IMPROVING NERVE REGENERATION WITH CHITOSAN BLENDED MICRO-GROOVED MEMBRANES

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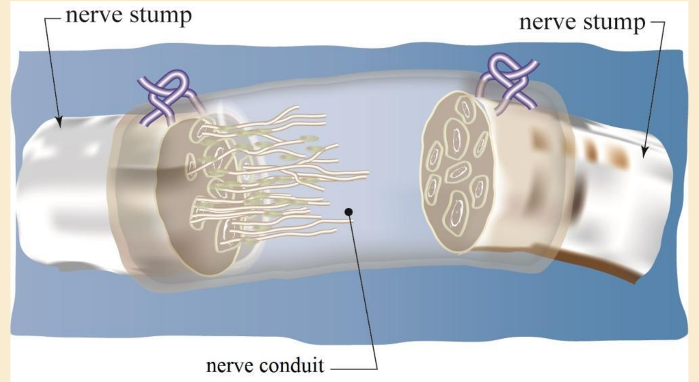
1 INTRODUCTION

Cells can perceive physico-mechanical stimuli from the environment, and respond to them, in a process called **mechanotranduction** [1].

Peripheral nerve injuries (PNIs), are a critical problem around the world, affecting more than one million people every year [2]. There are still no efficient therapeutic treatments for PNIs.



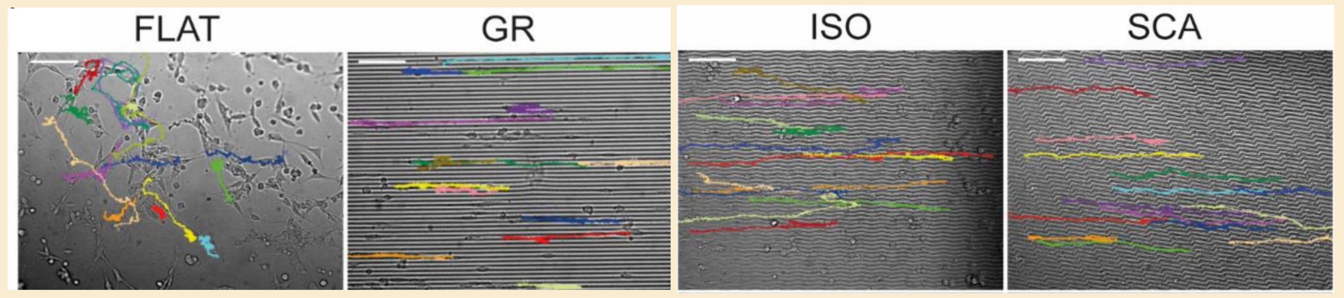
Nerve guidance conduits (NGCs), artificial scaffolds for nerve regeneration, represents a new strategy in the treatment of PNIs [3].

Chitosan is emerging as a promising FDA-approved biopolymer for tissue engineering thanks to its properties of biocompatibility and biodegradability [4]. Plain chitosan nerve conduits are already in use in Europe [5].

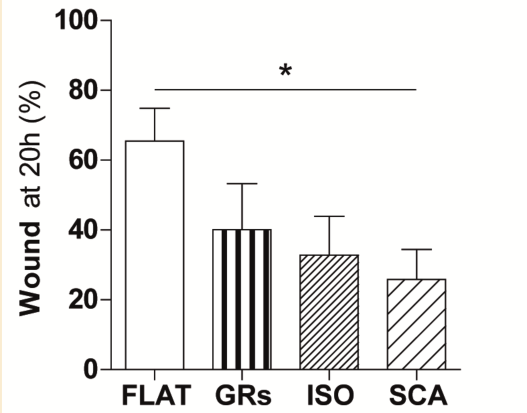
We already demonstrated that **chitosan micro-grooved membranes** orient Schwann cells, with various degrees depending on the asymmetry of the pattern [6].

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

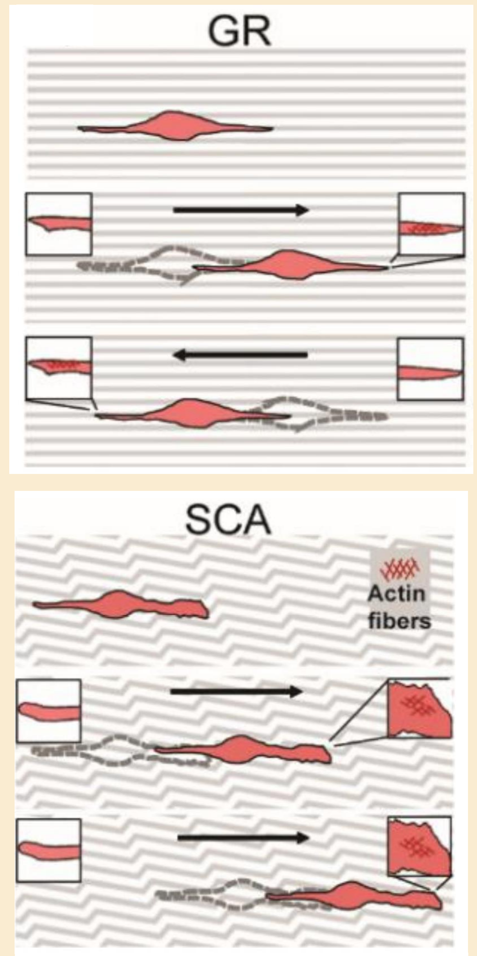
2 THE ASYMMETRY ROLE



Single cell migration



Wound-healing quantification



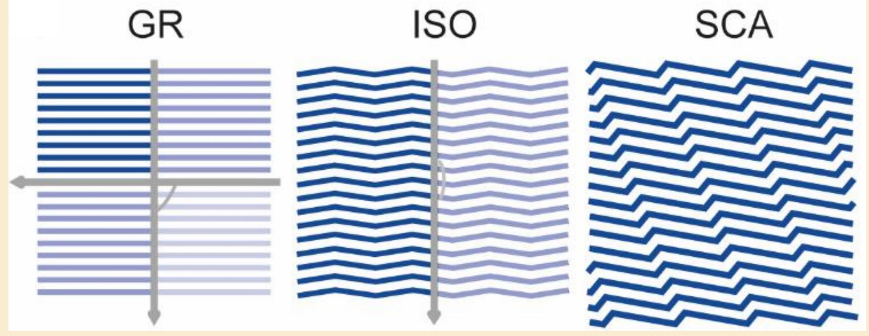
Model of the asymmetric development and directional migration of RT4-SCs on SCA chitosan substrates. The symmetry of the pattern influences these processes, promoting or demoting differently the spreading dynamics at the two cell edges on GR or SCA. The black arrows indicate the cell migration direction.

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

3 MATERIALS AND METHODS

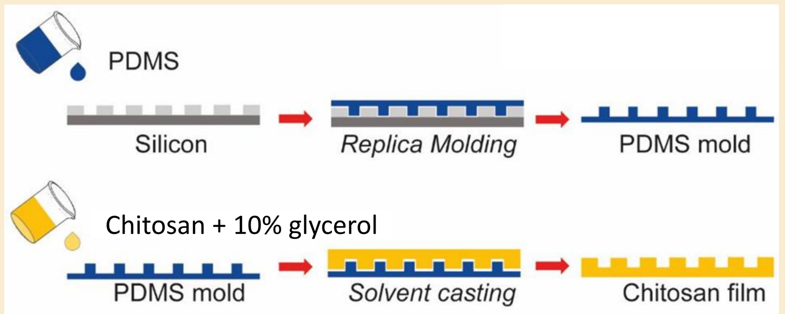
In this work, we develop, and test in vitro chitosan-glycerol blended microstructured substrates, aiming to improve the effectiveness of our micro-grooved chitosan membranes.

- **Glycerol** is mixed in the chitosan solution at a 10% v/v concentration.
- The **membranes' microstructure** is assessed through optical microscopy and atomic force microscopy (AFM).
- The **mechanical properties** of the membranes are studied via stretching tests, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).
- **Cell-material interaction** is tested using RT4-D6P2T Schwann cell line cultured on the surface of the microstructured membranes.
- We show **collective cell migration** experiments.

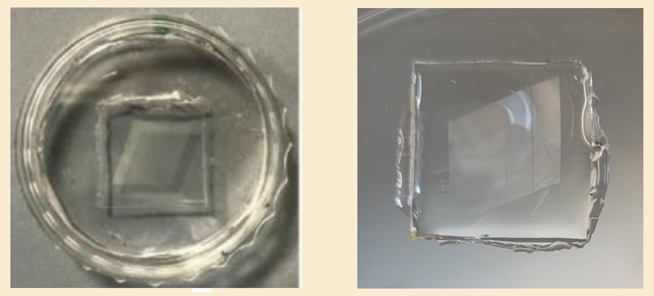


CAD designs of the three patterns: gratings (GR), isosceles triangles (ISO), and scalene triangles (SCA).

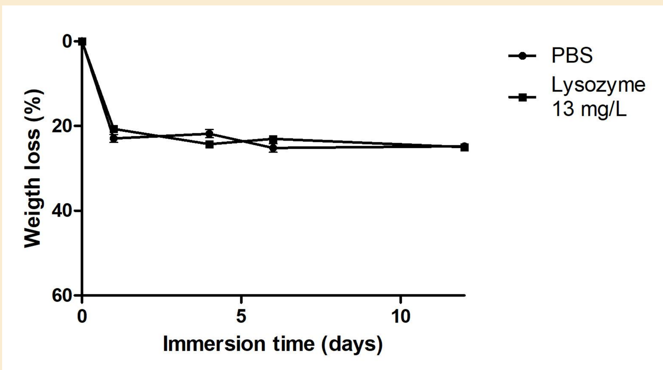
4 THE CHITOSAN-GLYCEROL BLENDED MEMBRANES



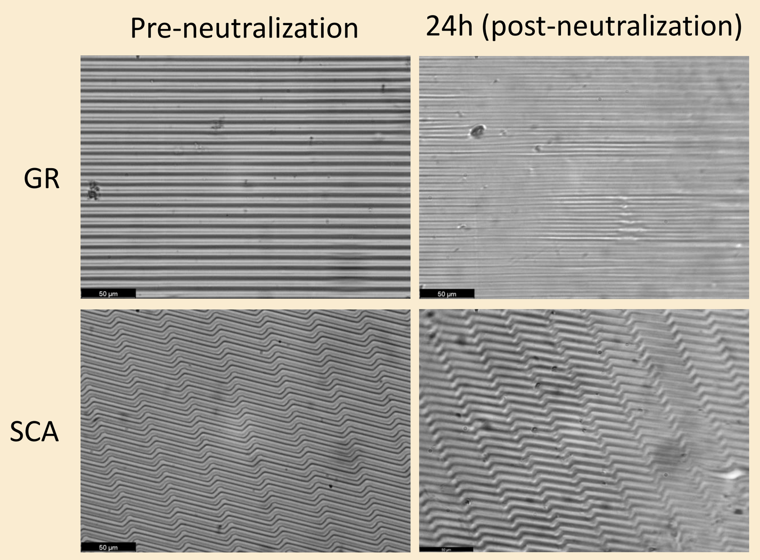
The microtextured blended chitosan membrane fabrication process with two molds by replica molding and solvent casting.



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

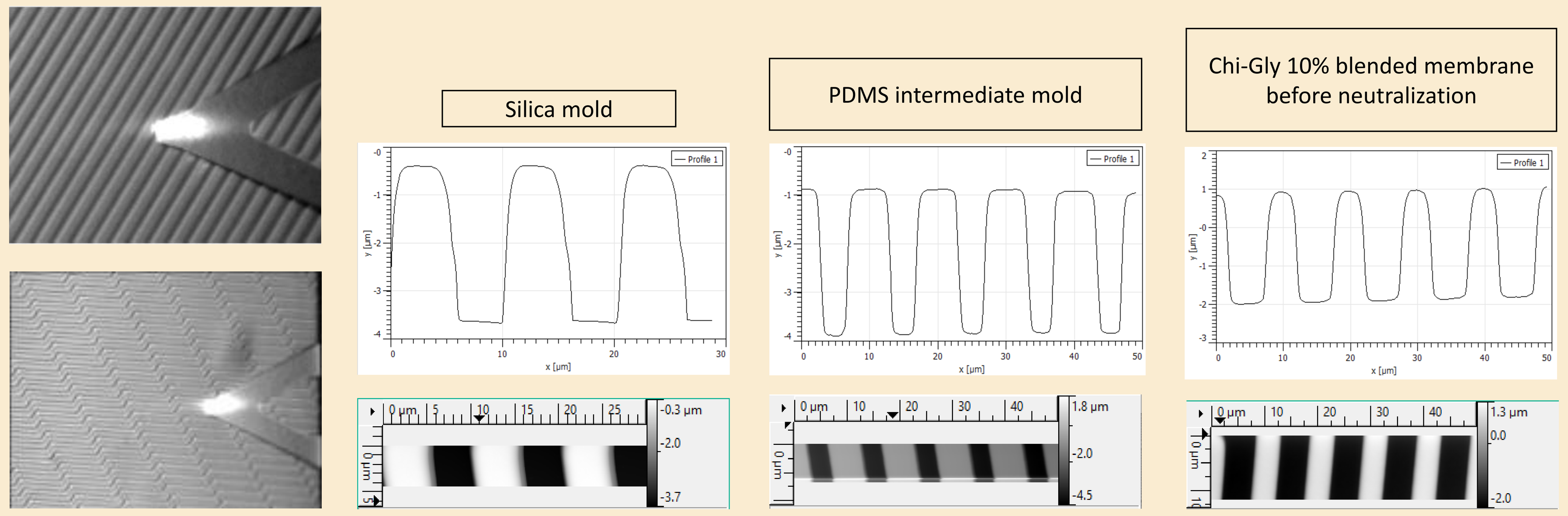


Weight loss of blended chitosan membranes at 37 °C for different time periods; after storage in PBS and after storage in 13 mg/L of lysozyme solution.

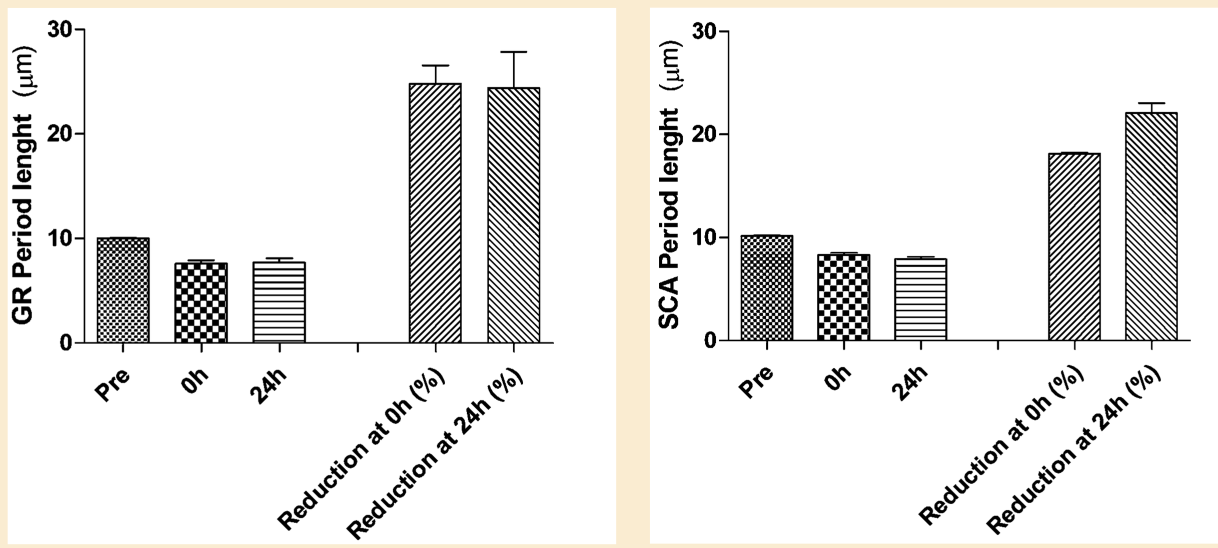


Optical microscopy images of the patterns before and after the neutralization process.

5 MICROSCOPICAL CHARACTERIZATION



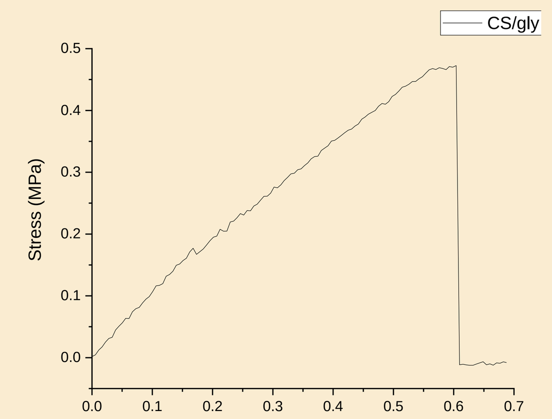
Atomic force microscopy (AFM) measurements of the lateral dimensions of the silica and PDMS molds and of the chitosan-glycerol blended membranes, before the neutralization process.



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

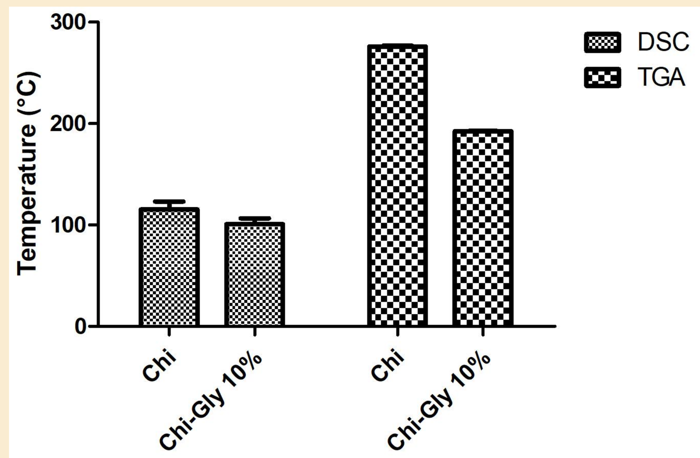
6 MECHANICAL PROPERTIES

	Young's modulus (MPa)	Stress at break (MPa)	Strain at break (%)
Chitosan medium weigth	5.0 ± 1	5.0 ± 1	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).

The value of 0.752 MPa for the Young's modulus is similar to that of the peripheral nerve.

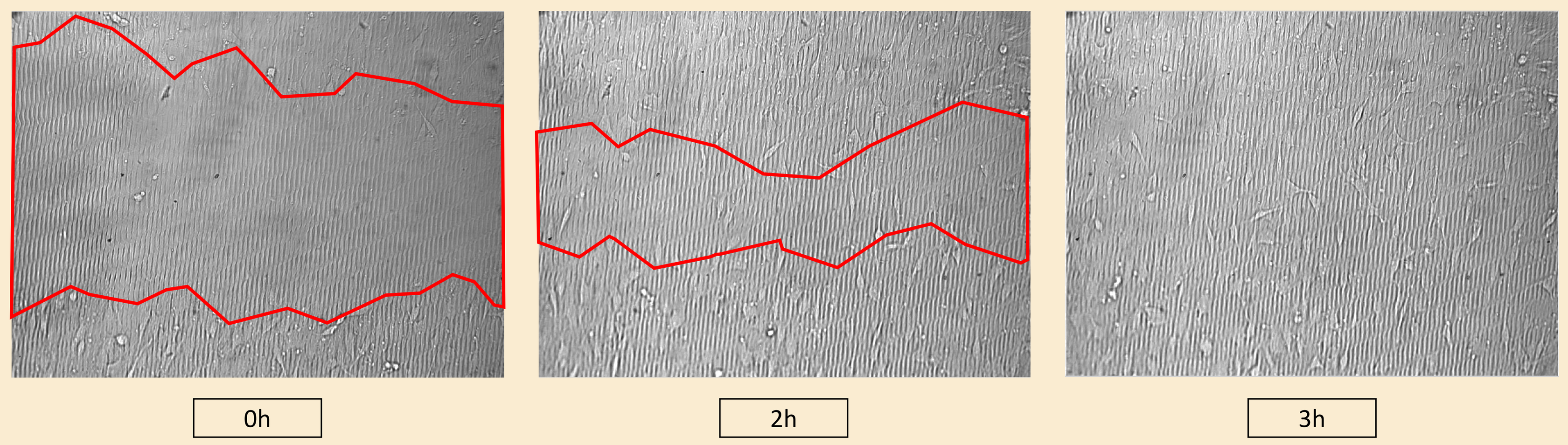


Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) of chitosan and chitosan-glycerol blended membranes.

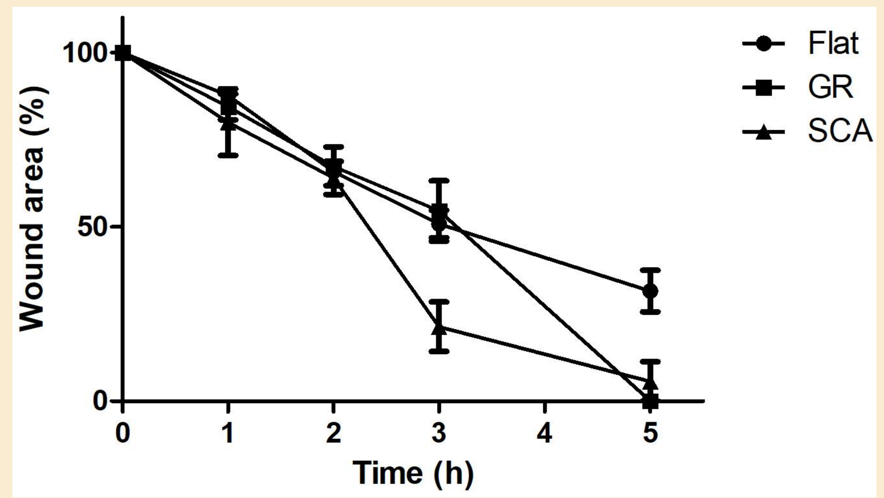
8 CONCLUSIONS

- The membranes presented **precise and stable directional and asymmetric micro-topographies, the dimensions are in the contact guidance range**.
- By blending the chitosan with glycerol, we **lowered the stiffness of the material**, which is now similar to that of a **peripheral nerve**.
- The blending with glycerol **does not seem to affect the biocompatibility of the membranes, nor their guidance capability**.
- **SCA promoted a better performance** in wound healing experiments.
- Overall, **this method is simple, reliable** and allows to produce precise micro-structured, chitosan-glycerol blended membranes.

7 MATERIAL-CELLS IN VITRO INTERACTION



Collective cell migration of RT4 D6P2T Schwann cell line. RT4 cells completely close the wound after 3/5 hours.



Wound healing quantification.

References:
[1] Tonazzini, I.; Cecchini, M. Front. *Nanomed.* 2017
[2] Sullivan, R.; Dailey, T.; Duncan, K.; Abel, N.; Borlongan, C.V. *Int. J. Mol. Sci.* 2016
[3] Du, J.; Chen, H.; Qing, L.; Yang, X.; Jia, X. *Biomater. Sci.* 2018
[4] Foster, L.J.; Ho, S.; Hook, J.; Basuki, M.; Marcal, H. *PLoS ONE* 2015
[5] Shapira, Y.; Tolmasov, M.; Nissan, M.; Reider, E.; Koren, A.; Biron, T.; Bitan, Y.; Livnat, M.; Ronchi, G.; Geuna, S.; et al. *Microsurgery* 2016
[6] Scaccini, L.; Mezzena, R.; De Masi, A.; Gagliardi, M.; Gambarotta, G.; Cecchini, M.; Tonazzini, I. Int. J. Mol. Sci. 2021