Robotic bioprinting of advanced skin and bladder tissue models using collagen proteins as functional components of a chitosan-agarose bioink

Karol Staszczyk[1], Szymon Mania[1], Adrianna Banach-Kopeć[1], Justyna Prajsnar[1,2], Daria Sowik[1,3], Paulina Parcheta-Szwindowska[4], Emilia Iłowska[5], Przemysław Karpowicz[5], Sylwia Rodziewicz-Motowidło[3], Robert Tylingo[1]

[1] Department of Chemistry, Technology and Biotechnology of Food, Faculty of Chemistry, Gdansk University of Technology, 11/12 Narutowicza Street, 80-233 Gdansk, Poland, [2] Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Niezapominajek Str. 8, 30-239 Krakow, Poland, [3] Department of Biomedical Chemistry, Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdańsk, Poland, [4] Department of Polymer Technology, Faculty of Chemistry, Gdansk University of Technology, 11/12 Narutowicza Street, 80-233 Gdansk, Poland, [5] Department of Biomedical Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdańsk, Poland

INTRODUCTION

Robotic bioprinting is a promising approach for the fabrication of functional *in vitro* soft tissue models, enabling precise spatial deposition of biomaterials and cells. Multi-axis robotic systems offer high flexibility and control, which is particularly valuable for complex tissues such as skin and urinary bladder wall analogs. The aim was to establish a reliable 3D biofabrication system for future applications in regenerative medicine, organoid development, and therapy testing. The study compares collagen-based materials obtained from salmon skin and bovine tendons by acid extraction and decellularization. Their amino acid composition, structure, thermal properties, influence on bioink rheology, and post-crosslinking scaffold hardness were evaluated.

COLLAGENS PREPARATION

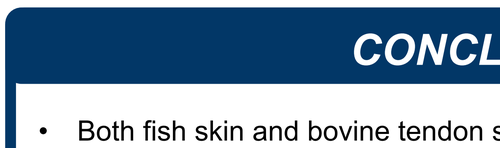
Raw Material Preparation

Acid extraction
Mechanical cleaning
Rinsing with water
Defatting using a detergent

Salmon skin
Bovine tendons

Decellularization
Mechanical cleaning
PBS washing at 4 °C
Pre-cleaning to eliminate blood

Removal of Non-Collagenous Proteins



Cell Disruption



Collagen Extraction

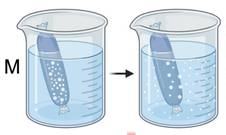
- Treatment with 0.5 M hydroxyacetic acid
- Raw material : solution ratio = 1:10 (w/v)
- Duration: 72 h
- Temperature: 4 °C
- Filtration through nylon cloth (100 µm)

Chemical Decellularization

- Hypotonic Tris-HCl / EDTA treatment
- Non-ionic detergent exposure
- Ionic detergent treatment (SDS) for removal of cellular remnants

Dialysis

Stage I: Dialysis against 0.1 M CH_3COOH for 24 h



Stage II: Dialysis against distilled water for 6–8 days

Post-treatment Purification

- Extensive PBS rinsing to remove detergents
- Enzymatic treatment with trypsin-EDTA
- Peracetic acid / ethanol sterilization and neutralization

Gelatin Preparation



60 °C, 20 min

- Collection of the purified collagen solution
- Heating: 60 °C / 20 min
- Cooling

Freeze-Drying

- Freezing: −20 °C for 24 h
- Freeze-drying:
  - 0.94 mbar, condenser
  - temperature −78 °C



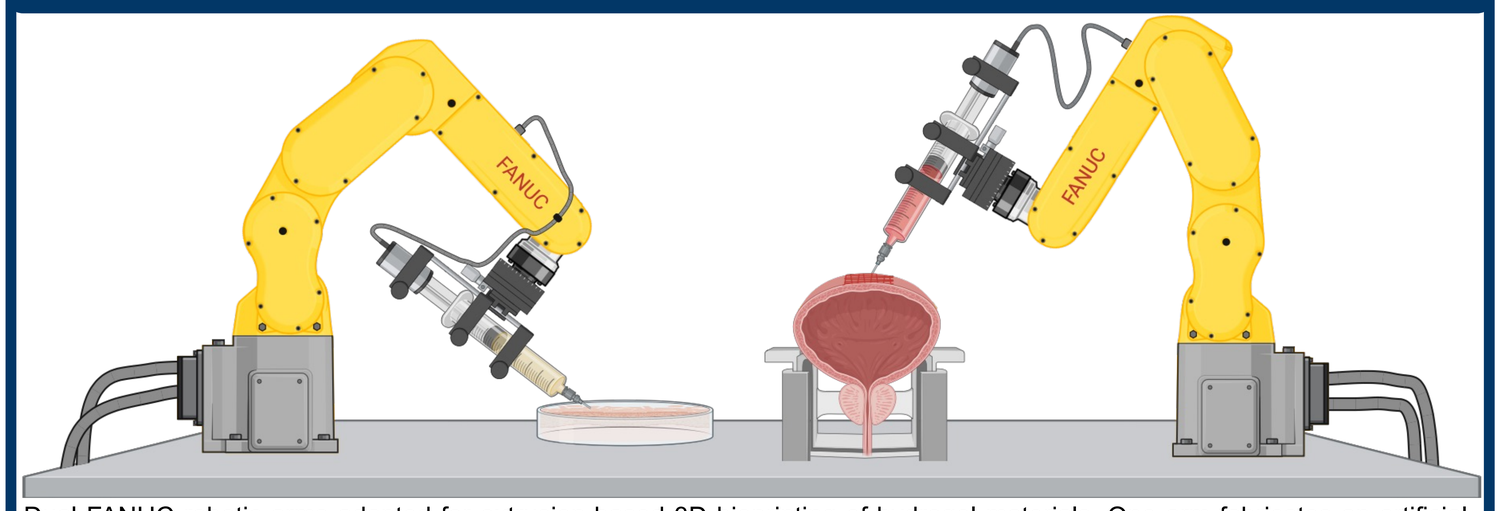
FC – collagen derived from fish skin
BT – collagen derived from bovine tendons

aFC/aBT – acid-extracted FC/BT
dFC/dBT – decellularized FC/BT

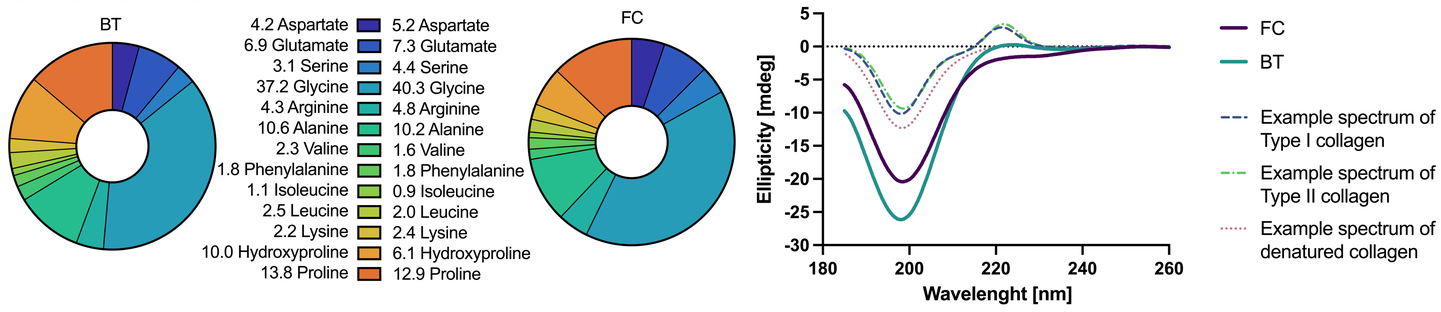
CONCLUSIONS

- Both fish skin and bovine tendon sources provided collagen with a typical amino acid profile rich in glycine, proline, and hydroxyproline, confirming their suitability as functional bioink components.
- Structural analyses indicated partial denaturation and a shift toward gelatin-like forms, which may improve printability and hydrogel processability while preserving the biologically relevant protein character.
- aBT showed slightly higher thermal stability than the corresponding aFC, suggesting potential advantages for mechanically more demanding constructs.

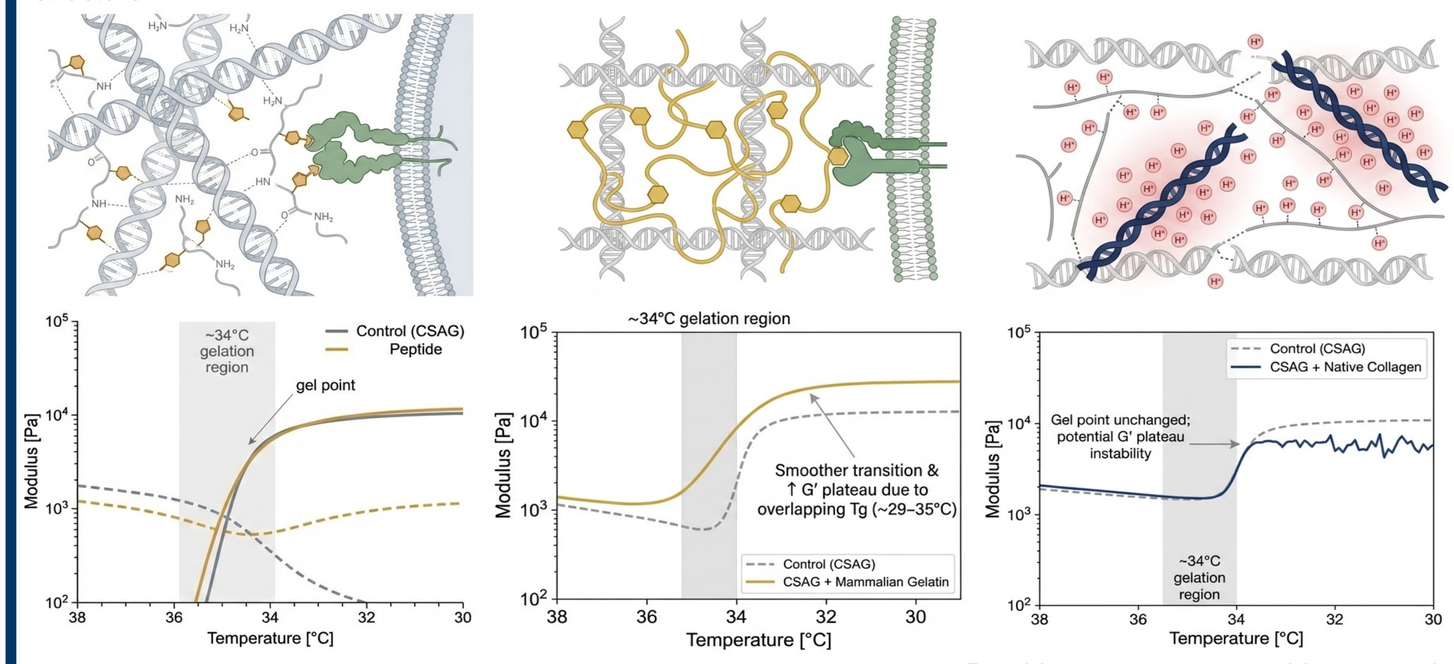
RESULTS



Dual FANUC robotic arms adapted for extrusion-based 3D bioprinting of hydrogel materials. One arm fabricates an artificial skin model using collagen derived from fish skin, while the second prints an artificial bladder model using collagen extracted from bovine tendons

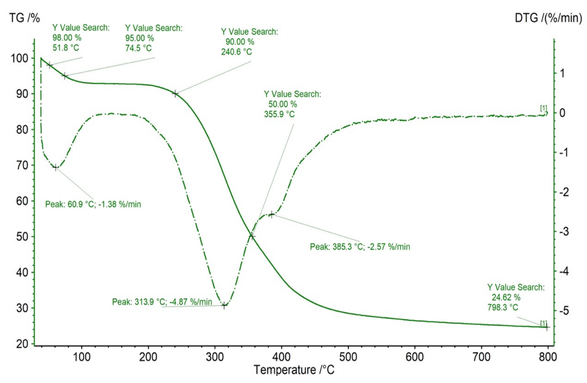


Comparative characterization of BT and FC showed similar amino acid profiles dominated by glycine, proline, and hydroxyproline, confirming the typical composition of collagen. Circular dichroism spectra revealed a weakened positive band and an intensified negative signal, indicating partial denaturation and disruption of the native triple-helix structure.

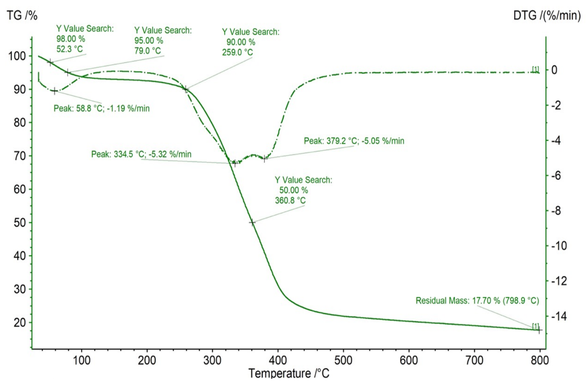


Additive Variant		Tg Impact (G'=G'')	G' Plateau	Key Biological Benefits	Process Risk
PB1 Peptide		Minimal shift	Minimal change	Targeted integrin activation	Low
Bovine Gelatin		Early rise (slight ↑)	Strong ↑	Multi-motif adhesion, mitigates oxidative stress	Low
Fish Gelatin		Minimal shift	Moderate ↑	Good adhesion, delayed structural effect	Low
Native Bovine Collagen		Little change	Moderate ↑	Complex fibrillar ECM mimic	High - pH disruption
Native Fish Collagen		Blurred transition	Unstable	Good ECM mimic	Very High - pH & thermal denaturation

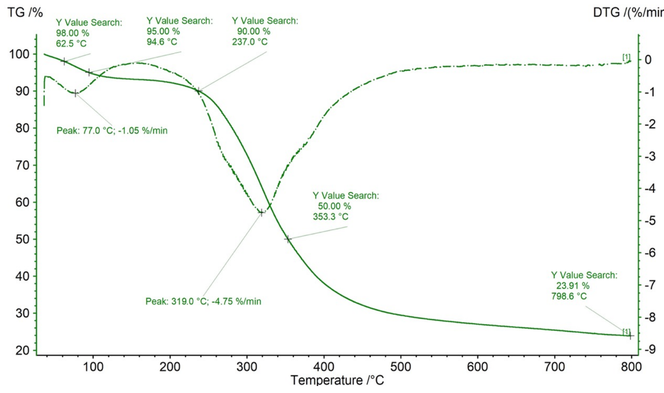
Peptides may provide precise biochemical cues without substantially altering the thermal gelation behavior or hydrogen bonding of the agarose-based matrix. By contrast, gelatin can undergo cooperative co-gelation with the primary network, potentially reinforcing the hydrogel while providing bioactive sites that support fibroblast spreading at neutral pH. In systems requiring acidic collagen solubilization, the local pH decrease may protonate chitosan, disrupt the hydrogen-bonded CS–AG network, and compromise phase stability.



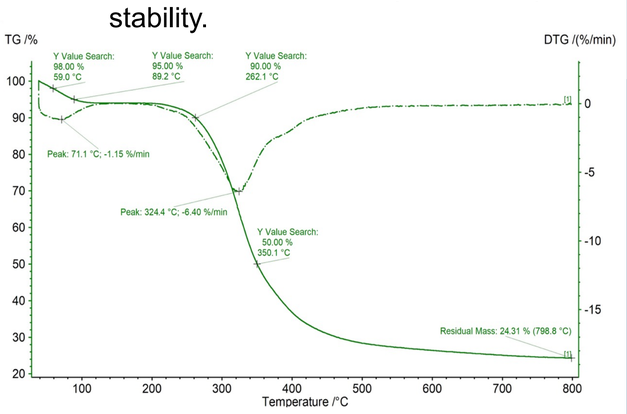
Thermogravimetric analysis of aFC. The main decomposition between 240–400 °C corresponded to breakdown of collagen chains, while further mass loss was linked to carbonization.



Thermogravimetric analysis of aBT. Compared with aFC, aBT showed slightly higher degradation temperatures and lower final residue, indicating better thermal stability and lower mineral residue.



Thermogravimetric analysis of dFC. The slightly lower onset temperature suggests similar thermal behavior, with minor differences arising from the extraction method and retained matrix components.



Thermogravimetric analysis of dBT. Compared with aBT, dBT showed lower degradation temperatures and a higher final residue, indicating reduced thermal stability and greater residual non-collagenous content.

This work was created as a result of research projects no. UMO-2024/53/B/ST8/00861 and UMO-2024/53/B/ST8/03047, financed by the National Science Centre.