Importance of Cell Alignment in Cardiac Tissue Engineering

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Abstract

Native myocardium is a structurally anisotropic and electromechanically integrated tissue in which cardiomyocytes, extracellular matrix fibers, vascular structures, and conduction pathways are organized across multiple length scales. A key feature of this architecture is not a simple stack of discrete layers with slight misalignment, but a continuous transmural variation in predominant myofiber angle from the endocardial to the epicardial surface. This organization supports anisotropic electrical propagation, regional deformation, ventricular torsion, wall thickening, and efficient blood ejection. Conventional two-dimensional culture systems often produce randomly oriented and immature cardiomyocytes, limiting their ability to reproduce native myocardial mechanics or disease-associated remodeling. This narrative review revises and expands the discussion of cardiomyocyte alignment in cardiac tissue engineering, with emphasis on nanofiber-based scaffolds, electrospinning, biomaterial selection, electrical stimulation, mechanical conditioning, bioprinting, and vascularization strategies. We compare spheroids, organoids, conventional scaffolds, aligned nanofibers, three-dimensional bioprinting, and biophysical stimulation approaches in terms of alignment mechanism, maturation readouts, advantages, limitations, and translational challenges. Particular attention is given to scaffold design parameters, including fiber diameter, degree of alignment, porosity, stiffness, degradation behavior, conductivity, surface functionalization, and extracellular matrix coating. We also discuss why alignment strategies should be adapted to the target ventricular region, because left and right ventricular myocardium differ in geometry, loading condition, fiber architecture, and contractile pattern. Finally, we highlight remaining barriers to clinical translation, including vascularization, oxygen and nutrient diffusion, host-tissue electrical integration, arrhythmia risk, scalability, reproducibility, and standardized functional assessment.

Keywords: cardiac tissue engineering; cardiomyocyte alignment; myocardial anisotropy; nanofiber scaffolds; electrospinning; electrical stimulation; mechanical conditioning; three-dimensional bioprinting

1. Introduction

Cardiac tissue engineering aims to generate myocardial constructs that reproduce not only cardiomyocyte survival and spontaneous beating but also the structural and functional organization required for physiologically meaningful contraction. Native myocardium is not a random assembly of contractile cells. It is an anisotropic tissue in which cardiomyocytes and extracellular matrix components are arranged in preferred directions, enabling coordinated force transmission and electrical propagation. This architectural organization is central to myocardial deformation and pump function, and it has long been recognized that cardiac mechanics cannot be understood without considering tissue structure and regional strain patterns (1).

Myocardial alignment is sometimes simplified as a set of discrete layers with different fiber directions. That description is useful as a rough teaching model, but it can also imply an artificial discontinuity within the ventricular wall. In the healthy ventricle, the predominant myofiber angle changes gradually across the wall, a pattern described in classic anatomic work and supported by modern imaging and multiscale computational studies (2-9). This continuous architecture contributes to ventricular torsion, regional wall thickening, and

heterogeneous but coordinated deformation during systole rather than uniform contraction of all myocardial regions.

Cardiomyocyte alignment is also important in vitro. Cells cultured on standard flat surfaces often spread and orient randomly, and such cultures rarely reproduce the anisotropic conduction velocity, sarcomere organization, and contractile directionality observed in native myocardium. Scaffold architecture, microscale patterning, aligned nanofibers, electrical pacing, mechanical loading, and three-dimensional bio fabrication have therefore been developed to guide cardiomyocyte orientation and maturation. Earlier work using anisotropic scaffolds demonstrated that engineered microarchitecture can influence cardiac tissue alignment and functional output (10), and studies of cell shape and cytoskeletal organization emphasized that geometry is not merely a morphological feature but a regulator of myofibrillar architecture and tissue function (11).

Among currently available approaches, nanofiber-based scaffolds are especially attractive because they provide contact guidance at a scale comparable to extracellular matrix fibrils. Cardiomyocytes can elongate along aligned fibers, organize sarcomeres parallel to the scaffold axis, and establish more anisotropic contractile behavior. However, nanofiber scaffolds are not sufficient by themselves to reproduce the full complexity of myocardium. Electrical stimulation can enhance coupling and calcium handling, mechanical stimulation can reinforce load-dependent maturation, and bioprinting can help control multicellular spatial organization and tissue geometry (12-14). This review therefore treats nanofiber technology as an important component of a broader integrated strategy rather than as a stand-alone or universally superior method.

The goal of this review is to provide a clearer, more critical, and more accurate synthesis of how alignment should be understood and engineered in cardiac tissue regeneration, disease modeling, and drug testing. We first define the review scope, then summarize native myocardial architecture, compare major tissue engineering approaches, discuss nanofiber scaffold design in detail, and finally consider how structural guidance can be combined with electrical, mechanical, and vascular cues to improve translational relevance.

2. Scope of the Review

This narrative review focuses on cardiomyocyte alignment in cardiac tissue engineering, especially nanofiber-based scaffolds, electrical and mechanical stimulation, bioprinting strategies, and vascularization. The review was designed to synthesize representative studies on myocardial architecture, scaffold design, cardiomyocyte maturation, anisotropic tissue organization, and translational challenges. It was not intended as a systematic review or meta-analysis.

Literature was identified through searches of PubMed/MEDLINE, Web of Science, and Google Scholar, supplemented by citation tracking from key articles. Searches covered database inception through May 2026. Search terms included combinations of: cardiac tissue engineering, cardiomyocyte alignment, myocardial anisotropy, ventricular fiber architecture, nanofiber scaffold, electrospinning, extracellular matrix, human pluripotent stem cell-derived cardiomyocytes, electrical stimulation, mechanical conditioning, mechanical loading, bioprinting, vascularization, cardiac patch, myocardium, and conduction.

English-language peer-reviewed original studies and reviews were prioritized. Classic anatomic and biomechanical studies were included when they were necessary to frame current concepts. Articles were selected for relevance to myocardial alignment, scaffold architecture, cardiomyocyte maturation, engineered anisotropy, vascularization, and translational feasibility. Because this was a narrative review, no formal risk-of-bias assessment, quality scoring, or quantitative meta-analysis was performed.

3. Native myocardial architecture and functional significance of alignment

The myocardium contains a hierarchy of structural organization from sarcomeres and myofibrils to cardiomyocytes, laminar sheets, extracellular matrix networks, and whole-ventricle geometry. Alignment within this hierarchy determines the direction in which active force is generated and transmitted. A key point in the revised manuscript is that myocardial structure should not be described as three fixed layers that simply point in different directions. Although endocardium, midwall, and epicardium are useful anatomical descriptors, ventricular myocardium exhibits a continuous transmural change in predominant fiber angle (2,3,9).

This architecture supports ventricular mechanics through coordinated regional deformation. During systole, the ventricle does not contract uniformly. Instead, longitudinal shortening, circumferential shortening, radial thickening,

shear, and torsion occur in spatially heterogeneous but integrated patterns. Recent modeling and imaging studies of right ventricular and left ventricular remodeling further demonstrate that changes in fiber architecture and regional material behavior can influence global ventricular performance (3-7). Therefore, cardiac tissue engineering should aim not only to align cells on a surface but also to reproduce meaningful anisotropy, regional organization, and electromechanical coupling.

The distinction between uniform contraction and coordinated regional contraction is important. Uniform contraction would imply that all parts of the myocardium shorten by the same amount and in the same direction. Healthy myocardium instead generates efficient pump function by combining region-specific deformation with synchronized electrical activation. Left ventricular torsion, for example, arises from the interaction between transmural fiber rotation, wall geometry, loading, and tissue mechanics; it cannot be reproduced by a simple parallel sheet of cells (8). Studies using strain-based and computational approaches suggest that myocardial fiber architecture can be inferred from or strongly influences measured strain patterns (9). These findings provide a mechanistic rationale for reproducing alignment in engineered myocardium.

Alignment strategies also need to consider differences between the left and right ventricles. The left ventricle has a thick wall and generates systemic pressure, whereas the right ventricle operates under lower pressure, has a thinner crescent-like geometry, and exhibits distinct loading and deformation patterns. Right ventricular architectural remodeling in disease may involve changes in fiber structure, wall thickness, and regional mechanics that differ from left ventricular post-infarction remodeling (4,6,7). Therefore, a scaffold architecture optimized for a left ventricular patch may not automatically model right ventricular myocardium. Future engineered tissues should be designed according to the target ventricular region, disease state, and functional application.

In regenerative medicine, the importance of alignment extends beyond contraction. Anisotropic cell organization affects conduction velocity, calcium wave propagation, gap junction distribution, sarcomere alignment, and extracellular matrix deposition. Poorly organized constructs may beat spontaneously but fail to generate directional force, integrate electrically with host myocardium, or model disease-specific remodeling. For this reason, alignment should be treated as a measurable design parameter rather than a qualitative observation. Quantitative outcomes such as orientation index, angular dispersion, sarcomere length, connexin-43 localization, conduction velocity, calcium transient kinetics, twitch stress, and force-frequency response should be reported when evaluating engineered cardiac tissues.

4. Current strategies for engineering aligned cardiac tissue

Several tissue engineering strategies have been used to improve the organization and maturation of cardiac constructs. Spheroids and organoids promote cell-cell interactions and can support multicellular communication, but they generally provide limited control over long-range alignment unless combined with external patterning or mechanical constraints. Conventional scaffolds, including collagen gels and synthetic polymer matrices, provide three-dimensional support and tunable mechanical properties, but they do not necessarily impose a physiologically relevant orientation unless anisotropic features are introduced (15-18).

Aligned nanofibers guide cardiomyocyte elongation through contact guidance. This is one of the most direct approaches for controlling cell orientation at the microscale and nanoscale. However, dense electrospun mats can limit cell infiltration, reduce effective cell-cell contact, and restrict tissue thickness. Scaffold-free methods such as cell sheets and myocardial tubes preserve high cell density and cell-cell coupling, but they require additional strategies to generate controlled three-dimensional geometry, perfusion, and transmural alignment (18). Albumin-based and other protein-derived fibrous scaffolds have shown that biologically active materials can support functional cardiac tissue formation, but the balance between mechanical strength, degradation, and biological activity must be carefully controlled (19).

Microfabricated scaffolds and nanocomposite materials have also been introduced to improve anisotropy and electrical behavior. Carbon-based or conductive components can enhance electrical signal propagation in some settings, but electroactive materials require careful assessment of cytotoxicity, degradation products, long-term

stability, and arrhythmogenic risk (20). Engineered anisotropy can be induced through micropatterned hydrogels, aligned fibers, structural pores, and printed architectures, and these strategies can be combined with human pluripotent stem cell-derived cardiomyocytes to improve disease modeling and drug testing (21-24).

Table 1 summarizes major strategies for producing aligned or functionally mature engineered myocardium. The key message is that no single method is sufficient for all purposes. Spheroids emphasize cell-cell interaction, scaffolds provide mechanical support, nanofibers provide directional guidance, bioprinting controls multicellular spatial placement, electrical stimulation enhances electrophysiological maturation, and mechanical loading promotes mechanobiological adaptation. A mature cardiac construct will likely require rational integration of several of these approaches.

Table 1 summarizes major strategies for producing aligned or functionally mature engineered myocardium, including spheroid-based approaches, conventional scaffolds, aligned nanofibers, electroactive scaffolds, and electrical stimulation strategies. Previous studies have demonstrated that spheroids enhance cell–cell interactions and tissue organization, scaffolds provide mechanical support and tunable environments, aligned nanofibers promote anisotropic cellular alignment, electroactive materials improve electrical coupling, and external stimulation approaches facilitate electrophysiological maturation. However, no single strategy is sufficient to achieve all requirements of functional cardiac tissue engineering; therefore, integrated approaches combining structural, electrical, and mechanical cues are increasingly being explored [6–7, 12–18, 20-40, 41]

Table 1. Comparison of major strategies for engineering aligned and mature cardiac tissue.

Strategy	**Primary mechanism**	**Main advantages**	**Main limitations**	**Useful readouts**	**Translational issues**	**Key refs**
Spheroids/organoids	Self-assembly and cell-cell adhesion	High cell density; multicellular interactions; useful for screening	Limited long-range alignment; diffusion limits in larger aggregates	Beating frequency, viability, calcium transients, gene expression	Standardization, size control, vascularization	16,17,24
Conventional scaffolds	Three-dimensional (3D) matrix support and tunable stiffness	Mechanical support; compatible with hydrogels and polymers	Alignment depends on additional cues; scaffold may separate cells	Twitch force, stiffness, sarcomere organization	Degradation, immune response, reproducibility	15,25
Aligned nanofibers	Contact guidance along fiber axis	Strong control of cardiomyocyte elongation and anisotropy	Dense mats may limit infiltration and coupling	Orientation index, sarcomere alignment, conduction anisotropy	3D scaling, vascularization, manufacturing control	21-23,26,27,41

Electroactive scaffolds	Structural guidance plus conductive pathways	May improve electrical coupling and synchronized activation	Conductive additives may raise safety and degradation concerns	Connexin-43, conduction velocity, action potential propagation	Cytotoxicity, long-term stability, arrhythmia risk	20,28
Electrical stimulation	Paced activation and electrophysiological conditioning	Improves coupling, calcium handling, and maturation	Excessive field strength or frequency may injure cells	Capture threshold, calcium kinetics, connexin-43, force-frequency response	Device integration, parameter standardization	12-14,29,30,32,33
Mechanical stimulation	Stretch, load, and mechanotransduction	Promotes sarcomere maturation, force generation, extracellular matrix (ECM) remodeling	Overstretch may induce injury or stress signaling	Twitch stress, passive tension, sarcomere length, ECM markers	Physiological loading, reproducibility, scaling	6,7,31,32,34
Bioprinting	Spatial placement of cells and bioinks	Controls geometry and multicellular organization	Resolution, cell viability, and functional maturation remain challenging	Print fidelity, viability, vascular channels, force output	Perfusion, thickness, good manufacturing practice (GMP) manufacturing	35-38
Cell sheet/scaffold-free tissues	Preserved cell-cell junctions and high cellular density	Good coupling; avoids permanent scaffold residues	Thickness and perfusion remain limiting	Synchronous beating, gap junctions, graft integration	Layering, vascularization, handling strength	16,18, 39, 40

5. Nanofiber-based scaffolds for cardiomyocyte alignment

Nanofiber scaffolds are central to this review because their structure resembles aspects of the native extracellular matrix and because aligned fibers provide a clear directional cue for cells. Electrospinning is the most widely used method for producing micro- and nanoscale fibers from synthetic polymers, natural polymers, or composites. By controlling polymer concentration, solvent composition, voltage, flow rate, collector geometry, and environmental conditions, it is possible to tune fiber diameter, alignment, density, and scaffold thickness (26,27). These parameters strongly influence cell attachment, elongation, infiltration, and maturation.

Fiber diameter is important because cardiomyocytes sense topographical features through focal adhesions and cytoskeletal organization. Very small fibers may mimic extracellular matrix fibrils but can form dense mats that restrict cell penetration. Larger fibers or melt-electro written architectures can provide more open pores and better

three-dimensional cellular infiltration, although they may offer less nanoscale biochemical mimicry. Porosity and pore interconnectivity should therefore be reported together with fiber diameter and orientation, rather than describing scaffolds only as aligned or random. Scaffold stiffness is also critical because cardiomyocytes respond to mechanical resistance; excessive stiffness can impair contraction, whereas overly soft scaffolds may not transmit force efficiently.

Synthetic polymers such as polycaprolactone, polylactic acid, and related copolymers offer reproducible processing, controllable degradation, and mechanical strength, but their surfaces may require biochemical modification to support cardiomyocyte adhesion. Natural polymers such as collagen, gelatin, fibrin, chitosan, and albumin can provide cell-recognizable ligands and improved biological compatibility, but they may have lower mechanical stability, batch variability, and faster degradation (19,25,26). Hybrid scaffolds are attractive because they combine the processability of synthetic polymers with the bioactivity of natural components.

Electroactive materials and nanocomposites may improve electrical coupling, but their advantages must be balanced against potential safety concerns. Carbon nanotubes, graphene derivatives, conductive polymers, and metallic nanostructures have been investigated as conductivity-enhancing components. These materials can support synchronized beating in some experimental systems, yet long-term biocompatibility, inflammatory response,

degradation, and arrhythmogenic effects remain unresolved translational issues (20,29-31). Therefore, conductivity should be treated as one scaffold property among many, not as an automatic marker of clinical suitability.

Surface functionalization is another important design variable. Coating aligned nanofibers with fibronectin, laminin, collagen, gelatin, or peptide motifs can improve initial cell adhesion and sarcomere organization. However, coating density, stability, and spatial uniformity can alter the effective biochemical cue experienced by cells. A scaffold with excellent fiber alignment but unstable or nonuniform surface chemistry may produce inconsistent biological results. Future studies should report not only the scaffold material but also coating method, coating concentration, sterilization procedure, residual solvent removal, and mechanical characterization.

The primary limitation of nanofiber sheets is three-dimensional scaling. Thin sheets can align cardiomyocytes well, but native myocardium is thick, vascularized, electrically integrated, and mechanically loaded. Stacking multiple aligned sheets may increase thickness but can also create diffusion barriers and weak interfaces between layers. The challenge is therefore to develop nanofiber architectures with open interconnected pores, controllable multilayer orientation, and compatibility with perfusion or vascularization. Combining nanofiber alignment with bioprinting, sacrificial channels, or vascular cell organization may be a promising direction (32, 33, 34, 35, 42, 43).

Nanofiber-based approaches should also be evaluated against rigorous functional endpoints. Improved alignment alone does not guarantee mature myocardium. Studies should assess contractile force, conduction anisotropy, calcium handling, sarcomere organization, metabolic maturation, and response to pacing or drugs. A scaffold designed for regenerative therapy should additionally be tested for mechanical integrity, degradation behavior, immune compatibility, electrical integration with host tissue, and scalability. These considerations make nanofiber scaffolds powerful but technically demanding platforms rather than simple culture substrates.

6. Electrical and mechanical stimulation for maturation and alignment

Cardiomyocytes in vivo mature under continuous electrical activation and mechanical loading. In vitro, electrical stimulation has been widely used to improve functional assembly of engineered myocardium. Early studies showed that field stimulation of cardiac myocytes cultured on scaffolds improved contractile and conductive properties (12). Subsequent platforms, including biowire systems, demonstrated that three-dimensional culture combined with programmed electrical stimulation can enhance maturation of human pluripotent stem cell-derived cardiomyocytes (13). More advanced protocols using progressive intensity or frequency training have further shown that electrical conditioning can improve sarcomere organization, excitation-contraction coupling, and metabolic maturation (14).

Electrical stimulation must be optimized rather than applied generically. Parameters such as pulse amplitude, pulse duration, frequency, stimulation waveform, electrode material, medium conductivity, and tissue size all influence the biological response. Appropriate pacing can improve connexin-43 organization, calcium transient kinetics, action potential propagation, and synchronous contraction. Inappropriate stimulation can cause abnormal membrane polarization, pH changes near electrodes, reactive species generation, or cell injury. Therefore, stimulation conditions should be reported in detail, and maturation should be evaluated by functional endpoints rather than by beating synchronization alone (30, 36-39).

Mechanical stimulation is equally important. Native cardiomyocytes experience passive stretch, active shortening, cyclic loading, and regional wall stress. Mechanical loading can influence sarcomere assembly, cytoskeletal organization, extracellular matrix deposition, and mechanotransduction pathways. Because myocardial fiber remodeling in vivo is linked to the local mechanical environment, combining aligned nanofibers with mechanical stimulation is especially relevant. In such systems, nanofibers can define an initial structural axis, while cyclic stretch or static tension can reinforce alignment, promote sarcomere maturation, and guide matrix remodeling (6-7, 40, 44).

Mechanical conditioning should be designed to match the intended application. A construct intended to model post-infarction left ventricular remodeling may require different stiffness, strain amplitude, and loading direction than one intended to model right ventricular pressure overload. Excessive load can activate stress responses, reduce viability, or alter phenotype in nonphysiological ways. Conversely, insufficient load may result in tissues that beat spontaneously but produce weak force. Future studies should report strain magnitude, frequency, direction, duration, passive tension, scaffold stiffness, and resulting contractile force.

Electrical and mechanical cues should not be considered independent. In native myocardium, electrical activation produces contraction, contraction changes mechanical load, and mechanical feedback affects electrophysiology. Engineered tissues that integrate aligned nanofibers, electrical pacing, and mechanical conditioning may better reproduce this feedback loop. However, such systems are experimentally more complex, and increased complexity can reduce reproducibility. Standardized reporting and quality control will therefore be essential for comparing studies across laboratories.

7. Integration with bioprinting, vascularization, and three-dimensional tissue engineering

Three-dimensional bioprinting offers a complementary strategy to nanofiber scaffolds because it can place cells, bioinks, and structural features in predefined geometries. Bioprinting can pattern cardiomyocytes, endothelial cells, fibroblasts, and extracellular matrix components, allowing more controlled construction of multicellular cardiac tissues than simple bulk casting. However, printed tissues often remain immature, and printing resolution may not be sufficient to reproduce nanoscale myofiber guidance without additional cues (32, 43, 45).

A major advantage of bioprinting is the possibility of incorporating vascular or perfusable channels. Oxygen diffusion limits the thickness of dense cardiac tissue, and thick engineered myocardium requires vascularization for survival and function. Strategies such as sacrificial printing, endothelialized channels, microfluidic perfusion, and prevascularized patches have therefore become important in the field (33, 34, 42). These approaches are particularly relevant for nanofiber-based constructs because highly aligned dense mats can improve cell orientation while simultaneously restricting diffusion and infiltration if not designed carefully.

Cell-sheet engineering and scaffold-free approaches provide another route toward dense cardiac tissues. Layered cell sheets can preserve cell-cell contact and extracellular matrix produced by the cells, and myocardial tubes fabricated from cell sheets have demonstrated the possibility of constructing beating tissues without permanent synthetic scaffolds (41). However, as with nanofiber sheets, tissue thickness, vascularization, and mechanical handling remain major challenges. Scaffold-free tissues may therefore benefit from integration with aligned templates, perfusion systems, or printed support structures.

The most promising future strategies are likely to combine structural, electrical, mechanical, and vascular design. For example, aligned nanofibers could guide local cardiomyocyte orientation, bioprinting could define three-dimensional tissue geometry and multicellular patterning, electrical stimulation could enhance coupling and conduction, mechanical loading could promote force generation, and endothelial networks could support oxygen and nutrient supply. Such integrated systems should be developed with clearly defined target applications, such as drug screening, disease modeling, ventricular patch therapy, or fundamental studies of mechanobiology.

Integration also requires careful consideration of measurement. A construct with aligned cells but no measurable force, poor conduction, or inadequate survival is not functionally mature. Conversely, a construct that contracts strongly but lacks correct regional anisotropy may not model native ventricular mechanics. Therefore, the evaluation framework should include structural metrics, electrophysiological metrics, mechanical metrics, metabolic markers, and long-term stability. Figure 1 illustrates major fabrication approaches, whereas Figure 2 illustrates electrical and mechanical conditioning systems that can be combined with scaffold-based constructs.

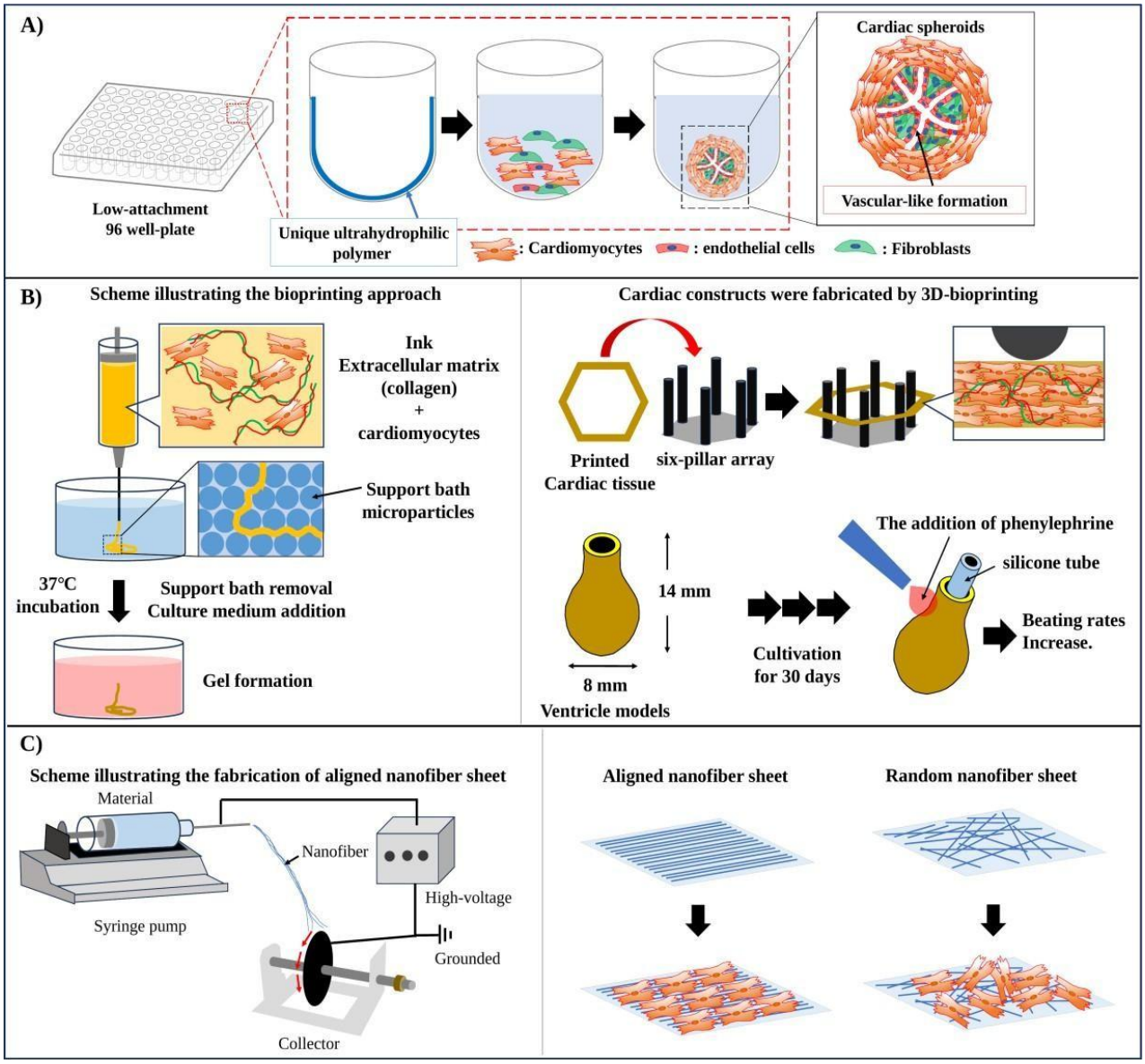

Figure 1. Representative cardiac tissue fabrication strategies**. (A)** Scaffold-free spheroid formation promotes cell-cell contact among cardiomyocytes, endothelial cells, and fibroblasts but provides limited long-range control of tissue alignment. **(B)** Three-dimensional bioprinting can define construct geometry and multicellular patterning using collagen-based or other bioinks, but printed cardiac tissues often require additional maturation cues**. (C)** Aligned nanofiber scaffolds provide contact guidance and promote cardiomyocyte elongation along the fiber axis; however, three-dimensional scaling and vascularization remain important challenges.

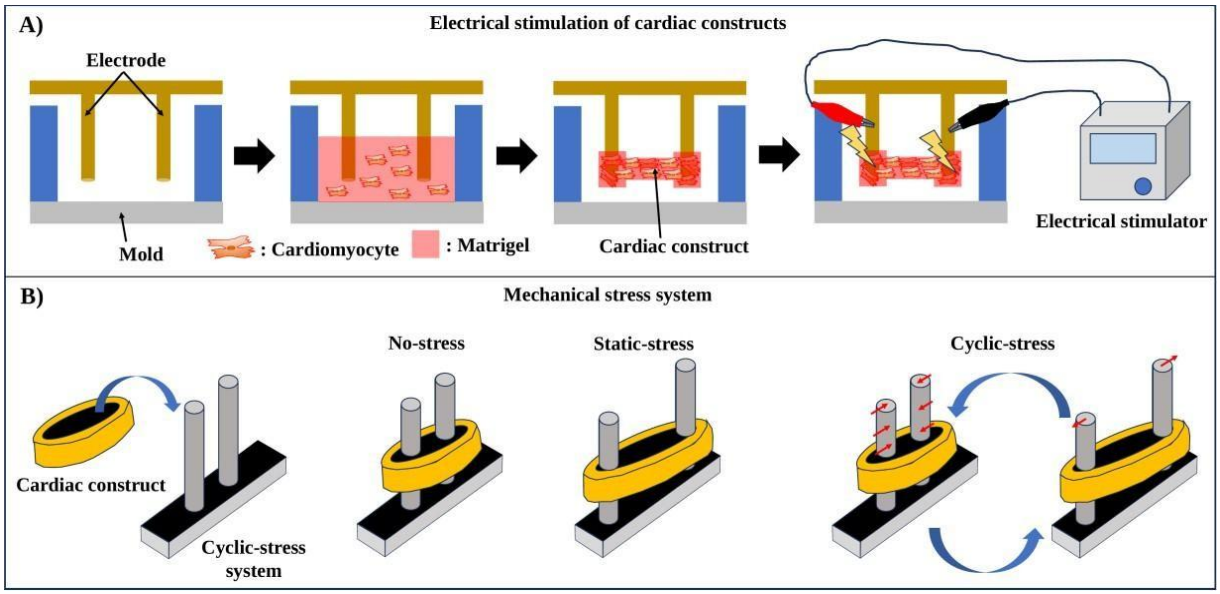

Figure 2. Electrical and mechanical conditioning systems for engineered cardiac tissue. **(A)** Electrical stimulation can improve synchronized activation, calcium handling, gap junction organization, and contractile maturation when stimulation parameters are optimized. **(B)** Mechanical conditioning, including static tension or cyclic stretch, can promote sarcomere organization, force generation, and matrix remodeling, but excessive loading may damage cells or induce nonphysiological stress responses.

8. Translational challenges and future perspectives.

For clinical translation, the key challenge is to move from visually aligned cells to reproducible, safe, vascularized, and electromechanically integrated tissue. Alignment must be measured quantitatively and linked to function. Recommended structural readouts include nuclear orientation, actin or sarcomere orientation, angular dispersion, orientation index, scaffold fiber diameter, pore size, and thickness. Recommended functional readouts include conduction velocity, excitation threshold, calcium transient amplitude and decay, twitch force, passive stiffness, force-frequency response, and response to pharmacological agents.

Host-tissue electrical integration is a major barrier. A cardiac patch that beats independently but fails to synchronize with host myocardium may increase arrhythmia risk. Strategies to improve integration include tuning tissue conduction properties, promoting gap junction formation, designing anisotropic conduction pathways, and controlling graft geometry. However, excessive or poorly controlled conductivity could also be dangerous. Thus, electrical integration should be tested carefully in preclinical models before clinical application (46-48).

Vascularization remains another major limitation. Engineered myocardium thicker than a few hundred micrometers requires oxygen and nutrient delivery beyond passive diffusion. Endothelial cells, pericytes, vascular channels, perfusion bioreactors, and host vessel inosculation are all relevant. For regenerative therapy after myocardial infarction, vascular integration must occur rapidly enough to prevent graft hypoxia and cell death. For disease modeling and drug screening, perfusion is important for maintaining stable metabolic conditions and for reproducing drug transport.

Scalability and manufacturing reproducibility also require attention. Electrospinning is useful for producing aligned fibers, but batch-to-batch variation in fiber diameter, alignment, residual solvent, and coating quality can affect biological results. Bioprinting allows spatial control, but print fidelity, cell viability, bioink composition, and post-print maturation can vary. Electrical and mechanical bioreactors add additional variables (49). Therefore, standardized manufacturing and reporting criteria will be needed before these technologies can support clinical-grade tissue products.

Finally, the field should avoid assuming that a single alignment pattern is universally optimal. The appropriate architecture depends on whether the aim is to model healthy left ventricle, right ventricular pressure overload, myocardial infarction, arrhythmia, drug-induced cardiotoxicity, or regenerative patch therapy. Future engineered

tissues should incorporate region-specific geometry, disease-specific mechanical loading, and appropriate multicellular composition. In this context, nanofibers, scaffolds, bioprinting, and stimulation platforms should be considered modular tools that can be combined rationally rather than competing technologies (50).

9. Conclusion

Cardiomyocyte alignment is a central design principle in cardiac tissue engineering because cardiac function depends on anisotropic architecture, coordinated regional deformation, electrical propagation, and directional force transmission. The myocardium should be described as a continuously organized anisotropic tissue rather than as a simple three-layer structure with slight misalignment. Healthy contraction is not uniform; it is regionally heterogeneous but mechanically coordinated.

Nanofiber-based scaffolds are powerful tools for guiding cardiomyocyte orientation, especially when fiber diameter, alignment, porosity, stiffness, degradation, conductivity, and surface chemistry are carefully controlled. Nevertheless, nanofibers alone cannot reproduce the full complexity of native myocardium. Electrical stimulation, mechanical conditioning, bioprinting, cell-sheet engineering, and vascularization strategies provide complementary cues that are necessary for improving maturation and translational relevance.

Future progress will depend on integrated systems that combine structural guidance with functional conditioning and vascular support. The most important remaining challenges include three-dimensional scaling, oxygen and nutrient diffusion, host electrical integration, arrhythmia risk, immune compatibility, manufacturing reproducibility, standardized functional testing, and clinical scalability. Addressing these challenges will enable engineered myocardium to become more useful for disease modeling, drug screening, and regenerative therapy (51).

Abbreviations

a. 3D: Three-dimensional
b. ECM: Extracellular Matrix
c. GMP: Good Manufacturing Practice

Conflicts of Interest

The authors declare no conflicts of interest.

Availability of Data and Materials

No new datasets were generated or analyzed during the preparation of this review. All data and materials discussed in this article are derived from previously published studies cited in the reference list.

Author Contributions:

Conceptualization: T.K., K.A.; Methodology, literature review, investigation, writing—original draft preparation, visualization, project administration: T.K.; Supervision, critical revision of the manuscript: K.A.; Writing—review and editing: T.K., K.A. Both authors have reviewed and approved the final version of the manuscript

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AI Declaration

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References

1. Sengupta PP, Korinek J, Belohlavek M, Narula J, Vannan MA, Jahangir A, Khandheria BK. Left ventricular structure and function: basic science for cardiac imaging. *J Am Coll Cardiol.* 2006;48(10):1988–2001. doi: 10.1016/j.jacc.2006.08.030

2. Streeter DD Jr. Gross morphology and fiber geometry of the heart. In: Berne RM, Sperelakis N, Geiger SR, editors. Handbook of Physiology, Section 2: The Cardiovascular System. Bethesda: American Physiological Society; 1979. p. 61-112.

3. Li DS, Mendiola EA, Avazmohammadi R, Sachse FB, Sacks MS. A multi-scale computational model for the passive mechanical behavior of right ventricular myocardium. J Mech Behav Biomed Mater. 2023;142:105788. doi:10.1016/j.jmbbm.2023.105788.

4. Mendiola EA, da Silva Gonçalves Bos D, Leichter DM, Vang A, Zhang P, Leary OP, Gilbert RJ, Avazmohammadi R, Choudhary G. Right ventricular architectural remodeling and functional adaptation in pulmonary hypertension. *Circ Heart Fail.* 2023;16(2):e009768. doi:10.1161/CIRCHEARTFAILURE.122.009768

5. Mendiola EA, Neelakantan S, Xiang Q, Xia S, Zhang J, Avazmohammadi R. An image-driven micromechanical approach to characterize multiscale remodeling in infarcted myocardium. Acta Biomater. 2024;173:109-122. doi:10.1016/j.actbio.2023.10.027.

6. Avazmohammadi R, Mendiola EA, Soares JS, Li DS, Chen Z, Merchant S, et al. A computational cardiac model for the adaptation to pulmonary arterial hypertension in the rat. Ann Biomed Eng. 2019;47(1):138-153. doi:10.1007/s10439-018-02130-y.

7. Mendiola EA, Neelakantan S, Xiang Q, Merchant S, Li K, Hsu EW, et al. Contractile adaptation of the left ventricle post-myocardial infarction: predictions by rodent-specific computational modeling. Ann Biomed Eng. 2023;51(4):846-863. doi:10.1007/s10439-022-03102-z.

8. Mukherjee T, Mendiola EA, Neelakantan S, Shah DJ, Avazmohammadi R. Four-dimensional assessment of left ventricular torsion in mitral valve prolapse using CMR. In: Colliot O, Mitra J, editors. Medical Imaging 2024: Image Processing. Proc SPIE. 2024;12926:129260E. doi:10.1117/12.3007019.

9. Usman M, Mendiola EA, Mukherjee T, Mehdi RR, Ohayon J, Avazmohammadi R. On the possibility of estimating myocardial fiber architecture from cardiac strains. In: Functional Imaging and Modeling of the Heart. Lecture Notes in Computer Science. 2023;13958:74-83. doi:10.1007/978-3-031-35302-4_8.

10. Engelmayr GC Jr, Cheng M, Bettinger CJ, Borenstein JT, Langer R, Freed LE. Accordion-like honeycombs for tissue engineering of cardiac anisotropy. Nat Mater. 2008;7(12):1003-1010. doi:10.1038/nmat2316.

11. Parker KK, Tan J, Chen CS, Tung L. Myofibrillar architecture in engineered cardiac myocytes. Circ Res. 2008;103(4):340-342. doi:10.1161/CIRCRESAHA.108.182469.

12. Radisic M, Park H, Shing H, Consi T, Schoen FJ, Langer R, et al. Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. Proc Natl Acad Sci U S A. 2004;101(52):18129-18134. doi:10.1073/pnas.0407817101.

13. Nunes SS, Miklas JW, Liu J, Aschar-Sobbi R, Xiao Y, Zhang B, et al. Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. Nat Methods. 2013;10(8):781-787. doi:10.1038/nmeth.2524.

14. Ronaldson-Bouchard K, Ma SP, Yeager K, Chen T, Song LJ, Sirabella D, et al. Advanced maturation of human cardiac tissue grown from pluripotent stem cells. Nature. 2018;556(7700):239-243. doi:10.1038/s41586-018-0016-3.

15. Zimmermann WH, Melnychenko I, Eschenhagen T. Engineered heart tissue for regeneration of diseased hearts. Biomaterials. 2004;25(9):1639-1647. doi:10.1016/S0142-9612(03)00521-0.

16. Arai K, Kitsuka T, Nakayama K. Scaffold-based and scaffold-free cardiac constructs for drug testing. Biofabrication. 2021;13(4):042001. doi:10.1088/1758-5090/ac1578.

17. Kawaguchi N, Hatta K, Nakanishi T. 3D-culture system for heart regeneration and cardiac medicine. Biomed Res Int. 2013;2013:895967. doi:10.1155/2013/895967.

18. .**Shimizu T, Sekine H, Yamato M, Okano T.** Cell sheet-based myocardial tissue engineering: New hope for damaged heart rescue. *Current Pharmaceutical Design.* 2009;15(24):2807–2814. doi:10.2174/138161209788923822

19. Fleischer S, Shapira A, Regev O, Nseir N, Zussman E, Dvir T. Albumin fiber scaffolds for engineering functional cardiac tissues. *Biotechnol Bioeng.* 2014;111(6):1246–1257. doi:10.1002/bit.25185

20. Shin SR, Jung SM, Zalabany M, Kim K, Zorlutuna P, Kim SB, et al. Carbon-nanotube-embedded hydrogel sheets for engineering cardiac constructs and bioactuators. ACS Nano. 2013;7(3):2369-2380. doi:10.1021/nn305559j.

21. Bian W, Jackman CP, Bursac N. Controlling the structural and functional anisotropy of engineered cardiac tissues. Biofabrication. 2014;6(2):024109. doi:10.1088/1758-5082/6/2/024109.

22. Kai D, Prabhakaran MP, Jin G, Ramakrishna S. Guided orientation of cardiomyocytes on electrospun aligned nanofibers for cardiac tissue engineering. J Biomed Mater Res B Appl Biomater. 2011;98(2):379-386. doi:10.1002/jbm.b.31862.

23. Xu C, Inai R, Kotaki M, Ramakrishna S. Aligned biodegradable nanofibrous structure: a potential scaffold for blood vessel engineering. Biomaterials. 2004;25(5):877-886.

doi:10.1016/S0142-9612(03)00593-3.

24. Zhao Y, Rafatian N, Feric NT, Cox BJ, Aschar-Sobbi R, Wang E, et al. A platform for generation of chamber-specific cardiac tissues and disease modeling. Cell. 2019;176(4):913-927.e18. doi:10.1016/j.cell.2018.11.042.

25. Ding M, Andersson H, Martinsson S, Sabirsh A, Jonebring A, Wang QD, et al. Aligned nanofiber scaffolds improve functionality of cardiomyocytes differentiated from human induced pluripotent stem cell-derived cardiac progenitor cells. Sci Rep. 2020;10:13575. doi:10.1038/s41598-020-70547-4.

26. Zhao G, Zhang X, Lu TJ, Xu F. Recent advances in electrospun nanofibrous scaffolds for cardiac tissue engineering. Adv Funct Mater. 2015;25(36):5726-5738. doi:10.1002/adfm.201502142.

27. Kitsara M, Agbulut O, Kontziampasis D, Chen Y, Menasche P. Fibers for hearts: a critical review on electrospinning for cardiac tissue engineering. Acta Biomater. 2017;48:20-40. doi:10.1016/j.actbio.2016.11.014.

28. Boffito M, Sartori S, Ciardelli G. Polymeric scaffolds for cardiac tissue engineering: requirements and fabrication technologies. Polymers (Basel). 2018;10(5):544. doi:10.3390/polym10050544.

29. Kharaziha M, Shin SR, Nikkhah M, Topkaya SN, Masoumi N, Annabi N, et al. Tough and flexible CNT-polymeric hybrid scaffolds for engineering cardiac constructs. Biomaterials. 2014;35(26):7346-7354. doi:10.1016/j.biomaterials.2014.05.014.

30. Tandon N, Marsano A, Maidhof R, Numata K, Montouri-Sorrentino C, Cannizzaro C, et al. Surface-patterned electrode bioreactor for electrical stimulation. Lab Chip. 2010;10(6):692-700. doi:10.1039/b917743d.

31. Elkhoury K, Kodeih S, Enciso-Martinez E, Maziz A, Bergaud C. Advancing cardiomyocyte maturation: current strategies and promising conductive polymer-based approaches. Adv Healthc Mater. 2024;13(13):e2303288. doi:10.1002/adhm.202303288.

32. Cui H, Nowicki M, Fisher JP, Zhang LG. 3D bioprinting for organ regeneration. Adv Healthc Mater. 2017;6(1):1601118. doi:10.1002/adhm.201601118.

33. Noor N, Shapira A, Edri R, Gal I, Wertheim L, Dvir T. 3D printing of personalized thick and perfusable cardiac patches and hearts. Adv Sci (Weinh). 2019;6(11):1900344. doi:10.1002/advs.201900344.

34. Kolesky DB, Homan KA, Skylar-Scott MA, Lewis JA. Three-dimensional bioprinting of thick vascularized tissues. Proc Natl Acad Sci U S A. 2016;113(12):3179-3184. doi:10.1073/pnas.1521342113.

35. You Y, Xu F, Liu L, Chen S, Ding Z, Sun D. Construction of thick myocardial tissue through layered seeding in multi-layer nanofiber scaffolds. Polymers (Basel). 2024;16(18):2664. doi:10.3390/polym16182664.

36. Hirt MN, Boeddinghaus J, Mitchell A, Schaaf S, Bornchen C, Muller C, et al. Functional

improvement and maturation of rat and human engineered heart tissue by chronic electrical stimulation. J Mol Cell Cardiol. 2014;74:151-161. doi:10.1016/j.yjmcc.2014.05.009.

37. Ruan JL, Tulloch NL, Razumova MV, Saiget M, Muskheli V, Pabon L, et al. Mechanical stress conditioning and electrical stimulation promote contractility and force maturation of induced pluripotent stem cell-derived human cardiac tissue. Circulation. 2016;134(20):1557-1567. doi: doi:10.1161/CIRCULATIONAHA.114.014998

38. Shadrin IY, Allen BW, Qian Y, Jackman CP, Carlson AL, Juhas ME, et al. Cardiopatch platform enables maturation and scale-up of human pluripotent stem cell-derived engineered heart tissues. Nat Commun. 2017;8(1):1825. doi:10.1038/s41467-017-01946-x.

39. Tulloch NL, Muskheli V, Razumova MV, Korte FS, Regnier M, Hauch KD, et al. Growth of engineered human myocardium with mechanical loading and vascular coculture. Circ Res. 2011;109(1):47-59. doi:10.1161/CIRCRESAHA.110.237206.

40. Carlos-Oliveira M, Lozano-Juan F, Occhetta P, Visone R, Rasponi M. Current strategies of mechanical stimulation for maturation of cardiac microtissues. Biophys Rev. 2021;13:717-727. doi:10.1007/s12551-021-00841-6.

41. Sekine H, Shimizu T, Yang J, Kobayashi E, Okano T. Pulsatile myocardial tubes fabricated with cell sheet engineering. Circulation. 2006;114(1 Suppl):I87-I93. doi:10.1161/CIRCULATIONAHA.105.000273.

42. Zhang YS, Arneri A, Bersini S, Shin SR, Zhu K, Goli-Malekabadi Z, et al. Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip. Biomaterials. 2016;110:45-59. doi:10.1016/j.biomaterials.2016.09.003.

43. Hochleitner G, Jungst T, Brown TD, Hahn K, Moseke C, Jakob F, et al. Additive manufacturing of scaffolds with sub-micron filaments via melt electrospinning writing. Biofabrication. 2015;7(3):035002. doi:10.1088/1758-5090/7/3/035002.

44. Marsano A, Maidhof R, Luo J, Fujikara K, Konofagou EE, Banfi A, et al. The effect of controlled expression of VEGF by transduced myoblasts in a cardiac patch. *Biomaterials.* 2013;34(2):393–401. doi:10.1016/j.biomaterials.2012.09.**038**

45. Kalhori D, Zakeri N, Zafar-Jafarzadeh M, Moroni L, Solati-Hashjin M. Cardiovascular 3D bioprinting: a review on cardiac tissue development. Bioprinting. 2022;28:e00221. doi:10.1016/j.bprint.2022.e00221.

46. Radisic M, Yang L, Boublik J, Cohen RJ, Langer R, Freed LE, et al. Medium perfusion enables engineering of compact and contractile cardiac tissue. Am J Physiol Heart Circ Physiol. 2004;286(2):H507-H516. doi:10.1152/ajpheart.00171.2003.

47. Camelliti P, Borg TK, Kohl P. Structural and functional characterisation of cardiac fibroblasts. Cardiovasc Res. 2005;65(1):40-51. doi:10.1016/j.cardiores.2004.08.020.

48. Gerbin KA, Yang X, Murry CE, Coulombe KLK. Enhanced electrical integration of engineered human myocardium via intramyocardial versus epicardial delivery in infarcted rat hearts. PLoS One. 2015;10(7):e0131446. doi:10.1371/journal.pone.0131446.

49. Xu F, Jin H, Liu L, Yang Y, Cen J, Wu Y, et al. Architecture design and advanced manufacturing of heart-on-a-chip: scaffolds, stimulation and sensors. Microsyst Nanoeng. 2024;10:96. doi:10.1038/s41378-024-00692-7.

50. Broadwin M, Imarhia F, Oh A, Stone CR, Sellke FW, Bhowmick S, et al. Exploring electrospun scaffold innovations in cardiovascular therapy: a review of electrospinning in cardiovascular disease. Bioengineering (Basel). 2024;11(3):218. doi:10.3390/bioengineering11030218.

51. Bernava G, Iop L. Advances in the design, generation, and application of tissue-engineered myocardial equivalents. Front Bioeng Biotechnol. 2023;11:1247572. doi:10.3389/fbioe.2023.1247572.