Engineered Chitosan–Lignosulfonate Films as Sustainable Biomaterials for Regenerative Medicine

Marialaura Giannaccari[1], Daniele Massari[2], Nora Bloise[1,3], Matteo Gigli[2], Livia Visai[1,3]

[1]Molecular Medicine Department (DMM), Centre for Health Technologies (CHT), Unità di Ricerca (UdR) INSTM, Operative Unit (OU) of the Interuniversity Center for the Promotion of the 3Rs Principles in Teaching and Research (Centro 3R), University of Pavia, 27100 Pavia, Italy;

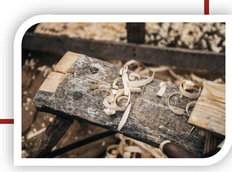
[2]Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155, 30172 Venezia Mestre, Italy;

[3]UOR6 Nanotechnology Lab., Department of Prevention and Rehabilitation in Occupational Medicine and Specialty Medicine, Istituti Clinici Scientifici Maugeri IRCCS,27100 Pavia, Italy.

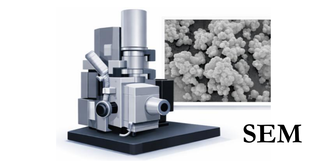
Email:marialaura.giannaccari01@universitadipavia.it

Aim of the work

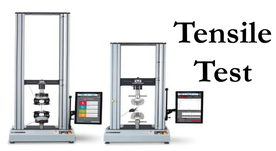
Lignin is a naturally biopolymer increasingly explored in biomedical applications due to biocompatibility. This study aimed to develop and characterize of eco-sustainable films based on deacetylated chitin nanocrystals (CsNCs), functionalized with different concentrations of lignosulfonate (L), as potential support for tissue regeneration.

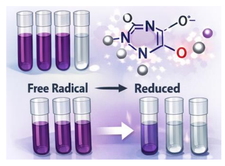


Material & Methods

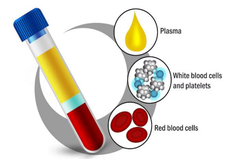












Results

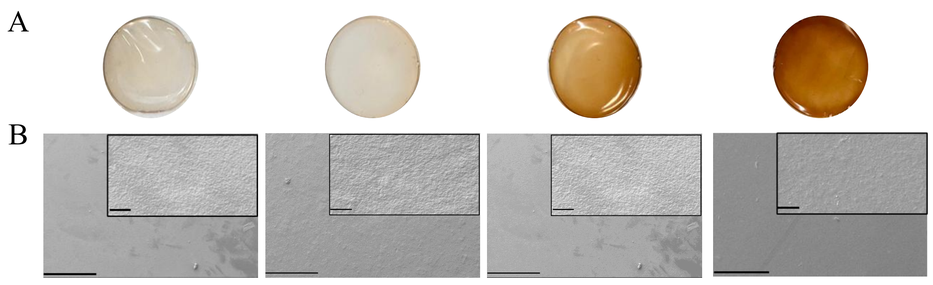


Figure 1..A) Macroscopic photographs of CsNC-based films containing different concentrations of lignin. **B)** Corresponding SEM images acquired after gold sputtering at an accelerating voltage of 3 kV (magnifications: 500×, scale bar = 100 µm; and 5.0k×, scale bar = 10 µm).

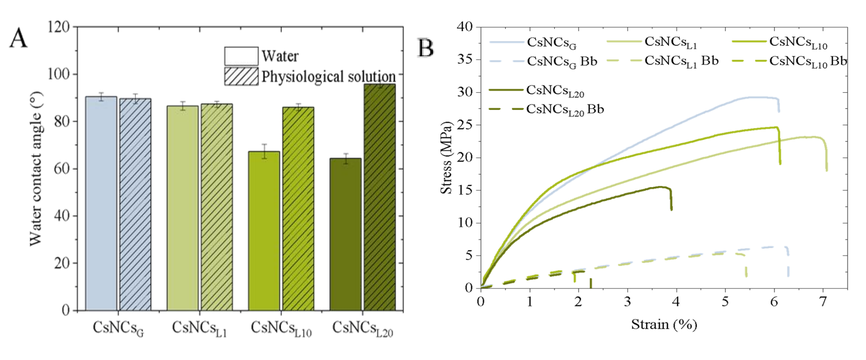


Figure 2. A) static contact angle of CsNCs-based films. **B)** Representative stress-strain curves of CsNCs-based films in air and submerged in a physiological solution at 37°C (Bb samples).

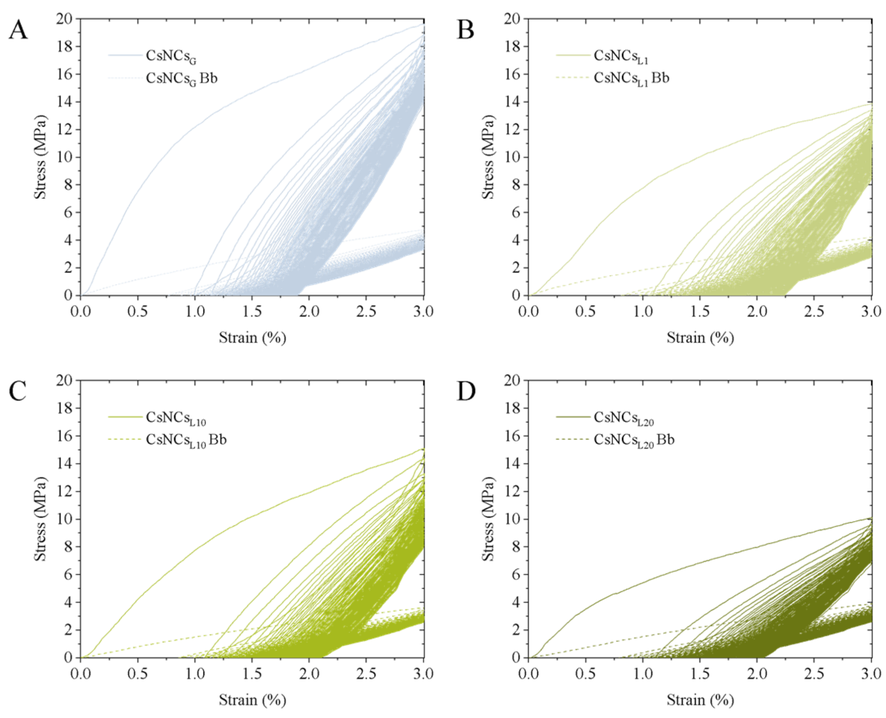


Figure 3. Cyclic stress-strain curves of CsNCs-based films. 100 cycles in air followed by 100 cycles submerged in a physiological solution at 37°C (Bb samples) after equilibration for 10 min in the same solution without any applied load.

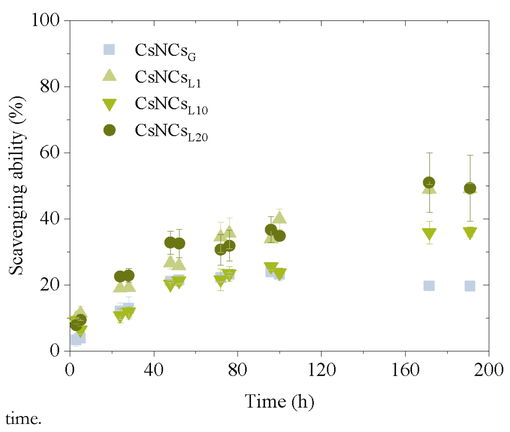


Figure 4. Antioxidant ability of CsNCs-based films over time.

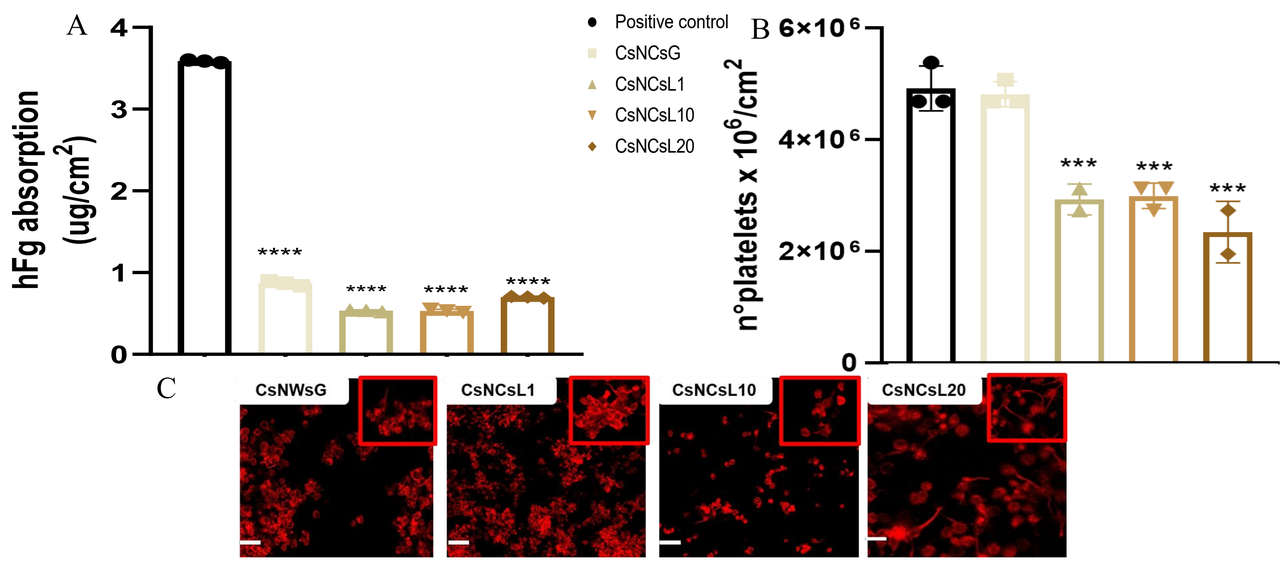


Figure 5. A) Absorption of human fibrinogen (hFg) starting from human plasma quantity detected through ELISA assay and expressed as µg/cm². Error bars indicate the standard deviation (SD) of the mean values of the replicates. CTRL represents hFbg absorption on high binding well plates,*****p<0.0001*. **B)** Platelets were obtained from human platelet-rich plasma (hPRP) and incubated for 1 h at 37 °C + 5% CO_2 on samples. Adhesion has been determined through LDH assay. Data were represented as number of adherent platelets per cm² of surface, ****p<0,0005* **C)** Representative CLSM images of human platelets stained with phalloidin-TRIC (in red) on the different films (scale bar: 20 µm).

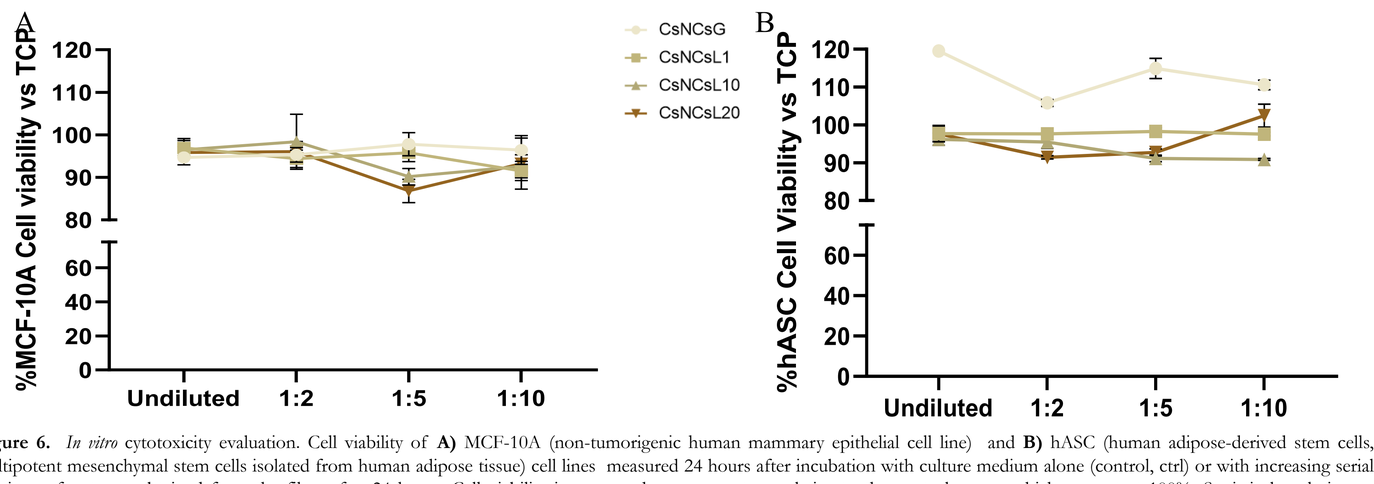


Figure 6. *In vitro* cytotoxicity evaluation. Cell viability of **A)** MCF-10A (non-tumorigenic human mammary epithelial cell line) and **B)** hASC (human adipose-derived stem cells, multipotent mesenchymal stem cells isolated from human adipose tissue) cell lines measured 24 hours after incubation with culture medium alone (control, ctrl) or with increasing serial dilutions of extracts obtained from the films after 24 hours. Cell viability is expressed as a percentage relative to the control group, which was set at 100%. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's post hoc test. No statistically significant differences were observed for MCF-10A cells. In contrast, hASC cells showed statistically significant differences ($p < 0.0001$) among most groups, except between CsNCsL1 and CsNCsL20.

Conclusion

The films demonstrated strong potential as sustainable biomaterials, combining enhanced wettability, antioxidant, and antibacterial properties, along with good cytocompatibility and hemocompatibility. Future work will focus on *in vivo* validation, targeted functionalization, and scalable production to enable clinical translation in tissue regeneration.

Acknowledgements