ELECTROSPUN WOOL KERATIN SCAFFOLDS COMBINED WITH GOLD NANOPARTICLES: A NEW STRATEGY FOR BONE REGENERATION

Marta Piccioni[1,2], Alessio Varesano[1], Riccardo Andrea Carletto[1], Elisa Restivo[2], Nora Bloise[2,3], Livia Visai[2,3], Claudia Vineis[1]

[1]STIIMA-CNR, Biella, Italy

[2]Molecular Medicine Department (DMM), Interuniversity Center for the Promotion of the 3Rs Principles in Teaching and Research (Center 3R), University of Pavia, Italy

[3]UOR6 Nanotechnology Laboratory, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy

Contact e-mail: marta.piccioni@stiima.cnr.it
marta.piccioni01@universitadipavia.it

PhD Research Symposium
12 November 2025
Aula 1.11 - Campus della Salute
PhD School in Translational and Precision Medicine
UNIVERSITÀ DI PAVIA
Dipartimento di Medicina Molecolare

Introduction

A current challenge in the regenerative medicine field is the discovery of new strategies for bone regeneration. Bone possesses a complex anatomical structure that makes it difficult to replicate finely. The present work, exploiting **tissue engineering,** provides an alternative to conventional methods (bone grafts) to cure bone defects, focused on producing electrospun keratin nanofiber-based scaffolds. **Wool keratin** is an attractive biomaterial to stimulate bone regeneration due to its biocompatibility, biodegradability and capacity to improve cell adhesion (1). Also, the addition of **gold nanoparticles** (GNPs) can enhance the scaffolds' antibacterial performance (2).

Methods

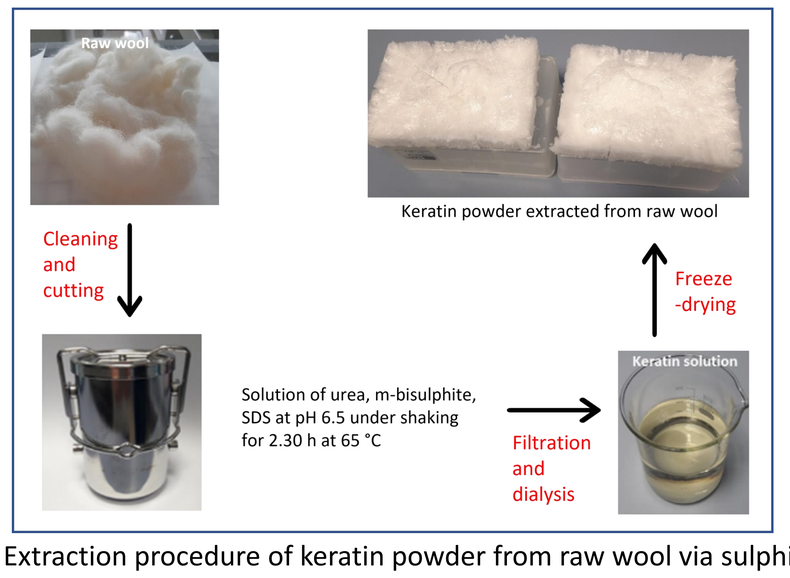

Figure 1. Extraction procedure of keratin powder from raw wool via sulphitolysis.

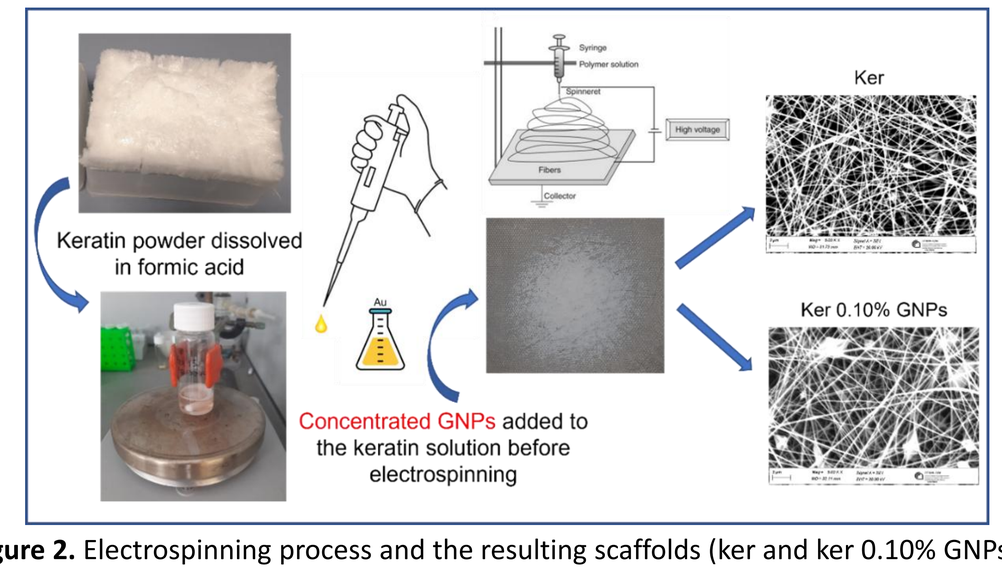

Figure 2. Electrospinning process and the resulting scaffolds (ker and ker 0.10% GNPs).

Keratin powder (15% wt.) extracted from waste wool fibers (Fig. 1) was dissolved in formic acid ≥95% (density 1.22 g/mL) and kept overnight under mild stirring in controlled laboratory conditions (20 °C and 65% RH).
Then, different concentrations of GNPs (0.10 – 0.15 – 0.20%) were added to the keratin solution before electrospinning (Fig. 2).
The obtained solution was electrospun for 1h on polypropylene non-woven 17 g/m^2 in a prototype lab applying specific electrospinning conditions: 25 kV voltage; 15 cm working distance; 0.4 mm internal needle diameter; 0.003 mL/min flow rate.
At the end of the process, the electrospun nanofibers with and without GNPs were thermally stabilized in an oven at 180°C for 4h to make them water-insoluble. Preliminary tests (cell viability and evaluation of the scaffold's bacterial adhesion) were performed on NF-ker and on NF-ker + 0.10% GNPs membranes.

Results and Discussion

The sample's biocompatibility was assessed through a CCK8 assay. Human osteoblast SaOs-2 cells were cultured for 1,3 and 7 days on the different scaffolds and on a tissue culture plate (TCP) control.

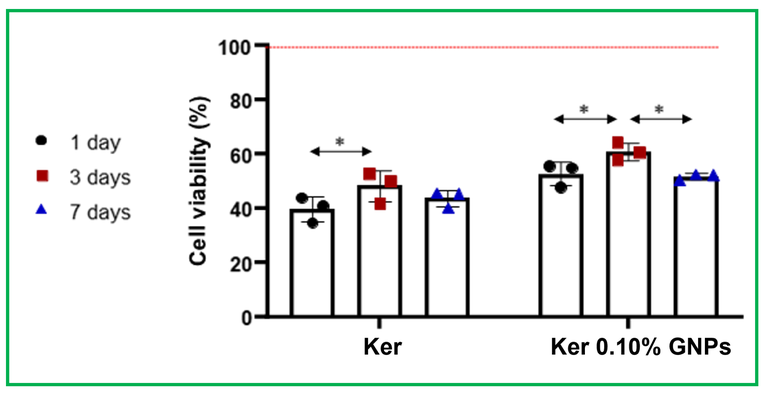

Figure 3. Outcomes expressed as a percentage of cell viability on materials with respect to TCP control set as 100% (red hatching).

The quantitative data, reported in the graph above (Fig. 3), display that cell viability is higher on GNPs-keratin membrane compared to the only keratin: GNPs promote SaOs-2 cell growth on keratin-based scaffolds. Furthermore, a reduction in cell growth is visible after 7 days of incubation on the samples: this evidence is probably due to the initial differentiation phase.

The anti-adhesive properties of electrospun keratin scaffolds were assessed to investigate the antimicrobial effect of GNPs.
Gram-negative *Escherichia coli* ATCC 25922 and Gram-positive *Staphylococcus aureus* ATCC 25923 were seeded on the samples and incubated at 37°C for 3h. The surviving bacteria were diluted and plated on agar plates. After incubation at 37°C for 24h, the colonies formed from adherent cells were counted and expressed as CFUs (colony-forming units)/mL.
GNPs in keratin-based scaffolds reduced the adhesion of *E. coli* (ca. 20%) and *S. aureus* (ca. 90%) after 3h, than pure keratin (Fig. 4A-B).

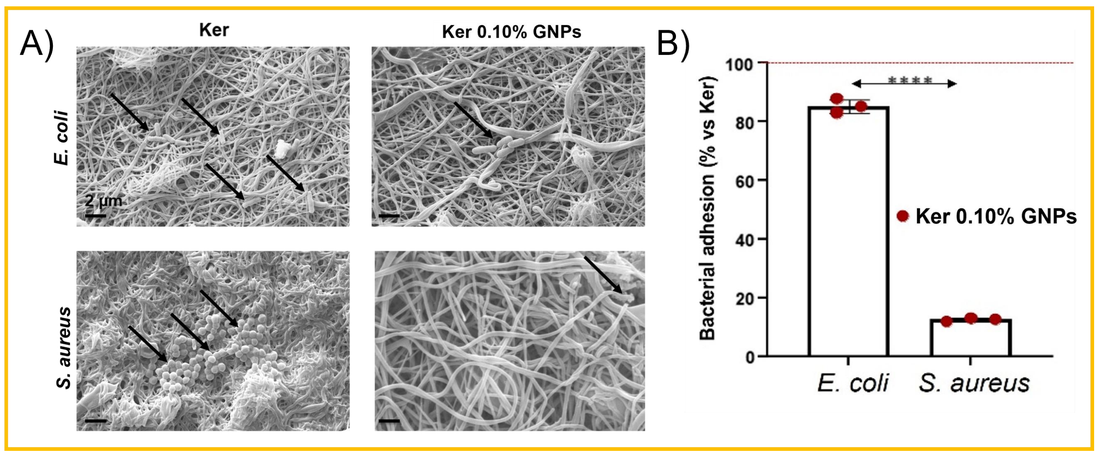

Figure 4. A) SEM images of bacterial adhesion after 3h. B) Bacterial adhesion (in %) on ker 0.10% GNPs compared to ker set as 100% (red hatching).

Conclusions

GNPs in keratin-based scaffolds stimulate cell adhesion and growth on the material's surface, enhancing also their antibacterial properties. These prior findings suggest that **electrospun keratin scaffolds added with GNPs** may represent a **promising biomaterial for bone tissue regeneration**, paving the way for future studies.

REFERENCES

(1) Sanchez Ramirez, D. O., Vineis, C., Cruz-Maya, I., Tonetti, C., Guarino, V., & Varesano, A. (2022). Wool Keratin Nanofibers for Bioinspired and Sustainable Use in Biomedical Field. *Journal of Functional Biomaterials*, *14*(1), 5. https://doi.org/10.3390/jfb14010005

(2) Okkeh, M., Bloise, N., Restivo, E., De Vita, L., Pallavicini, P., & Visai, L. (2021). Gold Nanoparticles: Can They Be the Next Magic Bullet for Multidrug-Resistant Bacteria? *Nanomaterials*, *11*(2), 312. https://doi.org/10.3390/nano11020312

ACKNOWLEDGEMENT: This study was supported by MICS Extended Partnership and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.3 – D.D. 1551.11-10-2022, PE00000004).