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Evaluation of a Biomimetic and Electroactive 3D Bioprinted Cardiac Patch: From Bioink Optimization to Functional Regeneration in a Perfusion System

Ayşenur Atak[1], Ayşen Güngör[2], Ayça Bal Öztürk[2,3], Banu Mansuroğlu[1], Yüksel Çetin[4*]

[1] Molecular Biology and Genetics, Yildiz Technical University, Istanbul, Türkiye
[2] Department of Stem Cell and Tissue Engineering, Institute of Health Sciences, Istinye University, Istanbul, Türkiye
[3] Department of Analytical Chemistry, Faculty of Pharmacy, Istinye University, Istanbul, Türkiye
[*4] Life Sciences, Biotechnology R&D, TUBITAK MAM, Kocaeli, Türkiye
*Corresponding author: Yüksel Çetin (e-mail: yuksel.cetin@tubitak.gov.tr)

INTRODUCTION & AIM

The escalating demand for tissue transplantation, compounded by donor shortages and immune rejection, has driven the development of biomimetic 3D cardiac patches utilizing decellularized bovine pericardium (dECM) to replicate the native extracellular matrix. However, achieving maximal DNA removal while preserving essential collagen and glycosaminoglycan (GAG) integrity remains a critical challenge in decellularization protocols (Zhang et al., 2023). The aim of this study was to establish a comprehensive framework for producing a multifunctional, electroactive cardiac construct through a two-phase optimization strategy. In the first phase, five distinct decellularization methods were systematically evaluated and compared using quantitative biochemical assays (DNA extraction, collagen, and GAG retention) alongside qualitative histological staining and scanning electron microscopy (SEM) to identify the most effective approach (Liu et al., 2024). In the second phase, the optimized dECM was blended with silk fibroin, alginate, and gold nanoparticles (AuNPs) to formulate five different hybrid bioinks, which were rigorously characterized for their printability (PR), rheological properties, chemical structures FTIR, electrical conductivity, cell viability, and swelling kinetics. This multi-parametric approach aims to deliver a structurally robust, highly biocompatible, and electrophysiologically active 3D-bioprinted patch capable of supporting cardiac maturation within a dynamic perfusion environment, offering significant translational potential for cardiovascular regenerative medicine.

METHOD

In this study, bovine pericardium tissues were decellularized using five protocols based on physical, chemical, and enzymatic approaches to develop biocompatible hydrogel. The protocols included the use of various detergents: M1 (10 mM SDC), M2 (1% SDS + 1% Triton X-100), M3 (0.1% SDC), M4 (1% ASB-14), and M5 (0.5% SDS + 1% Triton X-100). The resulting decellularized extracellular matrices (dECMs) were characterized through histological staining—H&E for cellular residues, Safranin O for proteoglycans, Fast Green for collagen, and Alcian Blue for glycosaminoglycans—along with microstructural analysis using scanning electron microscopy (SEM) for morphological evaluation. Biochemical assays were conducted to quantify DNA, RNA, collagen, and GAG content. Following optimization, the selected functional dECM was incorporated into with silk fibroin (SF) and alginate (alg) bioinks. Five bioink formulations were prepared: G1 (10% SF + 2.5% alg), G2 (10% SF + 2.5% alg + 2% dECM), G3 (10% SF + 2.5% alg + 3.5% dECM), G4 (10% SF + 2.5% alg + 5% dECM), and G5 (10% SF + 2.5% alg + 5% dECM + 0.1 mg/ml AuNPs). The printability (PR) and rheological properties of the bioinks were systematically assessed to determine their extrusion performance. The crosslinked hydrogel scaffolds were characterized by FTIR to analyze chemical networks, swelling kinetics analysis in PBS, and electrical conductivity measurements. Finally, the *in vitro* biocompatibility of the fabricated scaffolds were rigorously evaluated via the direct contact method using Human Umbilical Vein Endothelial Cells (HUVECs) (Bernava et al., 2026).

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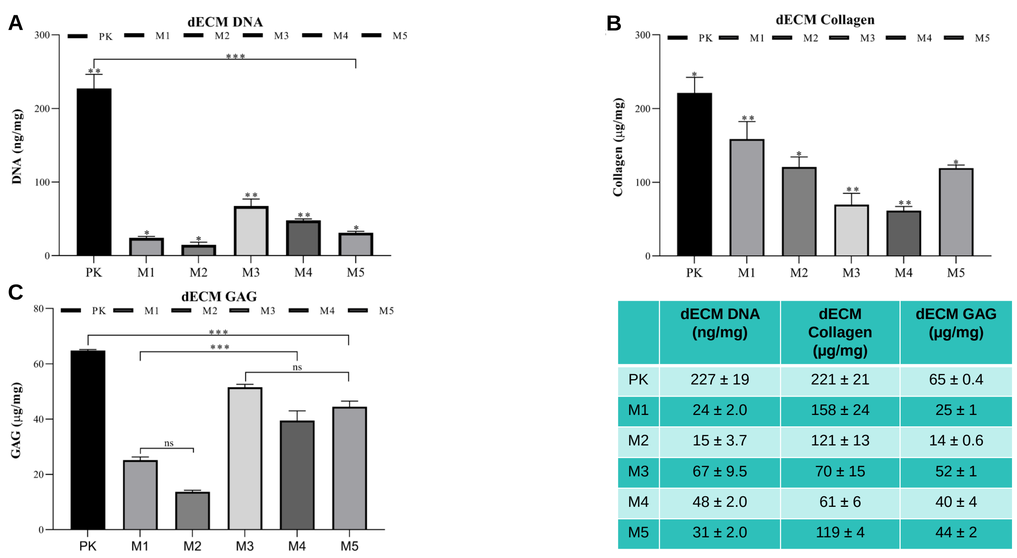
RESULTS & DISCUSSION

❑ Molecular Characterization of dECM

➢ ***Quantification of DNA, Collagen & GAG Content of dECM***



	dECM DNA (ng/mg)	dECM Collagen (µg/mg)	dECM GAG (µg/mg)
PK	227 ± 19	221 ± 21	65 ± 0.4
M1	24 ± 2.0	158 ± 24	25 ± 1
M2	15 ± 3.7	121 ± 13	14 ± 0.6
M3	67 ± 9.5	70 ± 15	52 ± 1
M4	48 ± 2.0	61 ± 6	40 ± 4
M5	31 ± 2.0	119 ± 4	44 ± 2

Figure 1. Quantification of **(A)** DNA , **(B)** collagen and **(C)** GAG contents in native pericardium and dECM.

❑ Structural Characterization of dECM

➢ ***Histological Analysis – H&E, Alician Blue, Fast Green and Safranin O Staining***

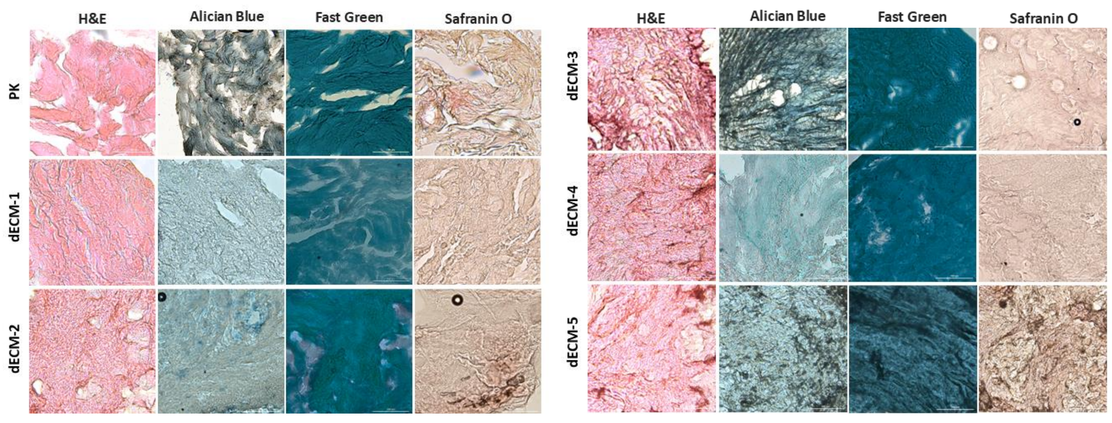


Figure 2. To evaluate the effects of different decellularization methods on genetic material removal and ECM preservation, H&E, Alcian Blue, Fast Green, and Safranin O stainings were performed. (Magnification: 40X)

➢ ***Microstructural Characterization of dECM- SEM***

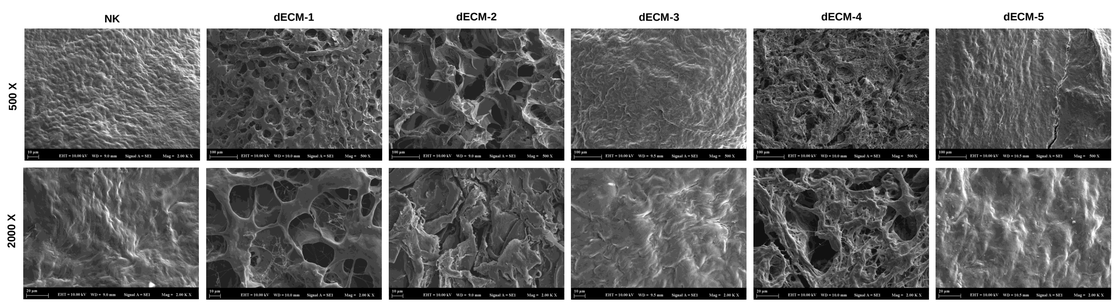


Figure 3. SEM images of NK (Magnification: 2000X) and dECM (Magnification: 500X and 2000X).

❑ Bioink Optimization & Characterization

➢ ***3D-Bioprintability & Rheological Behavior of Bioinks***

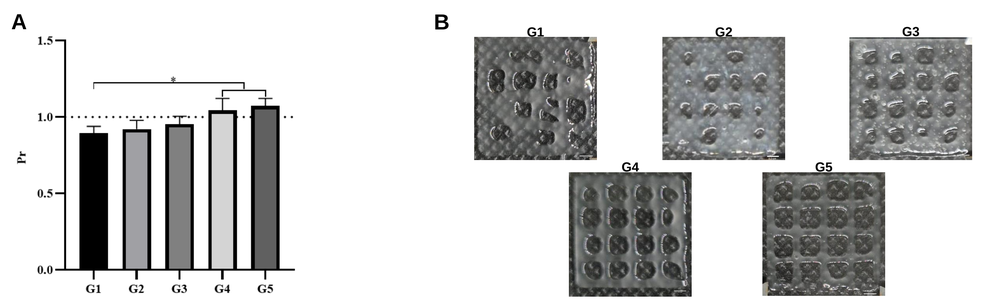


Figure 4. (A) Quantitative printability (Pr) analysis of different bioink formulations (G1–G5). The dotted line represents the ideal printability value (Pr = 1). Significant differences are indicated by asterisks (*p < 0.05). **(B)** Gross morphological images of the 3D-bioprinted lattices (G1–G5). The top panel shows the initial optimization groups with varying filament spreading, while the bottom panel demonstrates optimized groups with well-defined square pore geometries and enhanced shape fidelity. Scale bars represent 0.2 cm.

❑ Structural, Functional & Biocompatibility Characterization of Hydrogels

➢ ***Chemical Network & Structural Analysis via FTIR***

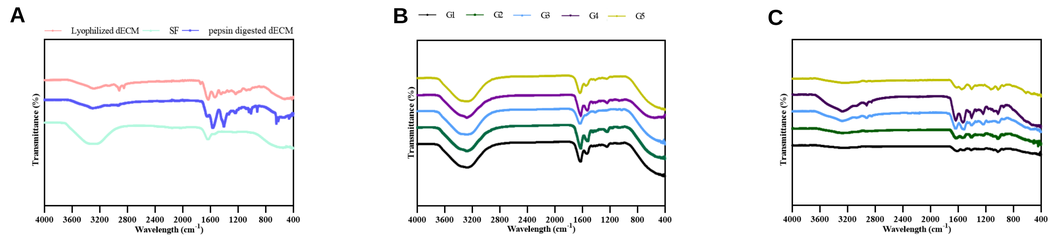


Figure 5. (A) FTIR profiles of baseline materials: Lyophilized dECM, SF, and pepsin-digested dECM. **(B)** FTIR spectra of hydrogel formulation groups (G1–G5) before lyophilization. **(C)** FTIR spectra of hydrogel formulation groups (G1–G5) after lyophilization.

➢ ***Swelling Kinetics and Water Absorption Capacity***

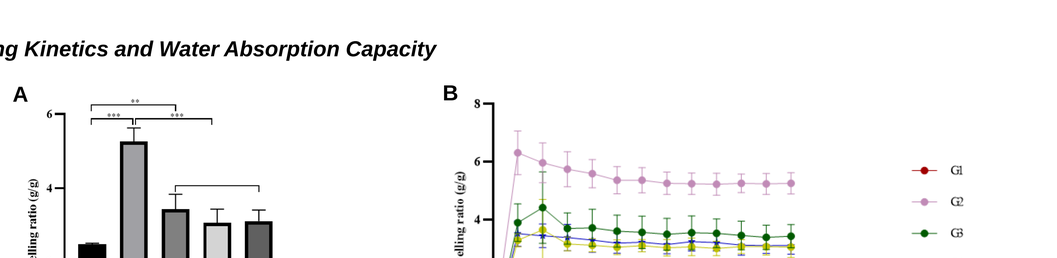


Figure 6. (A) Comparative evaluation of maximum equilibrium swelling ratios (g/g) among different formulation groups (G1–G5). Significant differences are indicated by asterisks ($p < 0.01$, ***$p < 0.001$). **(B)** Time-dependent swelling kinetics (0 to 24 h) of the crosslinked hydrogels in PBS at physiological conditions.

➢ ***Electrical Conductivity Analysis***

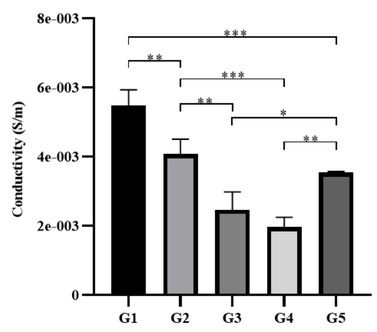


Figure 7. Comparative electrical conductivity (S/m) across hydrogel groups (G1–G5), illustrating the impact of varying dECM concentrations and gold nanoparticle (AuNP) incorporation (G5) on electroactive properties. Significant differences are indicated by asterisks (*p < 0.05, * *p < 0.01, * * *p < 0.001).

➢ ***In Vitro Cytocompatibility & Cell Viability Analysis***

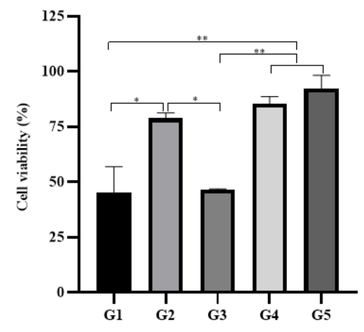


Figure 8. Quantitative evaluation of cell viability (%) in HUVECs following exposure to different hydrogel formulation groups (G1–G5) via the direct contact method. Significant differences are indicated by asterisks (*p < 0.05, * *p < 0.01).

CONCLUSIONS

- Among the tested methods, M5 (0.5% SDS + 1% Triton X-100) was established as the most comprehensive protocol for bovine pericardium by achieving a high DNA removal efficiency of 86.35% (31 ± 2.0 ng/mg) while demonstrating the most balanced retention of both structural collagen (119 ± 4 µg/mg) and GAG (44.44 ± 2.03 µg/mg) matrix components.
- G5 achieved a near-ideal printability value (Pr ≈ 1.07), successfully forming 3D lattices with well-defined square pore geometries and superior structural integrity.
- G5 reached a stable hydration equilibrium within 2 hours (3.07 ± 0.35 g/g) and maintained its structural dimensions over 24 hours, proving highly advantageous for structural stability in dynamic environments.
- Due to the synergistic effect of the 5% dECM matrix and gold nanoparticles, G5 emerged as the most promising cardiac patch candidate by significantly boosting electrical conductivity (>3.5 x 10^{-3}S/m) and supporting maximum HUVEC cell viability (91.95 ± 5.92%).

FUTURE WORK/ REFERENCES

The engineered electroactive **G5 hybrid hydrogel** holds significant potential as a biomimetic patch for treating myocardial infarction and restoring damaged cardiac tissues. The high electrical conductivity provided by gold nanoparticles offers a critical advantage for achieving functional electrophysiological integration with host myocardium, minimizing post-infarct arrhythmia risks. Future directions involve utilizing this cell-free scaffold for post-printing cell seeding with functional cardiomyocytes or stem cells to monitor tissue maturation under dynamic perfusion systems.

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