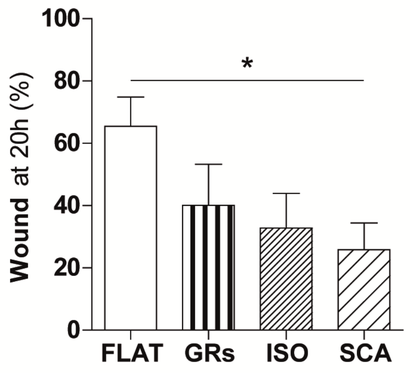


CAD designs of the three patterns: gratings (GR), isosceles triangles (ISO), and scalene triangles (SCA).

3. Previous results

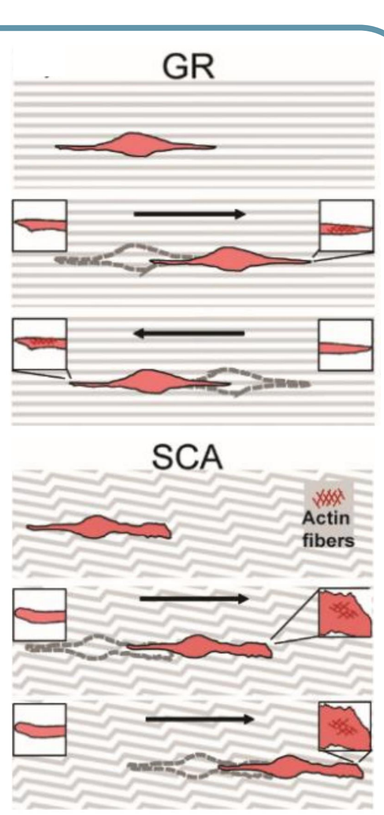


Single cell migration.



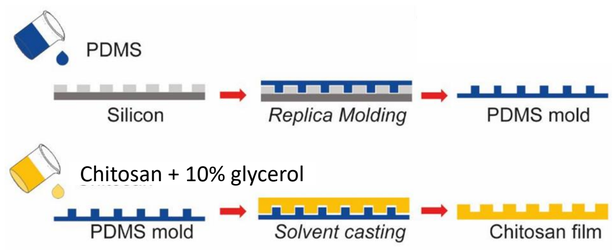
Wound-healing quantification

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.



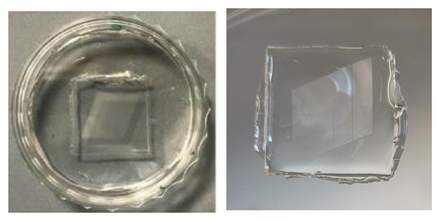
Scaccini, L.; Mezzena, R.; De Masi, A.; Gagliardi, M.; Gambarotta, G.; Cecchini, M.; Tonazzini, I. Chitosan Micro-Grooved Membranes with Increased Asymmetry for the Improvement of the Schwann Cell Response in Nerve Regeneration. Int. J. Mol. Sci. 2021, 22, 7901. https://doi.org/10.3390/ijms22157901

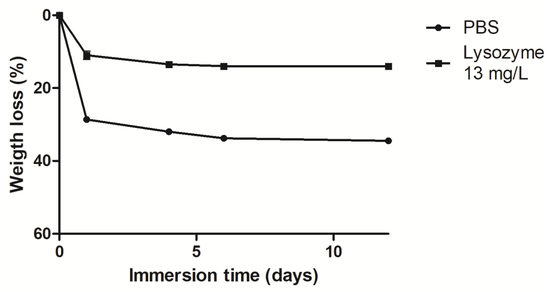
4. Chitosan-glycerol blended membranes



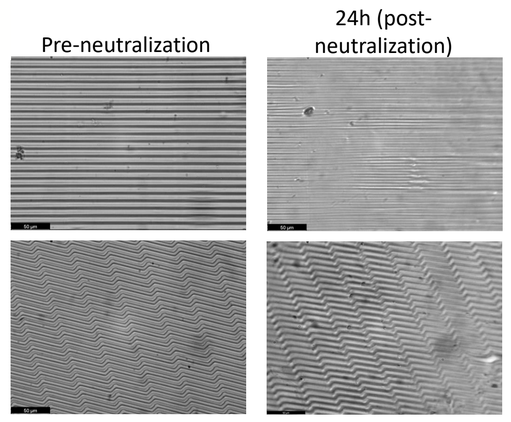
The microtextured blended chitosan membrane fabrication process with two molds by replica molding and solvent casting.

Images of a PDMS mold (left) and a chitosan-glycerol blended micropatterned membrane (right).



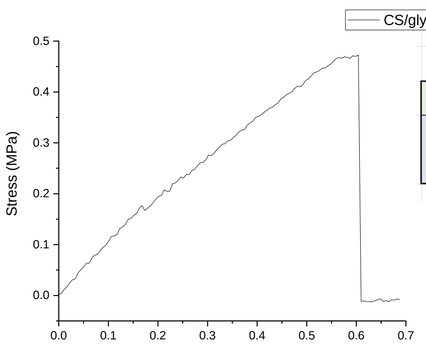


Weight loss of blended chitosan membranes at 37 °C for different time periods; after storage in PBS and after storage in 13 mg/L of lysozyme solution



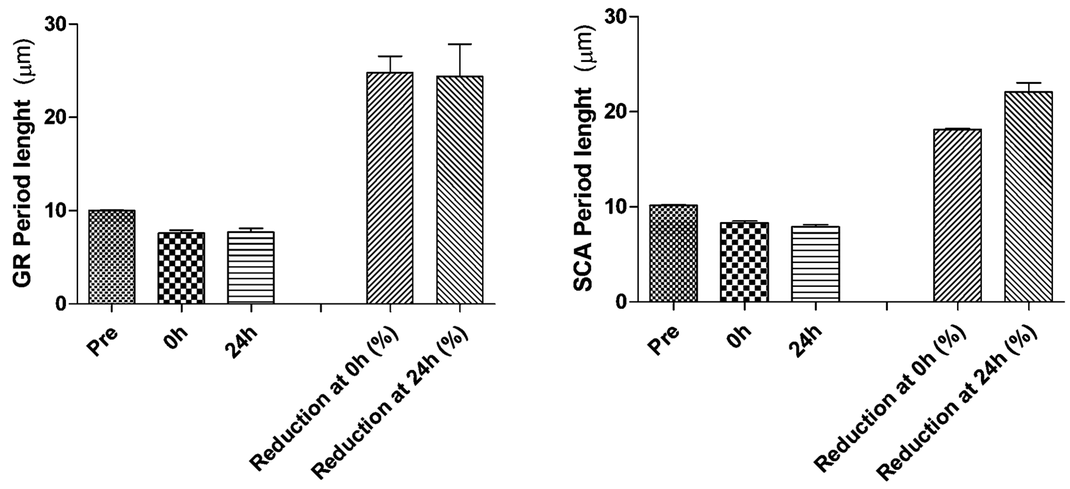
Optical microscopy images of the patterns before and after the neutralization process.

5. Mechanical and physical properties



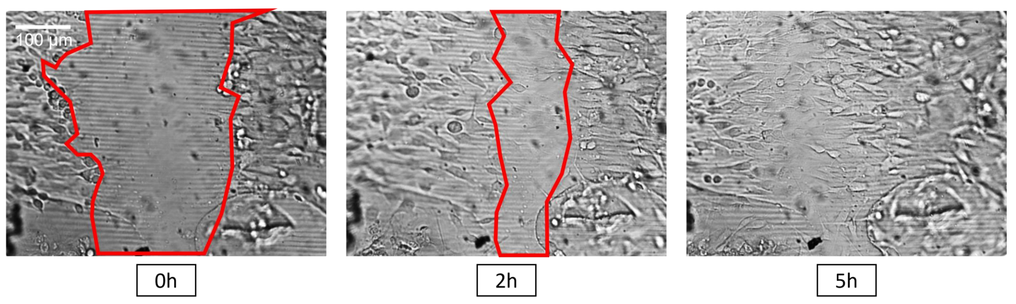
	Young's modulus (MPa)	Stress at break (MPa)	Strain at break (%)
Chitosan medium weigth	5.0 ± 1.0	5.0 ± 1.0	130 ± 11
Chitosan medium weigth + 10% glycerol	0.752 ± 0.087	0.824 ± 0.18	105 ± 58

Stress-strain curve for chitosan-glycerol blended membranes (left) and comparison of mechanical properties of chitosan-glycerol blended membranes and chitosan only membranes (upper).



Reduction in dimensions of micro-grooved chitosan-glycerol blended membranes after the neutralization process.

6. Cell-material interactions



Collective cell migration of RT4 D6P2T Schwann cell line. RT4 cells completely close the wound after 5 hours.

7. Conclusions

- The membranes presented **precise and stable directional and asymmetric micro-topographies.**
- The addition of glycerol **lowered the stiffness of the material.**
- The neutralization process leads to a **shrinkage of the membranes** of around 20%.
- The blending with glycerol **does not seem to affect the biocompatibility of the membranes, nor their guidance capability.**

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