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Tissue engineering: A systematic review about 3D printing applications in cardiac scaffold improvement, cell viability, and porosity control

Ingeniería de tejidos: Una revisión sistemática sobre aplicaciones de impresión 3D en la mejora de andamiajes cardíacos, viabilidad celular y control de porosidad

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Abstract

This study analyzed the impact of 3D bioprinting on cardiac tissue engineering, focusing on strategies to optimize cell viability, structural design, and functional vascularization. A systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses methodology, selecting recent articles (2020-2025) from databases such as *PubMed* and *Google Scholar*. The results highlighted that the use of composite bioinks, such as alginate and gelatin, maintained high cell viability of 99% or higher, while hybrid bioinks showed a strong dependence on hydrogel concentration. Furthermore, it was demonstrated that geometric design—particularly angular architecture—significantly influences mechanical properties, allowing for the replication of myocardial anisotropy. The prevascularization strategy using microvascular fragments proved superior for graft integration and survival *in vivo*. It was concluded that 3D bioprinting offers precise control over the biological and mechanical properties of printed tissues, although challenges related to complete cellular maturation and scalability for clinical applications remain. It is recommended that future research focus on the integration of complex vascular networks and controlled-release systems to improve long-term functionality.

Keywords: 3D bioprinting, cardiac tissue engineering, cell viability, scaffolds, vascularization

Resumen

Este estudio analizó el impacto de la bioimpresión 3D en la ingeniería de tejidos cardíacos, centrándose en estrategias para optimizar la viabilidad celular, el diseño estructural y la vascularización funcional. Se llevó a cabo una revisión sistemática siguiendo la metodología *Preferred Reporting Items for Systematic Reviews and Meta-Analyses*, seleccionando artículos recientes (2020-2025) de bases de datos como *PubMed* y *Google Scholar*. Los resultados destacaron que el uso de biotintas compuestas, como alginato y gelatina, mantuvo una alta viabilidad celular mayor o igual al 99%, mientras que las biotintas híbridas mostraron una dependencia fuerte de la concentración del hidrogel. Asimismo, se demostró que el diseño geométrico, especialmente la arquitectura angular, influye de manera significativa en las propiedades mecánicas, permitiendo replicar la anisotropía del miocardio. La estrategia de prevascularización mediante fragmentos microvasculares resultó mayor para la integración y la supervivencia del injerto *in vivo*. Se concluyó que la bioimpresión 3D ofrece un control preciso sobre las propiedades biológicas y mecánicas de los tejidos impresos, aunque se mantienen los desafíos relacionados con la maduración celular completa y la escalabilidad para aplicaciones clínicas. Se recomienda enfocar futuras investigaciones en la integración de redes vasculares complejas y sistemas de liberación controlada para mejorar la funcionalidad a largo plazo.

Palabras clave: bioimpresión 3D, ingeniería de tejidos cardíacos, viabilidad celular, andamiajes, vascularización

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1. Introduction

The heart is an extremely complex organ, capable of beating steadily throughout a lifetime (Bliley et al., 2024). However, when the heart suffers a severe injury, its capacity to regenerate is limited by the loss of cardiomyocytes (Spedicati et al., 2024; Bliley et al., 2024). In this regard, the lack of natural repair causes heart failure, a condition that affects millions of people worldwide (Pourchet et al., 2025; Bar et al., 2025).

In this respect, cardiac tissue engineering offers a solution to this problem through the development of patches (Spedicati et al., 2024) or the complete printing of the organ, making it possible to replace the damaged tissue and restore heart function. Achieving this requires a technology capable of building living structures with precision (Zhang et al., 2024), and that is where three-dimensional (3D) bioprinting comes in.

3D bioprinting creates customized scaffolds using biological materials and living cells (Mayoral et al., 2022). These are deposited layer by layer to recreate the shape and function of cardiac tissue (Ketabat et al., 2023; Bliley et al., 2024). This technique not only makes it possible to reproduce the shape of the heart, but also to control features that are important for cells to behave appropriately, for example, porosity, stiffness, and fiber orientation (Spedicati et al., 2024; Ketabat et al., 2023). This review focused on three key points that are being taken into account thanks to 3D printing (Table 1).

Table 1
Key points of the systematic review

Key point	Description
Cell viability and biomimetic microenvironment	For cells to survive the printing process, it is necessary to use bioinks that protect them from mechanical stress (Ketabat et al., 2023). This is an internal force that causes particles to resist change (Mayoral et al., 2022).
Porosity control and scaffold architecture	A scaffold that is truly effective must resemble real cardiac tissue. The use of 3D printing makes it possible to design parts with tuned porosity and aligned fibers (Ketabat et al., 2023). This directly influences how the cells will be distributed and how they will function within the patch or organ (Zhang et al., 2024).
Vascularization and functional improvement	Achieving printed tissues with blood vessels that keep them alive is a major challenge (Pourchet et al., 2025). In this regard, strategies are being developed to facilitate the integration of the patch with the patient's circulatory system.

These advances showed how 3D printing is changing the way solutions to repair the heart are designed. Although there are still unresolved challenges, the fact that progress is being made in this field is evidence that a major step is being taken toward future clinical applications.

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2. RESEARCH METHOD

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method was applied.

2.1. Phase 1: Objective, tools, criteria, and focus

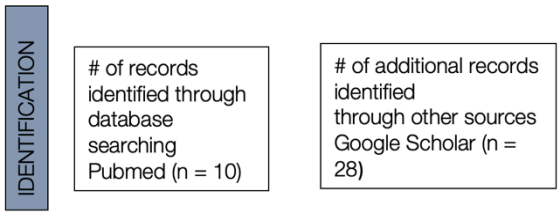
2.1.1. Main objective

This study analyzed how 3D bioprinting is being used in cardiac tissue engineering, focusing on strategies to improve cell viability, the structural design of the scaffold, and functional vascularization, in order to develop patches or structures capable of restoring the function of damaged cardiac muscle.

2.1.2. Sources and tools used

For the collection and management of the information, scientific databases such as PubMed and Google Scholar were used. Subsequently, relevant articles were selected using the Rayyan platform, where filters were applied to remove duplicates (Figure 1).

Figure 1
Search criteria for PubMed and Google Scholar



2.1.3. Inclusion and exclusion criteria

Articles published between 2020 and 2025, in English, addressing experimental and theoretical results on 3D bioprinting applied to cardiac tissues were considered.

2.1.4. Area of focus

This review focused on advances related to the bioprinting of cardiac patches and 3D structures that mimic heart tissue. This made it possible to assess how different techniques, materials, and designs influence the functionality and survival of cells.

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2.2. Phase 2: Data extraction

For the analysis, information was compiled from published research describing the use of 3D bioprinting applied to the heart. The data were extracted considering technical and biological criteria (Table 2).

Table 2
Technical and biological terms evaluated

Term	Description
Printing technique	Method used, such as extrusion, Freeform Reversible Embedding of Suspended Hydrogels (FRESH), inkjet, etc., and the structural precision they provide.
Bioink composition	Main materials (natural or hybrid) and additives used to protect and enhance cell viability.
Scaffold design and porosity	Fiber orientation, pore size, and layer thickness, which influence cell distribution and mechanical properties.
Vascularization strategies	Incorporation of microchannels or prevascularization to keep the tissue alive.
Mechanical properties	Comparison with native cardiac tissue to determine its elasticity and strength.
Functional evaluation	Analysis of contraction, electrical synchronization, and expression of cardiac markers.

Based on these aspects, it was important to summarize the variables analyzed in the selected studies (Table 3).

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Table 3
Variables analyzed in 3D bioprinting studies for cardiac tissue engineering

Category	Variable analyzed	Purpose/Relevance
Printing technique	Extrusion, inkjet, FRESH, and microextrusion.	Determine structural precision and effect on cell viability.
Bioink used	Gelatin, alginate, collagen, methacryloyl gelatin (GelMA), and polycaprolactone (PCL).	Assess biocompatibility and mechanical properties.
Additives or biochemical factors	*Arginylglycylaspartic acid (RGD), fibrin, and growth factors.*	Improve cell adhesion, proliferation, and differentiation.
Cell viability	Percentage of living cells after printing.	Measure the impact of the process on cell survival.
Scaffold design	Pore size, fiber orientation, layer thickness.	Analyze their influence on the distribution and function of cells.
Mechanical properties	Young's modulus, elasticity, and stiffness.	Compare with values of native cardiac tissue.
Vascularization	Presence of microchannels or prevascularization.	Assess the capacity of the printed tissue to remain alive and integrate.
Functional evaluation	Contraction, electrical synchronization, and cardiac markers.	Verify whether the printed tissue mimics myocardial function.

2.3. Phase 3: Comparative analysis

Once the information had been compiled, a comparative analysis was carried out to identify patterns and relationships among materials, techniques, and biological results. The study was structured around three important points:

- Cell viability and biomimetic microenvironment.
- Porosity and scaffold architecture.
- Vascularization and functional integration.

This analysis determined the combinations of bioinks, printing parameters, and design strategies that proved most efficient for maintaining tissue function in the printed heart.

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2.4. Phase 4: Conclusion and future challenges

Finally, it was important to identify which challenges still limit the clinical application of 3D bioprinting in the cardiac field (Table 4).

Table 4
Clinical limitations

Limitation	Description
Cell maturation and synchronization	Printed cells must be made to behave as mature and fully functional cardiac tissue in order to avoid future problems.
Mechanical integration	It must be ensured that the printed patch can withstand the contractile forces of the heart without losing its elasticity.
Stable vascularization	Functional microvascular networks that allow long-term cell survival need to be developed.

These three aspects represent the most important challenges that need to be resolved so that 3D bioprinting of cardiac tissue does not fall behind and can be applied in real medical settings. The data were organized according to the methodology, following the same order as section 3. During the selection process, records that did not meet the criterion of being original experimental studies were discarded. Works focused on compiling and analyzing previously published information on cardiac tissue engineering and biomaterials were excluded (Figure 2).

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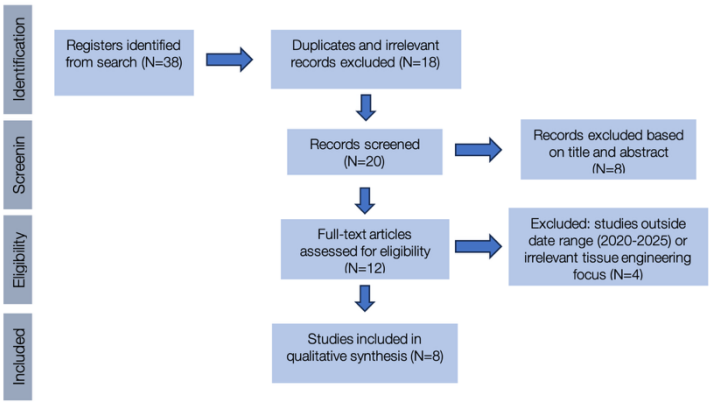
Figure 2
PRISMA flow diagram of the study selection



3. Results

3.1. Cell viability and biomimetic microenvironment

Different types of bioink were analyzed to determine their impact on cell survival after printing (Table 5).

Table 5
Types of bioink analyzed

Bioink	Description
Composite hydrogel	The combination of alginate and gelatin proved effective for cell survival. More specifically, the Alg3Gel1 formulation, which refers to a 3:1 ratio, maintained cell viability above 99% for both cardiomyocytes and endothelial cells (cells that line the interior of blood vessels) over 21 days of culture.
Factor release	The use of functionalized bioinks was reported for the release of bioactive agents. The design of a cardiac patch composed of an RGD-modified alginate core and an alginate sulfate shell was the one that made it possible to encapsulate and release extracellular vesicles in a controlled manner, preserving the integrity of the vesicles after printing.
Synthetic-natural hybrids	In hybrid scaffolds of poly(ε-caprolactone) and methacryloyl gelatin, hydrogel concentration was observed to influence viability. Compared with 10% methacryloyl gelatin concentrations, the 5% and 7% concentrations showed higher viability of cardiac fibroblasts (Figure 3).

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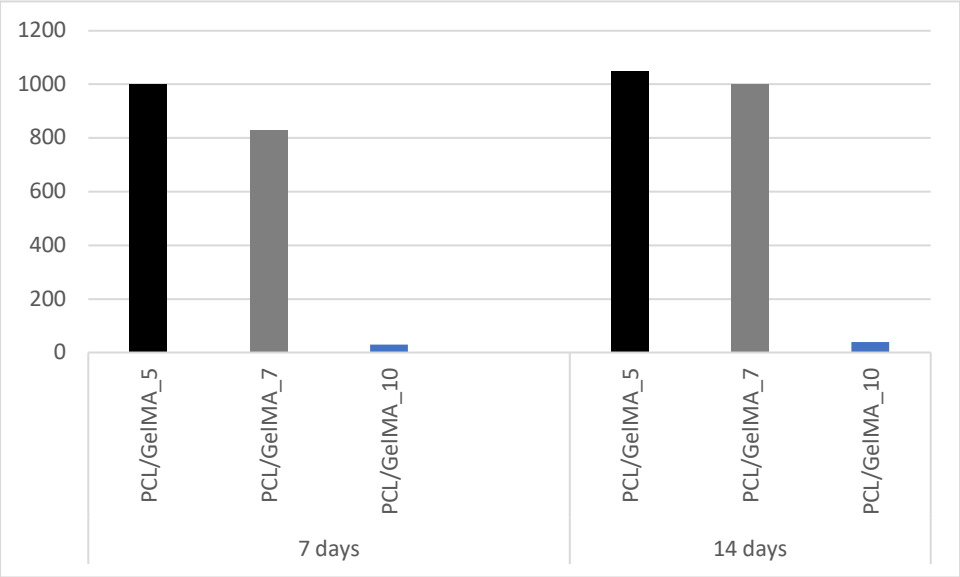
Figure 3
Viability chart



Thanks to a support bath based on k-carrageenan sub-microgels, cell-laden constructs were printed while maintaining viability above 92.1% in mesenchymal stem cells on the first day after printing.

3.2. Porosity, scaffold architecture, and mechanical properties

The mechanical properties needed to mimic different states of cardiac tissue were achieved through structural design and the appropriate choice of materials. Compared with the traditional lattice design (0°/90°), scaffolds with what is known as an angular design showed a higher elastic modulus and a lower swelling rate. In addition, poly(ε-caprolactone) scaffolds were fabricated with wavy filaments to provide elasticity to the construct (Figure 4).

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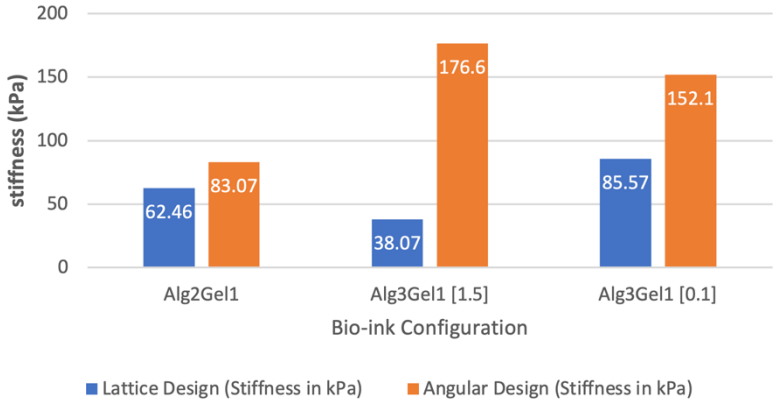
Figure 4

Comparison of the elastic modulus between lattice and angular designs

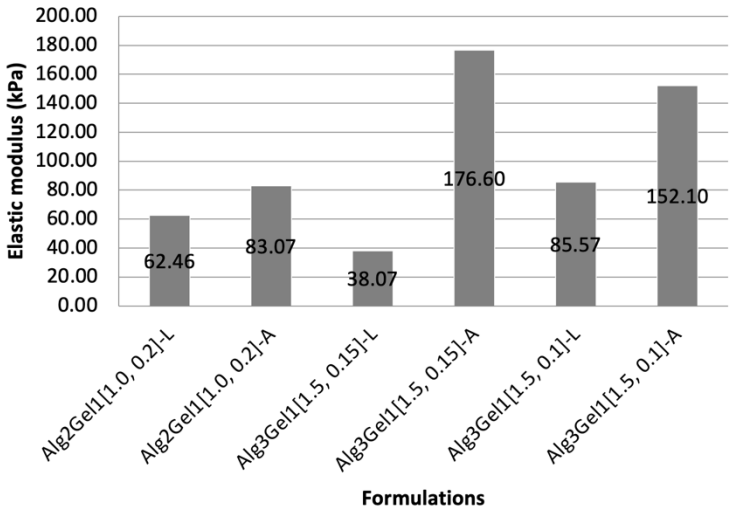


Note. Adapted from Ketabat et al. (2023).

The results indicated that the angular design systematically outperforms the lattice design across all the bioink formulations evaluated. This finding suggests that the angular orientation of the fibers allows a load distribution that better mimics myocardial anisotropy, providing the stiffness required to withstand the contractile forces of the heart without compromising the structural integrity of the patch (Figure 5).

Figure 5

Comparison of the elastic modulus of scaffolds with lattice and angular designs



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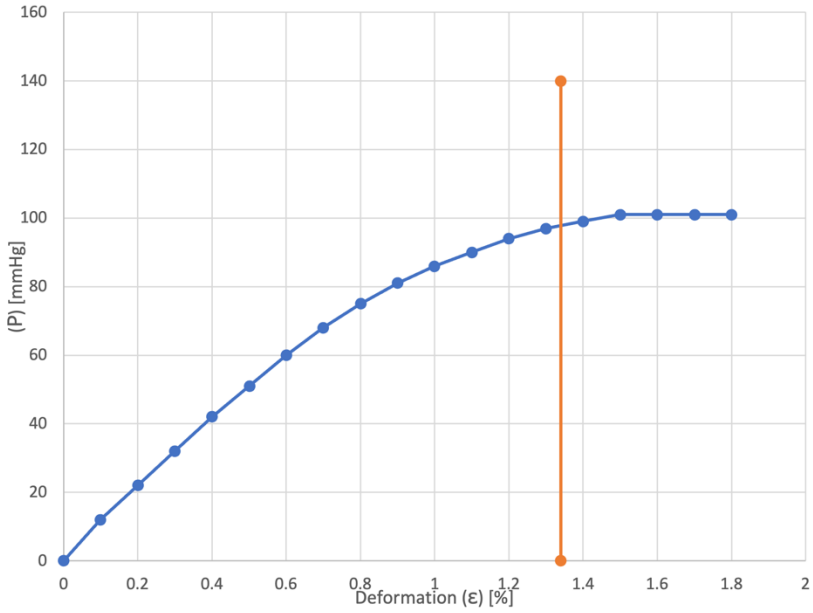
3.2.1. Tissue stiffness mimicry

To simulate fibrotic cardiac tissue, poly(ε-caprolactone) / methacryloyl gelatin scaffolds were tuned to reach a Young's modulus between 6.8 and 10.5 megapascals (MPa). For cardiac patches intended for transplantation in early studies, constructs based on alginate-RGD and gelatin showed an initial elastic modulus between four and five kilopascals on the first day after printing. Regarding pressure resistance, a customized vascular graft was identified, fabricated by a hybrid technique of fused deposition modeling and electrospinning, which demonstrated a burst pressure of 101 ± 15 mmHg, exceeding the average systolic pressure in newborns (Figure 6).

Figure 6
Porosity in a hybrid vascular patch



3.2.2. Resolution and porosity

The fabrication of complex structures such as heart valves and scale heart models was facilitated by the use of k-carrageenan support media. These made it possible to reach a printing resolution of up to 33 micrometers (μm). In this regard, pores develop at 10 μm on the inner surface and 80-100 μm on the outer surface of vascular patches. A porosity gradient was achieved through electrospinning, where the pores of the inner surface allowed nutrient diffusion without cell leakage and the outer surface promoted cell migration.

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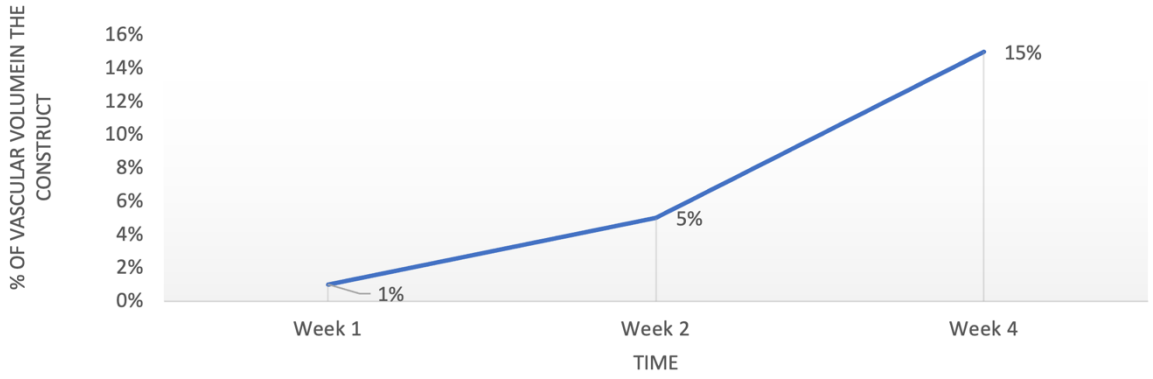
3.3. Vascularization and functional integration

Important results were obtained regarding the capacity of printed tissues to become vascularized and display biological functionality. In this regard, prevascularization with microfragments was carried out alongside non-vascularized controls, which degraded, in contrast to the vascularized cardiac patches, which showed a dense vascular network connected to the host and graft survival of up to four weeks. Incorporating adipose tissue-derived microvascular fragments into a fibrin / gelatin bioink resulted in rapid and effective vascularization (Figure 7).

Figure 7

Evolution of vascular density in vivo



Note. Representation of the formation of functional vascular networks in cardiac patches prevascularized with microvascular fragments (MVF).

The analysis of biological integration capacity revealed that the prevascularization strategy is a determining factor for the survival of printed tissue. In this regard, the patches that incorporated adipose tissue-derived microvascular fragments showed good progression: while in the first week vascular presence was only beginning, by the fourth week a dense and functional network connected directly to the host system had consolidated. This growth not only prevented the graft degradation observed in the non-vascularized controls, but also allowed long-term patch survival and the detection of spontaneous synchronized beating, which confirms that rapid vascularization is the cornerstone of the functional maturation of printed myocardium (Figure 8).

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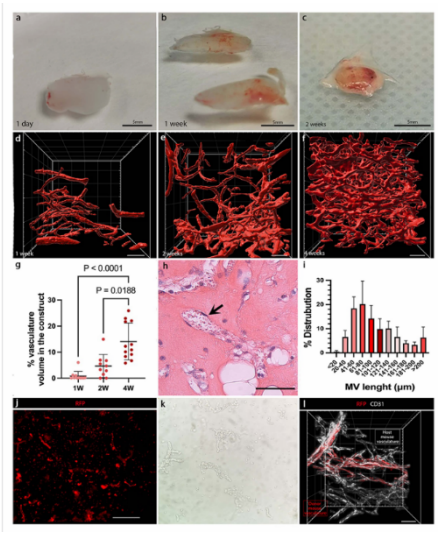
Figure 8

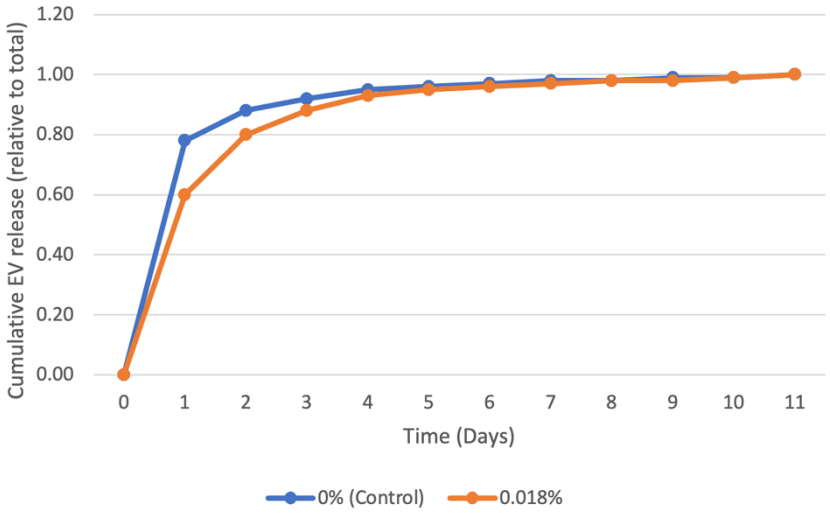
3D micro-CT reconstructions showing the vascular network formed in vivo in cardiac patches prevascularized with microvascular fragments



Alginate sulfate extended the release of extracellular vesicles to 11 days, releasing 88% of the load by day four, compared with 92% in controls without the sulfated polymer (Figure 9).

Figure 9

Chart of cumulative release of extracellular vesicles from alginate sulfate scaffolds



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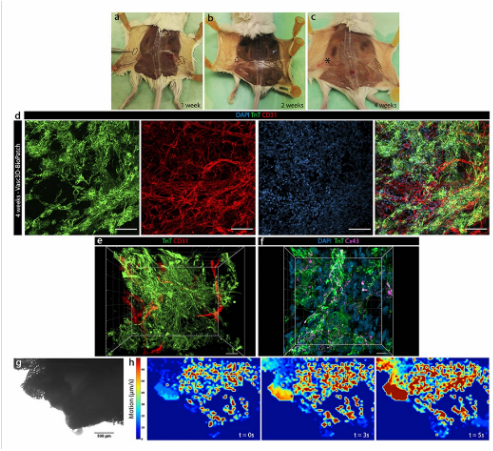
3.3.1. Functional evaluation and maturation

Cardiac patches printed with microvascular fragments showed spontaneous and synchronized beating after four weeks of viable implantation. In this regard, the detection of connexin 43 between adjacent cardiomyocytes in the vascularized patches indicated electrical coupling (Figure 10).

Figure 10

Immunofluorescence images showing the expression of connexin 43 (Cx43) in vascularized cardiac patches, indicating the formation of functional electrical junctions



In poly(ε-caprolactone) / methacryloyl gelatin scaffolds, cyclic mechanical stimulation induced the overexpression of smooth muscle α-actin in cardiac fibroblasts, suggesting activation toward a myofibroblast phenotype. Mesenchymal stem cells differentiated on vascular scaffolds responded with functional contractility to stimulation with endothelin-1.

4. Discussion

The results allow for an objective assessment of the current state of cardiac bioprinting. Cell viability was observed to be above 99%. This establishes a critical standard for graft success, demonstrating that alginate and gelatin formulations act as effective protective microenvironments. The results also highlighted a direct correlation between scaffold architecture and functional response. In other words, the capacity to reach burst pressures of 101 ± 15 mmHg and to maintain electrical synchronization through the expression of connexin 43 suggests that printed tissues not only mimic the shape of the heart, but are close to achieving real biological integration in in vivo models. These quantitative values reinforced the viability of 3D bioprinting as a solid alternative to the limitations of traditional regenerative medicine (Table 6).

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Table 6
Synthesis of performance parameters and quantitative findings

Parameter analyzed	Best reported result	Reference
Cell viability	> 99% (with Alg3Gel1 bioink)	Ketabat et al. (2023)
Printing resolution	33 µm (with k-carrageenan support)	Zhang et al. (2024)
Mechanical strength	101 ± 15 mmHg (burst pressure)	Mayoral et al. (2022)
Survival in vivo	four weeks (with prevascularization)	Pourchet et al. (2025)
Stiffness (elastic modulus)	176.60 kPa (angular design)	Ketabat et al. (2023)

However, in synthesizing these results, variability was identified in the effect estimates, especially in mechanical stiffness and long-term cell viability. This heterogeneity was attributed to the diversity of bioink formulations and the differences in printing parameters among the key studies (Ketabat et al., 2023; Spedicati et al., 2024). Although the confidence intervals for cell viability remained close across alginate / gelatin formulations, the in vivo vascularization data showed greater dispersion due to differences in the animal models used. Therefore, there is a risk of bias from methodological heterogeneity that must be considered when applying these findings to a clinical setting.

4.1. Cell viability and biomimetic microenvironment

The choice of bioink is not only about initial survival, but about a balance between mechanical support and a microenvironment that allows long-term growth (Spedicati et al., 2024). Additives such as methacryloyl gelatin and RGD provide cell adhesion sites, explaining why cells spread and organized instead of remaining as inactive spheres (Zhang et al., 2024; Ketabat et al., 2023).

4.2. Porosity, scaffold architecture, and mechanical properties

3D bioprinting does not only create shapes; it programs mechanical properties into the scaffold through architecture, a level of control that is practically impossible with traditional molding techniques (Spedicati et al.,

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2024). Stiffness mimicry demonstrated the versatility of 3D bioprinting for modulating mechanical properties and creating disease models or regeneration patches (Zhang et al., 2024; Ketabat et al., 2023).

4.3. Vascularization and functional integration

Prevascularization with microvascular fragments is a superior strategy for long-term graft survival (Pourchet et al., 2025). On the other hand, the release of extracellular vesicles is a promising option for paracrine therapy (Bar et al., 2025).

4.4. Limitations and future challenges

Despite the advances achieved, significant limitations remain. The incomplete maturation of cardiomyocytes derived from pluripotent stem cells is an obstacle to tissue functionality (Pourchet et al., 2025; Bliley et al., 2024). In the future, new strategies could be explored, such as the use of bioactive agents for the immobilization of growth factors, which has already proven successful in bone regeneration (Keremu et al., 2025) and could be adapted for cardiac tissues.

5. Conclusions

This systematic review analyzed the use of 3D bioprinting for cardiac tissue engineering, focusing on the objectives of cell viability, scaffold architecture, and vascularization. The findings showed that the bioink selection process is a highly important factor. Combinations of natural hydrogels such as alginate / gelatin were shown to maintain cell viability above 99% in cardiomyocytes and endothelial cells. On the other hand, hybrid bioinks such as poly(ε-caprolactone) / methacryloyl gelatin show a direct correlation between hydrogel concentration and viability, where concentrations of 5 to 7% favor cell survival compared with concentrations of 10%.

It was also confirmed that 3D bioprinting offers precise control that directly impacts mechanical function. The versatility of the angular design makes it possible to modulate mechanical properties in order to mimic healthy tissue or fibrotic cardiac tissue, thus demonstrating its usefulness for disease models and regenerative models. Despite all these advances, challenges remain for clinical application, such as the complete maturation of cardiomyocytes, the immune response, and large-scale production. Therefore, future lines of research should focus on the integration of multiscale vascular networks.

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