SYNTHESIS AND CHARACTERIZATION OF TYPE I COLLAGEN-BASED SCAFFOLDS CROSS-LINKED WITH NATURAL POLYPHENOLS EXTRACTED FROM GREEN TEA

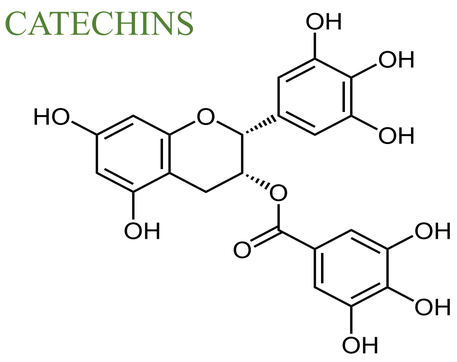
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INTRODUCTION

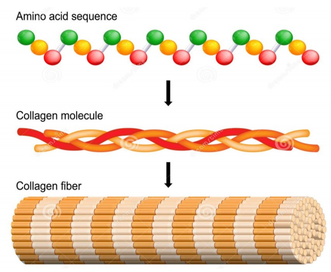
Collagen, thanks to its high biocompatibility, low immunogenicity, and ability to mimic the extracellular matrix (ECM), is a promising candidate for soft tissue repair. Despite these advantages, its poor mechanical strength and rapid degradation in vivo limit its use in high-stress environment. To overcome these limitations, various strategies have been explored to enhance collagen's mechanical stability and expand its use in biomedical applications. [1]

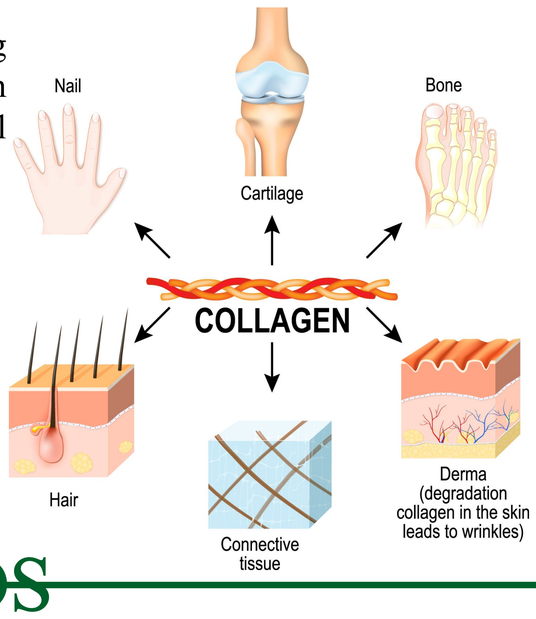
Polyphenols are natural compounds with strong antioxidant and anti-inflammatory properties, largely due to their hydroxyl groups. Among them, catechins, especially epigallocatechin gallate (EGCG), stand out for their ability to neutralize free radicals and chelate metals. EGCG's trihydroxylated structure and gallate group enhance its radical-scavenging activity. Polyphenols can also crosslink type I collagen, both covalently and non-covalently, making them useful in biomaterial development. [2]

CATECHINS

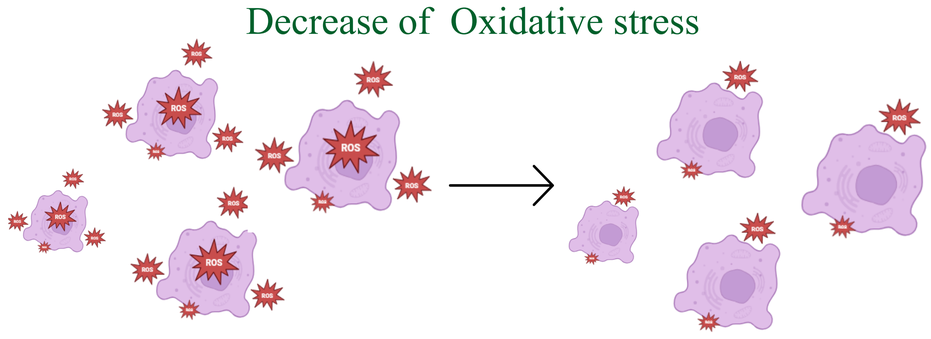


COLLAGEN STRUCTURE

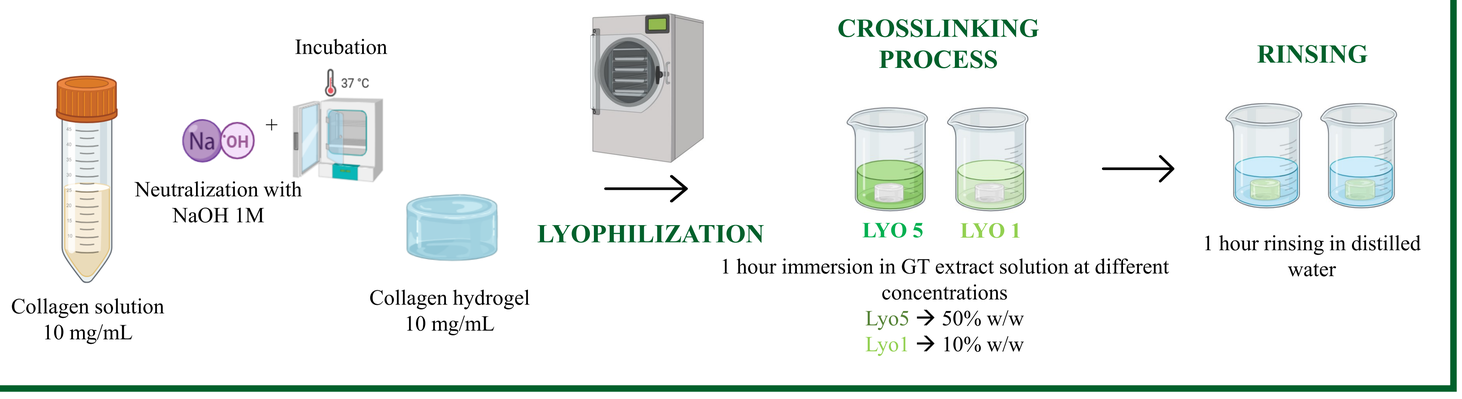




Decrease of Oxidative stress



MATERIALS & METHODS



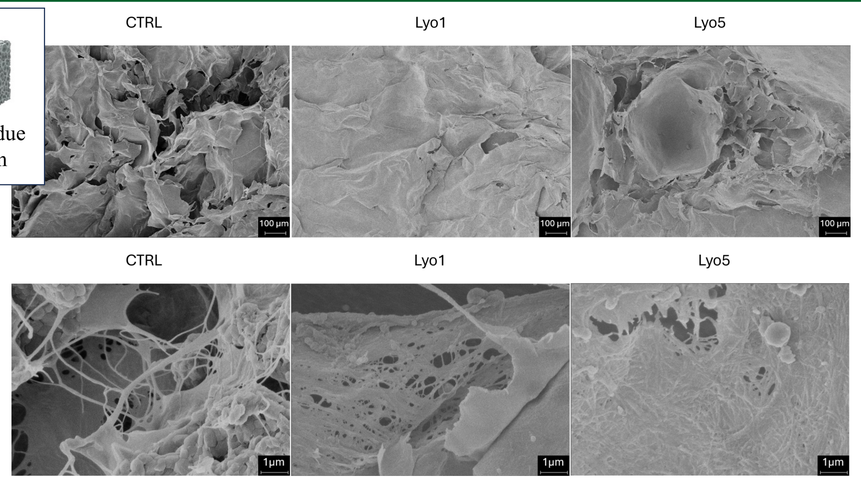
RESULTS

CHEMICAL CHARACTERIZATION

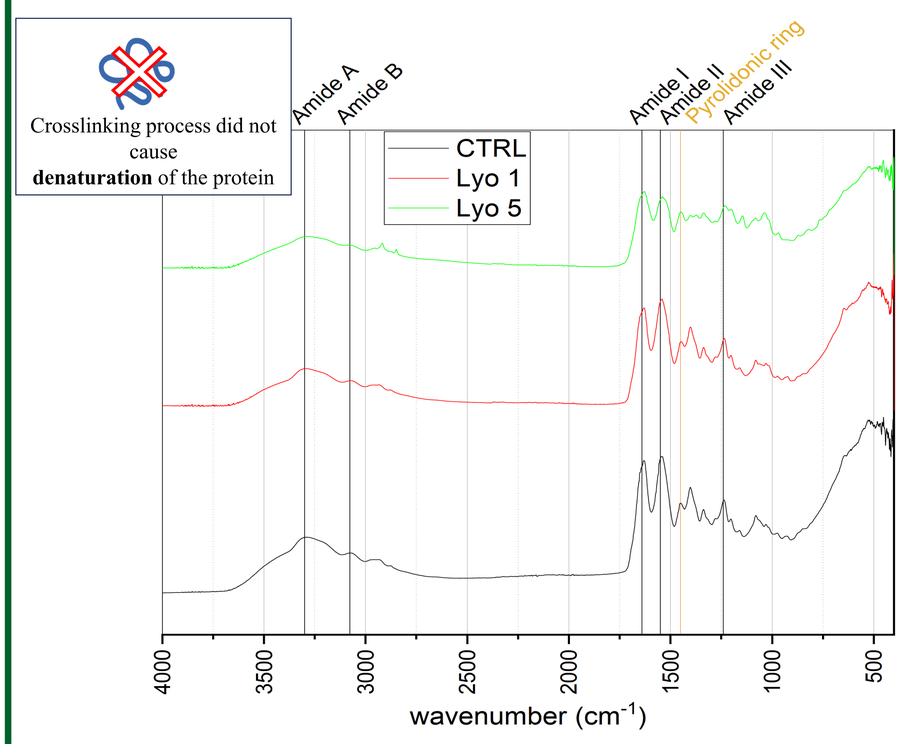
Type I collagen-based scaffolds were successfully functionalized with a green tea extract (GT) at varying concentrations.
Samples were characterized through:

- **FE-SEM** analysis to evaluate if the internal structure is modified by the increasing amount of extract.
- **ATR-FTIR** analysis to see if the collagen triple helical structure was preserved after functionalization
- **Zeta potential** analysis to evaluate how the presence of polyphenols influenced the surface chemistry and reactivity

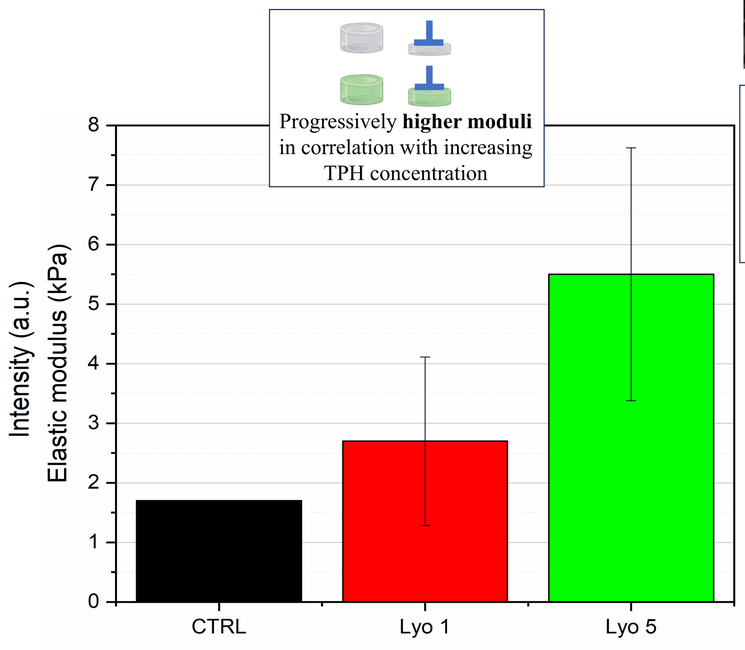
Difference in **pore size** due to the functionalization



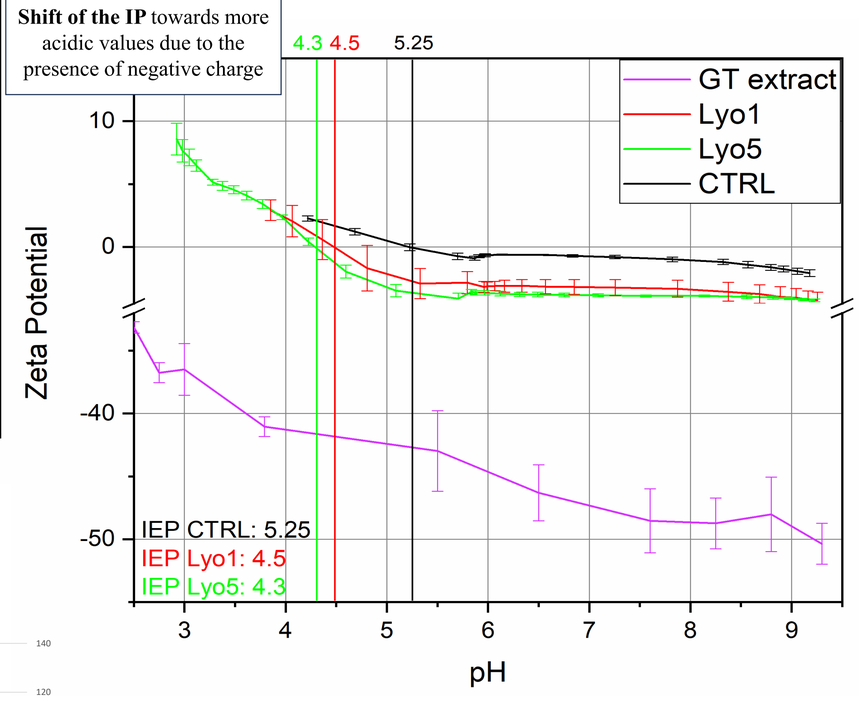
Crosslinking process did not cause **denaturation** of the protein



Progressively **higher moduli** in correlation with increasing TPH concentration



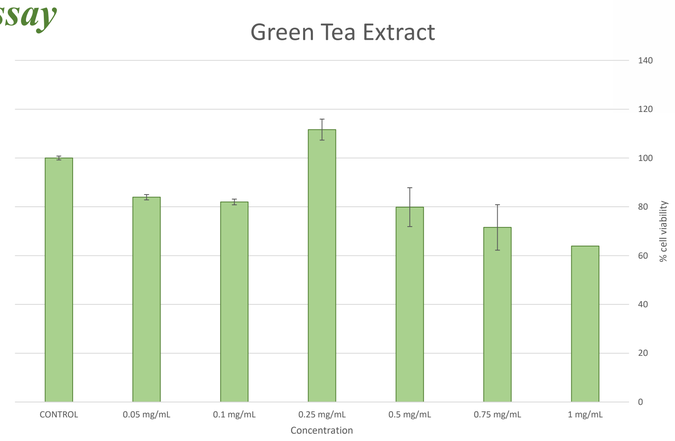
Shift of the IP towards more acidic values due to the presence of negative charge



BIOLOGICAL CHARACTERIZATION

Cell viability assays were performed on THP-1 cells treated with the GT extract to assess its cytotoxicity and to find the concentrations that do not compromise cell survival.

MTT assay



To be continued…

CONCLUSIONS

Type I collagen-based scaffolds were successfully functionalized with a GT extract at varying concentrations. The comprehensive physicochemical characterization confirmed that GT extract act as effective natural cross-linkers capable of improving the mechanical properties (elastic modulus in compression), and morphological integrity (FE-SEM) of collagen scaffolds in a concentration-dependent way. ATR-FTIR analysis demonstrated that the collagen triple helical structure was preserved after functionalization. Zeta potential measurements indicated that the presence of polyphenols also influenced the surface chemistry and reactivity of the scaffolds under physiological pH, which could have implications for future surface functionalization or in vivo interactions. Possible application of these scaffolds could be in different tissue engineering applications, eventually coupled to additional cross-linking methods to further enhance their stiffness.

REFERENCES

[1] Ye, W. *et al.* Articular cartilage reconstruction with TGF-β1-simulating self-assembling peptide hydrogel-based composite scaffold. *Acta Biomater* **146**, 94–106 (2022).
[2] Szwedowski, D. *et al.* Genetics in Cartilage Lesions: Basic Science and Therapy Approaches. *International Journal of Molecular Sciences 2020, Vol. 21, Page 5430* **21**, 5430 (2020).