



Importance of Cell Alignment in Cardiac Tissue Engineering

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Abstract

Native myocardium is a structurally anisotropic, electromechanically integrated tissue in which cardiomyocytes, extracellular matrix fibers, vascular structures, and conduction pathways are organized across multiple length scales. Rather than a simple stack of discrete layers with slight misalignment, its defining architectural feature is a continuous transmural gradient in predominant myofiber angle from the endocardial to the epicardial surface—an organization that underlies anisotropic electrical propagation, regional deformation, ventricular torsion, wall thickening, and efficient blood ejection. Conventional two-dimensional culture systems, by contrast, typically yield randomly oriented, immature cardiomyocytes that fail to capture native myocardial mechanics or disease-associated remodeling. This narrative review revisits and extends the discussion of cardiomyocyte alignment in cardiac tissue engineering, focusing on nanofiber-based scaffolds, electrospinning, biomaterial selection, electrical stimulation, mechanical conditioning, bioprinting, and vascularization strategies. Spheroids, organoids, conventional scaffolds, aligned nanofibers, three-dimensional bioprinting, and biophysical stimulation approaches are compared with respect to alignment mechanisms, maturation readouts, advantages, limitations, and translational challenges, with particular attention to scaffold design parameters such as fiber diameter, degree of alignment, porosity, stiffness, degradation behavior, conductivity, surface functionalization, and extracellular matrix coating. We further argue that alignment strategies must be tailored to the target ventricular region, since left and right ventricular myocardium differ markedly in geometry, loading conditions, fiber architecture, and contractile patterns. Finally, we outline the barriers that still stand in the way of 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 seeks to generate myocardial constructs that reproduce not merely cardiomyocyte survival and spontaneous beating, but the structural and functional organization on which physiologically meaningful contraction depends. Native myocardium is far from a random assembly of contractile cells: it is an anisotropic tissue in which cardiomyocytes and extracellular matrix components are arranged along preferred directions, enabling coordinated force transmission and electrical prop-

agation. Because this architectural organization underlies myocardial deformation and pump function, it has long been recognized that cardiac mechanics cannot be understood in isolation from tissue structure and regional strain patterns [1].

Myocardial alignment is sometimes reduced to a set of discrete layers with different fiber directions—a useful teaching model, but one that risks implying an artificial discontinuity within the ventricular wall. In reality, the predominant myofiber angle changes gradually across the healthy ventricular wall, a pattern documented in classic

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anatomic work and reinforced by modern imaging and multiscale computational studies [2–9]. This continuous architecture underlies ventricular torsion, regional wall thickening, and the heterogeneous yet coordinated deformation that occurs during systole, rather than uniform contraction across all myocardial regions.

Alignment matters just as much in vitro. Cells cultured on standard flat surfaces tend to spread and orient randomly, and such cultures rarely capture the anisotropic conduction velocity, sarcomere organization, and contractile directionality seen in native myocardium. To guide cardiomyocyte orientation and maturation, researchers have therefore turned to scaffold architecture, microscale patterning, aligned nanofibers, electrical pacing, mechanical loading, and three-dimensional biofabrication. Earlier work with anisotropic scaffolds showed that engineered microarchitecture can shape both cardiac tissue alignment and functional output [10], while studies of cell shape and cytoskeletal organization established geometry as more than a morphological detail—it actively regulates myofibrillar architecture and tissue function [11].

Nanofiber-based scaffolds stand out among current approaches because they offer contact guidance at a scale comparable to that of extracellular matrix fibrils. Along aligned fibers, cardiomyocytes can elongate, organize their sarcomeres parallel to the scaffold axis, and develop more anisotropic contractile behavior.

Nanofiber scaffolds alone, however, cannot reproduce the full complexity of myocardium. Electrical stimulation can strengthen 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 accordingly treats nanofiber technology as one component within a broader integrated strategy, not as a stand-alone or universally superior method.

This review aims to offer 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. It proceeds by first defining the review's scope, then summarizing native myocardial architecture, comparing the major tissue engineering approaches, examining nanofiber scaffold design in detail, and finally considering how structural guidance can be combined with electrical, mechanical, and vascular cues to strengthen translational relevance.

2. Scope of the Review

This narrative review examines cardiomyocyte alignment in cardiac tissue engineering, with particular attention to nanofiber-based scaffolds, electrical and mechanical stim-

ulation, bioprinting strategies, and vascularization. It was designed to synthesize representative studies on myocardial architecture, scaffold design, cardiomyocyte maturation, anisotropic tissue organization, and translational challenges, rather than to serve as a systematic review or meta-analysis.

Relevant literature was identified through searches of PubMed/MEDLINE, Web of Science, and Google Scholar, supplemented by citation tracking from key articles, covering 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.

Priority was given to English-language, peer-reviewed original studies and reviews, with classic anatomic and biomechanical studies included where they helped frame current concepts. Articles were selected for their relevance to myocardial alignment, scaffold architecture, cardiomyocyte maturation, engineered anisotropy, vascularization, and translational feasibility. As a narrative rather than a systematic review, this work did not involve formal risk-of-bias assessment, quality scoring, or quantitative meta-analysis.

3. Native Myocardial Architecture and Functional Significance of Alignment

The myocardium is organized hierarchically, from sarcomeres and myofibrils up through cardiomyocytes, laminar sheets, extracellular matrix networks, and whole-ventricle geometry. Alignment at each level of this hierarchy determines the direction in which active force is generated and transmitted. A central point of this review is that myocardial structure should not be reduced to three fixed layers pointing in different directions.

The endocardium, midwall, and epicardium remain useful anatomical descriptors, but ventricular myocardium in fact exhibits a continuous transmural change in predominant fiber angle [2,3,9].

This architecture underlies ventricular mechanics through coordinated regional deformation: rather than contracting uniformly during systole, the ventricle undergoes longitudinal shortening, circumferential shortening, radial thickening, shear, and torsion in patterns that are spatially heterogeneous yet integrated. Recent modeling and imaging studies of right and left ventricular remodel-

eling further show that changes in fiber architecture and regional material behavior can shape global ventricular performance [3–7]. Cardiac tissue engineering should aim not merely to align cells on a surface, therefore, but to reproduce meaningful anisotropy, regional organization, and electromechanical coupling.

The distinction between uniform and coordinated regional contraction matters: uniform contraction would mean every part of the myocardium shortens by the same amount and in the same direction, yet healthy myocardium instead achieves efficient pump function by pairing region-specific deformation with synchronized electrical activation. Left ventricular torsion, for instance, emerges from the interplay between transmural fiber rotation, wall geometry, loading, and tissue mechanics, and cannot be reproduced by a simple parallel sheet of cells [8]. Strain-based and computational studies suggest that myocardial fiber architecture can be inferred from, can strongly shape, measured strain patterns [9], offering a mechanistic rationale for reproducing alignment in engineered myocardium.

Differences between the left and right ventricles further complicate alignment strategies. The left ventricle, with its thick wall, generates systemic pressure, whereas the right ventricle operates under lower pressure, adopts a thinner crescent-like geometry, and exhibits distinct loading and deformation patterns. Right ventricular remodeling in disease can involve changes in fiber structure, wall thickness, and regional mechanics that diverge from left ventricular post-infarction remodeling [4,6,7], meaning a scaffold optimized for a left ventricular patch will not necessarily model right ventricular myocardium. Future engineered tissues should instead be designed around the target ventricular region, disease state, and functional application.

In regenerative medicine, alignment matters for more than contraction alone. Anisotropic cell organization shapes conduction velocity, calcium wave propagation, gap junction distribution, sarcomere alignment, and extracellular matrix deposition; poorly organized constructs may beat spontaneously yet still fail to generate directional force, integrate electrically with host myocardium, or model disease-specific remodeling. Alignment should therefore be treated as a measurable design parameter rather than a qualitative impression, with quantitative outcomes such as orientation index, angular dispersion, sarcomere length, connexin-43 localization, conduction velocity, calcium transient kinetics, twitch stress, and force-frequency response reported whenever engineered cardiac tissues are evaluated.

4. Current Strategies for Engineering Aligned Cardiac Tissue

Multiple tissue engineering strategies have been developed to improve the organization and maturation of cardiac constructs. Spheroids and organoids foster cell-cell interactions and support multicellular communication, yet they generally offer limited control over long-range alignment unless paired with external patterning or mechanical constraints. Conventional scaffolds, such as collagen gels and synthetic polymer matrices, provide three-dimensional support and tunable mechanical properties but do not inherently impose a physiologically relevant orientation unless anisotropic features are deliberately introduced [15–18].

Aligned nanofibers guide cardiomyocyte elongation through contact guidance, making them among the most direct means of controlling cell orientation at the microscale and nanoscale. Dense electrospun mats, however, can limit cell infiltration, reduce effective cell-cell contact, and restrict achievable tissue thickness. Scaffold-free methods such as cell sheets and myocardial tubes preserve high cell density and cell-cell coupling but demand additional strategies to achieve controlled three-dimensional geometry, perfusion, and transmural alignment [18]. Albumin-based and other protein-derived fibrous scaffolds demonstrate that biologically active materials can support functional cardiac tissue formation, provided that mechanical strength, degradation, and biological activity are carefully balanced [19].

Microfabricated scaffolds and nanocomposite materials have also emerged to improve anisotropy and electrical behavior. Carbon-based or conductive components can enhance electrical signal propagation in certain settings, yet electroactive materials demand 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 paired with human pluripotent stem cell-derived cardiomyocytes to strengthen disease modeling and drug testing [21–24].

Table 1 compares the major strategies for producing aligned or functionally mature engineered myocardium, spanning spheroid-based approaches, conventional scaffolds, aligned nanofibers, electroactive scaffolds, electrical stimulation, mechanical stimulation, bioprinting, and cell sheet/scaffold-free tissues [6,7,12–18,20–43]. No single method proves sufficient for every purpose: spheroids favor cell-cell interaction, scaffolds supply mechanical

support, nanofibers impose directional guidance, bioprinting governs multicellular spatial placement, electrical stimulation drives electrophysiological maturation, and mechanical loading promotes mechanobiological adaptation. A mature cardiac construct will therefore likely demand the rational integration of several of these approaches, combining structural, electrical, and mechanical cues rather than depending on any one strategy alone.

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	[21–23,26,27,39]
Electroactive scaffolds	Structural guidance plus conductive pathways	May improve electrical coupling and synchronized activation	Conduction-active 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	[6,7,31,32,42]
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	[34–36,40,41,43]
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	[16,18,37,38]

Beyond the choice of method, **Table 1** also surfaces recurring translational themes—vascularization, scalability, reproducibility, and standardized functional readouts—that the following sections examine in greater depth.

5. Nanofiber-Based Scaffolds for Cardiomyocyte Alignment

Nanofiber scaffolds occupy a central place in this review because their structure echoes aspects of the native extracellular matrix, and aligned fibers offer cells a clear directional cue. Electrospinning remains the most widely used method for producing micro- and nanoscale fibers from synthetic polymers, natural polymers, or composites: by adjusting polymer concentration, solvent composition, voltage, flow rate, collector geometry, and environmental conditions, researchers can tune fiber diameter, alignment, density, and scaffold thickness [26,27]—parameters that strongly shape cell attachment, elongation, infiltration, and maturation.

Fiber diameter matters because cardiomyocytes sense topographical cues through focal adhesions and cytoskeletal organization. Very fine fibers may mimic extracellular matrix fibrils yet form dense mats that restrict cell penetration, whereas larger fibers or melt-electrowritten architectures open up more porous, three-dimensional structures with greater cell infiltrability at the cost of nanoscale biochemical mimicry. Porosity and pore interconnectivity should therefore be reported alongside fiber diameter and orientation, rather than reducing descriptions of scaffolds to “aligned” versus “random.” Scaffold stiffness is equally critical, since cardiomyocytes respond directly to mechanical resistance: excessive stiffness impairs contraction, while overly soft scaffolds fail to transmit force efficiently.

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

Electroactive materials and nanocomposites may strengthen electrical coupling, but their benefits must be weighed against real safety concerns. Carbon nanotubes, graphene derivatives, conductive polymers, and metal-

lic nanostructures have all been explored as conductivity-enhancing components, and while these materials can support synchronized beating in some experimental systems, their long-term biocompatibility, inflammatory potential, degradation behavior, and arrhythmogenic effects remain unresolved translational issues [20,29–31]. Conductivity, in short, should be treated as one property among many, not as an automatic marker of clinical suitability.

Surface functionalization is another critical design variable. Coating aligned nanofibers with fibronectin, laminin, collagen, gelatin, or peptide motifs can strengthen initial cell adhesion and sarcomere organization, yet variations in coating density, stability, and spatial uniformity can shift the effective biochemical cue that cells experience. A scaffold with excellent fiber alignment but unstable or nonuniform surface chemistry can therefore still yield inconsistent biological results. Future studies should report not just the scaffold material but also the coating method, coating concentration, sterilization procedure, residual solvent removal, and mechanical characterization.

Three-dimensional scaling is the primary limitation of nanofiber sheets. Thin sheets align cardiomyocytes effectively, but native myocardium is thick, vascularized, electrically integrated, and mechanically loaded, and stacking multiple aligned sheets to increase thickness can introduce diffusion barriers and weak interfaces between layers. The challenge, then, is to design nanofiber architectures with open, interconnected pores, controllable multilayer orientation, and compatibility with perfusion or vascularization—an aim that may be advanced by combining nanofiber alignment with bioprinting, sacrificial channels, or vascular cell organization [32–34,40,42,44].

Nanofiber-based approaches must also be judged against rigorous functional endpoints, since 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, and a scaffold intended for regenerative therapy should also be tested for mechanical integrity, degradation behavior, immune compatibility, electrical integration with host tissue, and scalability. Taken together, these demands make nanofiber scaffolds powerful yet technically exacting platforms, not simple culture substrates.

6. Electrical and Mechanical Stimulation for Maturation and Alignment

Cardiomyocytes mature *in vivo* under continuous electrical activation and mechanical loading, and electrical

stimulation has correspondingly become a widely used *in vitro* strategy for improving the functional assembly of engineered myocardium. Early studies showed that field stimulation of cardiac myocytes cultured on scaffolds improved contractile and conduction properties [12], and later platforms, including biowire systems, demonstrated that three-dimensional culture combined with programmed electrical stimulation can enhance the 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 demands careful optimization rather than generic application. Pulse amplitude, pulse duration, frequency, stimulation waveform, electrode material, medium conductivity, and tissue size all shape the biological response: appropriate pacing improves connexin-43 organization, calcium transient kinetics, action potential propagation, and synchronous contraction, whereas inappropriate stimulation can trigger abnormal membrane polarization, pH shifts near electrodes, reactive species generation, or outright cell injury. Stimulation conditions should therefore be reported in detail, with maturation judged by functional endpoints rather than by synchronized beating alone [30,35–37,43].

Mechanical stimulation matters just as much. Native cardiomyocytes experience passive stretch, active shortening, cyclic loading, and regional wall stress, and mechanical loading in turn shapes sarcomere assembly, cytoskeletal organization, extracellular matrix deposition, and mechanotransduction pathways. Because myocardial fiber remodeling *in vivo* tracks the local mechanical environment, pairing aligned nanofibers with mechanical stimulation is particularly relevant: nanofibers can establish an initial structural axis, while cyclic stretch or static tension reinforces alignment, drives sarcomere maturation, and guides matrix remodeling [6,7,38,45].

Mechanical conditioning should match the intended application. A construct designed to model post-infarction left ventricular remodeling may call for different stiffness, strain amplitude, and loading direction than does a construct designed to model right ventricular pressure overload. Excessive load can trigger stress responses, reduce viability, or drive a nonphysiological phenotype in directions, while insufficient load leaves tissues that beat spontaneously but generate weak force. Future studies should report strain magnitude, frequency, direction, duration, passive tension, scaffold stiffness, and the resulting contractile force.

Electrical and mechanical cues cannot be treated as independent variables. In native myocardium, electrical activation drives contraction, contraction alters mechanical load, and mechanical feedback in turn shapes electrophysiology. Engineered tissues that integrate aligned nanofibers, electrical pacing, and mechanical conditioning may reproduce this feedback loop more faithfully, though such systems are correspondingly more complex to build—and added complexity tends to erode reproducibility. Standardized reporting and quality control will therefore be essential for comparing results across laboratories.

7. Integration with Bioprinting, Vascularization, and Three-Dimensional Tissue Engineering

Three-dimensional bioprinting complements nanofiber scaffolds by placing cells, bioinks, and structural features within predefined geometries. It can pattern cardiomyocytes, endothelial cells, fibroblasts, and extracellular matrix components, enabling more controlled construction of multicellular cardiac tissues than simple bulk casting allows. Printed tissues, however, often remain immature, and printing resolution may fall short of reproducing nanoscale myofiber guidance without additional cues [32,41,44].

Bioprinting's chief advantage lies in its capacity to incorporate vascular or perfusable channels. Because oxygen diffusion limits the thickness of dense cardiac tissue, thick engineered myocardium requires vascularization to survive and function, making strategies such as sacrificial printing, endothelialized channels, microfluidic perfusion, and prevascularized patches increasingly important [33,40,42]. These approaches matter especially for nanofiber-based constructs, since highly aligned dense mats improve cell orientation but can simultaneously restrict diffusion and infiltration if not designed with care.

Cell-sheet engineering and scaffold-free approaches offer a further route to dense cardiac tissue. Layered cell sheets preserve cell-cell contact and the extracellular matrix the cells themselves produce, and myocardial tubes fabricated from cell sheets have shown that beating tissues can be built without permanent synthetic scaffolds [39]. As with nanofiber sheets, though, tissue thickness, vascularization, and mechanical handling remain major hurdles, so scaffold-free tissues may benefit from pairing with aligned templates, perfusion systems, or printed support structures.

Future strategies will most likely combine structural, electrical, mechanical, and vascular design. Aligned nanofibers, for example, could guide local cardiomyocyte orientation; bioprinting could define three-dimensional tissue geometry and multicellular patterning; electrical stimulation could strengthen coupling and conduction; mechanical loading could drive force generation; and endothelial networks could sustain oxygen and nutrient supply. Such integrated systems should be built around clearly defined applications, whether for drug screening, disease modeling, ventricular patch therapy, or fundamental studies of mechanobiology.

Integration also demands careful attention to measurement. A construct with aligned cells but no measurable force, poor conduction, or inadequate survival is not functionally mature, just as a construct that contracts strongly but lacks appropriate regional anisotropy may fail to model native ventricular mechanics. The evaluation framework should therefore span structural metrics, electrophysiological metrics, mechanical metrics, metabolic markers, and long-term stability. Figure 1 illustrates the major fabrication approaches, and Figure 2 illustrates electrical and mechanical conditioning systems that can be combined with scaffold-based constructs.

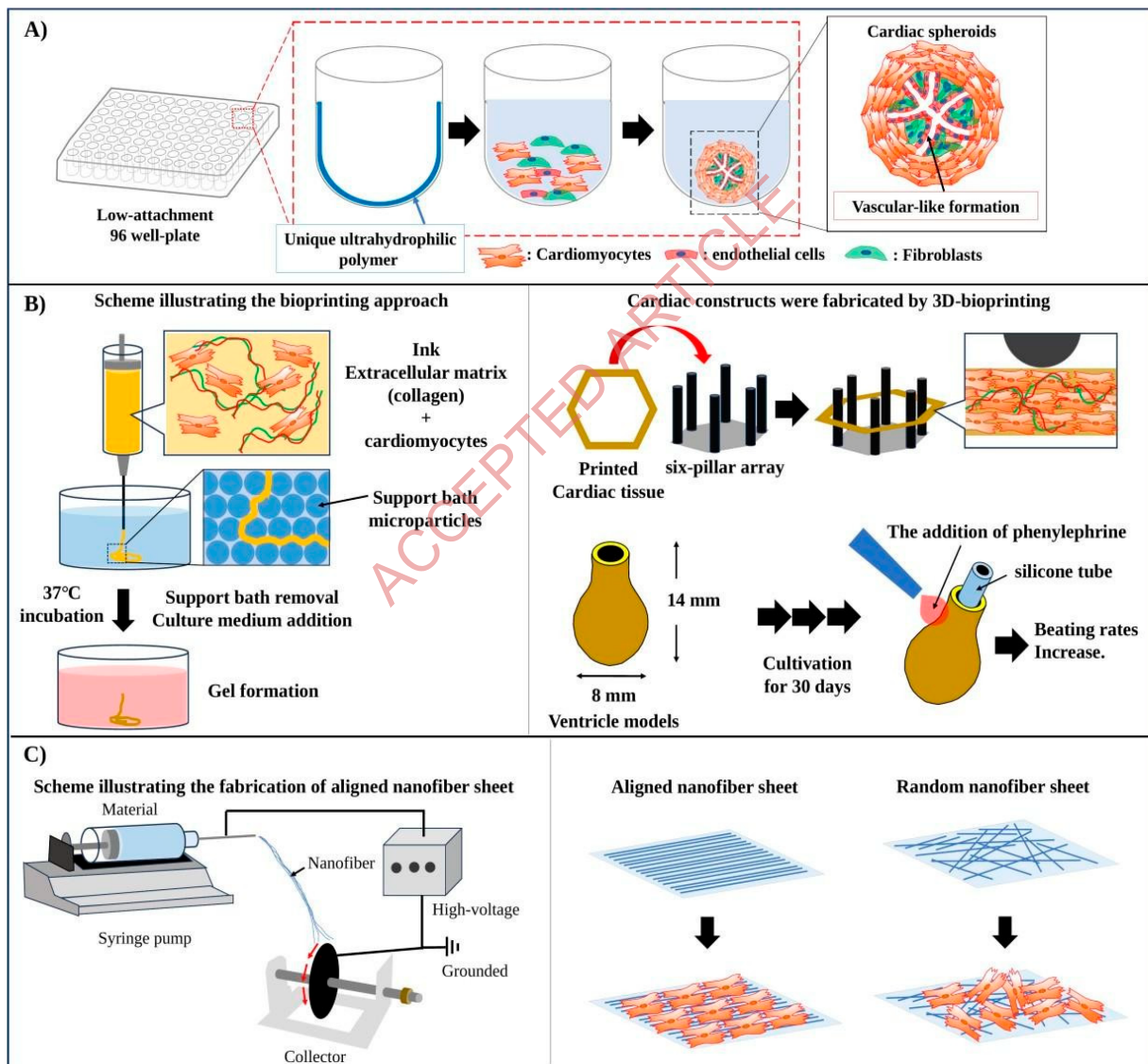


Figure 1: Representative cardiac tissue fabrication strategies. (A) Scaffold-free spheroid formation promotes cell-cell contact among cardiomyocytes, endothelial cells, and fibroblasts but provides limited long-range control of tissue alignment. (B) Three-dimensional bioprinting can define construct 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.

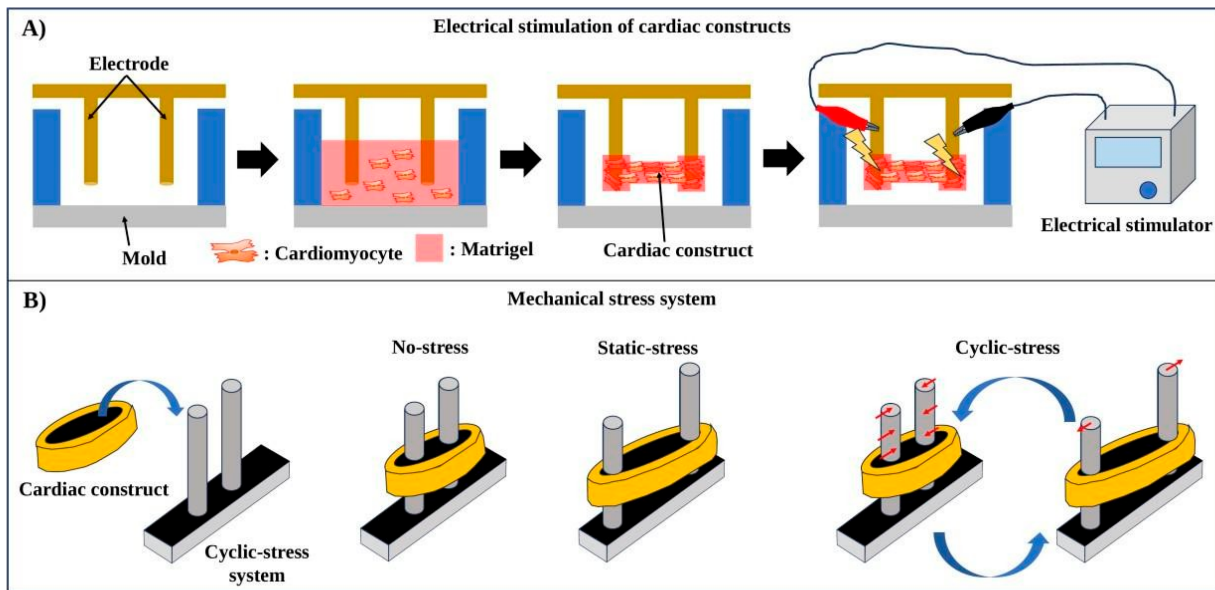


Figure 2: Electrical and mechanical conditioning systems for engineered cardiac tissue. **(A)** Electrical stimulation can improve synchronized activation, calcium handling, gap junction organization, and contractile maturation when stimulation parameters are optimized. **(B)** Mechanical conditioning, 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 central challenge is moving from visually aligned cells to tissue that is reproducible, safe, vascularized, and electromechanically integrated. Alignment, in other words, must be measured quantitatively and tied directly to function.

Structural readouts worth reporting include nuclear orientation, actin or sarcomere orientation, angular dispersion, orientation index, scaffold fiber diameter, pore size, and thickness. Functional readouts should include conduction velocity, excitation threshold, calcium transient amplitude and decay, twitch force, passive stiffness, force-frequency response, and responses to pharmacological agents.

Host-tissue electrical integration remains a major barrier: a cardiac patch that beats independently but fails to synchronize with host myocardium can raise arrhythmia risk. Strategies for improving integration include tuning tissue conduction properties, promoting gap junction formation, designing anisotropic conduction pathways, and controlling graft geometry, though excessive or poorly controlled conductivity carries its own dangers. Electrical integration should accordingly be tested with care in preclinical models before any clinical application [46–48].

Vascularization poses another major limitation. Once engineered myocardium exceeds a few hundred mi-

croimeters in thickness, oxygen and nutrient delivery can no longer rely on passive diffusion alone, making endothelial cells, pericytes, vascular channels, perfusion bioreactors, and host-vessel inosculation all essential considerations. For regenerative therapy after myocardial infarction, vascular integration must occur quickly enough to prevent graft hypoxia and cell death; for disease modeling and drug screening, perfusion is instead important for sustaining stable metabolic conditions and reproducing drug transport.

Scalability and manufacturing reproducibility demand attention as well. Electrospinning readily produces aligned fibers, but batch-to-batch variation in fiber diameter, alignment, residual solvent, and coating quality can skew biological results. Bioprinting offers spatial control, yet print fidelity, cell viability, bioink composition, and post-print maturation all vary, and electrical and mechanical bioreactors introduce further sources of variability [49].

Clinical-grade tissue products will therefore require standardized manufacturing and reporting criteria before these technologies can progress toward clinical translation.

Finally, the field should resist the assumption that any single alignment pattern is universally optimal. The right architecture depends on whether the goal is to model the healthy left ventricle, right ventricular pressure overload, myocardial infarction, arrhythmia, drug-induced cardiotoxicity, or regenerative patch therapy. Future engi-

neered tissues should build in region-specific geometry, disease-specific mechanical loading, and appropriate multicellular composition, treating nanofibers, scaffolds, bioprinting, and stimulation platforms as modular tools to be combined rationally rather than as competing technologies [50].

9. Conclusions

Cardiomyocyte alignment stands as a central design principle in cardiac tissue engineering, since cardiac function depends on anisotropic architecture, coordinated regional deformation, electrical propagation, and directional force transmission. Rather than a simple three-layer structure with slight misalignment, the myocardium is better described as a continuously organized anisotropic tissue in which healthy contraction is not uniform but regionally heterogeneous and mechanically coordinated.

Nanofiber-based scaffolds offer a powerful means of guiding cardiomyocyte orientation, provided that fiber diameter, alignment, porosity, stiffness, degradation, conductivity, and surface chemistry are all carefully controlled. Even so, nanofibers alone cannot capture the full complexity of native myocardium; electrical stimulation, mechanical conditioning, bioprinting, cell-sheet engineering, and vascularization strategies each supply complementary cues that maturation and translational relevance require.

Future progress hinges on integrated systems that unite structural guidance with functional conditioning and vascular support. The most pressing 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—challenges whose resolution will make engineered myocardium far more useful for disease modeling, drug screening, and regenerative therapy [51].

List of Abbreviations

3D	Three-dimensional
ECM	Extracellular Matrix
GMP	Good Manufacturing Practice
hPSC-CM	Human Pluripotent Stem Cell-derived Cardiomyocyte(s)
LV	Left Ventricle/Left Ventricular
MI	Myocardial Infarction
RV	Right Ventricle/Right Ventricular

Author Contributions

Conceptualization, writing—review and editing: T.K. and K.A.; Methodology, literature review, investigation, writing—original draft preparation, visualization, project administration: T.K.; Supervision, critical revision of the manuscript: K.A. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

This review generated no new datasets; all data and materials discussed derive from previously published studies cited in the reference list.

Conflicts of Interest

The authors declare no conflicts of interest.

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AI Declaration

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References

- [1] Sengupta, P.P.; Korinek, J.; Belohlavek, M.; Narula, J.; Vannan, M.A.; Jahangir, A.; Khandheria, B.K. Left Ventricular Structure and Function: Basic Science for Cardiac Imaging. *J. Am. Coll. Cardiol.* **2006**, *48*, 1988–2001. [CrossRef] [PubMed]
- [2] Streeter, D.D., Jr. Gross Morphology and Fiber Geometry of the Heart. In *Handbook of Physiology, Section 2: The Cardiovascular System*; Berne, R.M., Sperelakis, N., Geiger, S.R., Eds.; American Physiological Society: Bethesda, MD, USA, 1979; pp. 61–112.
- [3] Li, D.S.; Mendiola, E.A.; Avazmohammadi, R.; Sachse, F.B.; Sacks, M.S. A Multi-Scale Computational Model for the Passive Mechanical Behavior of Right Ventricular Myocardium. *J. Mech. Behav.*

- Biomed. Mater.* **2023**, *142*, 105788. [[CrossRef](#)] [[PubMed](#)]
- [4] Mendiola, E.A.; da Silva Gonçalves Bos, D.; Leichter, D.M.; Vang, A.; Zhang, P.; Leary, O.P.; Gilbert, R.J.; Avazmohammadi, R.; Choudhary, G. Right Ventricular Architectural Remodeling and Functional Adaptation in Pulmonary Hypertension. *Circ. Heart Fail.* **2023**, *16*, e009768. [[CrossRef](#)] [[PubMed](#)]
- [5] Mendiola, E.A.; Neelakantan, S.; Xiang, Q.; Xia, S.; Zhang, J.; Serpooshan, V.; Vanderslice, P.; Avazmohammadi, R. An Image-Driven Micromechanical Approach to Characterize Multiscale Remodeling in Infarcted Myocardium. *Acta Biomater.* **2024**, *173*, 109–122. [[CrossRef](#)] [[PubMed](#)]
- [6] Avazmohammadi, R.; Mendiola, E.A.; Soares, J.S.; Li, D.S.; Chen, Z.; Merchant, S.; Hsu, E.W.; Vanderslice, P.; Dixon, R.A.F.; Sacks, M.S. A Computational Cardiac Model for the Adaptation to Pulmonary Arterial Hypertension in the Rat. *Ann. Biomed. Eng.* **2019**, *47*, 138–153. [[CrossRef](#)] [[PubMed](#)]
- [7] Mendiola, E.A.; Neelakantan, S.; Xiang, Q.; Merchant, S.; Li, K.; Hsu, E.W.; Dixon, R.A.F.; Vanderslice, P.; Avazmohammadi, R. Contractile Adaptation of the Left Ventricle Post-Myocardial Infarction: Predictions by Rodent-Specific Computational Modeling. *Ann. Biomed. Eng.* **2023**, *51*, 846–863. [[CrossRef](#)] [[PubMed](#)]
- [8] Mukherjee, T.; Mendiola, E.A.; Neelakantan, S.; Shah, D.J.; Avazmohammadi, R. Four-Dimensional Assessment of Left Ventricular Torsion in Mitral Valve Prolapse Using CMR. In *Medical Imaging 2024: Image Processing*; Colliot, O., Mitra, J., Eds.; SPIE: Bellingham, WA, USA, 2024; Volume 12926. [[CrossRef](#)]
- [9] Usman, M.; Mendiola, E.A.; Mukherjee, T.; Mehdi, R.R.; Ohayon, J.; Alluri, P.G.; Sadayappan, S.; Choudhary, G.; 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; Springer: Cham, Switzerland, 2023; volume 13958, pp. 74–83. [[CrossRef](#)] [[PubMed](#)]
- [10] Engelmayer, G.C., Jr.; Cheng, M.; Bettinger, C.J.; Borenstein, J.T.; Langer, R.; Freed, L.E. Accordion-Like Honeycombs for Tissue Engineering of Cardiac Anisotropy. *Nat. Mater.* **2008**, *7*, 1003–1010. [[CrossRef](#)] [[PubMed](#)]
- [11] Parker, K.K.; Tan, J.; Chen, C.S.; Tung, L. Myofibrillar Architecture in Engineered Cardiac Myocytes. *Circ. Res.* **2008**, *103*, 340–342. [[CrossRef](#)] [[PubMed](#)]
- [12] Radisic, M.; Park, H.; Shing, H.; Consi, T.; Schoen, F.J.; Langer, R.; Freed, L.E.; Vunjak-Novakovic, G. Functional Assembly of Engineered Myocardium by Electrical Stimulation of Cardiac Myocytes Cultured on Scaffolds. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 18129–18134. [[CrossRef](#)] [[PubMed](#)]
- [13] Nunes, S.S.; Miklas, J.W.; Liu, J.; Aschar-Sobbi, R.; Xiao, Y.; Zhang, B.; Jiang, J.; Massé, S.; Gagliardi, M.; Hsieh, A.; et al. Biowire: A Platform for Maturation of Human Pluripotent Stem Cell-Derived Cardiomyocytes. *Nat. Methods* **2013**, *10*, 781–787. [[CrossRef](#)] [[PubMed](#)]
- [14] Ronaldson-Bouchard, K.; Ma, S.P.; Yeager, K.; Chen, T.; Song, L.; Sirabella, D.; Morikawa, K.; Teles, D.; Yazawa, M.; Vunjak-Novakovic, G. Advanced Maturation of Human Cardiac Tissue Grown from Pluripotent Stem Cells. *Nature* **2018**, *556*, 239–243. [[CrossRef](#)] [[PubMed](#)]
- [15] Zimmermann, W.H.; Melnychenko, I.; Eschenhagen, T. Engineered Heart Tissue for Regeneration of Diseased Hearts. *Biomaterials* **2004**, *25*, 1639–1647. [[CrossRef](#)] [[PubMed](#)]
- [16] Arai, K.; Kitsuka, T.; Nakayama, K. Scaffold-Based and Scaffold-Free Cardiac Constructs for Drug Testing. *Biofabrication* **2021**, *13*, 042001. [[CrossRef](#)] [[PubMed](#)]
- [17] Kawaguchi, N.; Hatta, K.; Nakanishi, T. 3D-Culture System for Heart Regeneration and Cardiac Medicine. *BioMed Res. Int.* **2013**, *2013*, 895967. [[CrossRef](#)] [[PubMed](#)]
- [18] Shimizu, T.; Sekine, H.; Yamato, M.; Okano, T. Cell Sheet-Based Myocardial Tissue Engineering: New Hope for Damaged Heart Rescue. *Curr. Pharm. Des.* **2009**, *15*, 2807–2814. [[CrossRef](#)] [[PubMed](#)]
- [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*, 1246–1257. [[CrossRef](#)] [[PubMed](#)]
- [20] Shin, S.R.; Jung, S.M.; Zalabany, M.; Kim, K.; Zorlutuna, P.; Kim, S.B.; Nikkhah, M.; Khabiry, M.; Azize, M.; Kong, J.; et al. Carbon-Nanotube-Embedded Hydrogel Sheets for Engineering Cardiac Constructs and Bioactuators. *ACS Nano* **2013**, *7*, 2369–2380. [[CrossRef](#)] [[PubMed](#)]
- [21] Bian, W.; Jackman, C.P.; Bursac, N. Controlling the Structural and Functional Anisotropy of Engineered Cardiac Tissues. *Biofabrication* **2014**, *6*, 024109. [[CrossRef](#)] [[PubMed](#)]
- [22] Kai, D.; Prabhakaran, M.P.; Jin, G.; Ramakrishna, S. Guided Orientation of Cardiomyocytes on Electrospun Aligned Nanofibers for Cardiac Tissue Engineering. *J. Biomed. Mater. Res. Part B* **2011**, *98*, 379–386. [[CrossRef](#)] [[PubMed](#)]
- [23] Xu, C.; Inai, R.; Kotaki, M.; Ramakrishna, S. Aligned Biodegradable Nanofibrous Structure: A Potential Scaffold for Blood Vessel Engineering. *Biomaterials* **2004**, *25*, 877–886. [[CrossRef](#)] [[PubMed](#)]
- [24] Zhao, Y.; Rafatian, N.; Feric, N.T.; Cox, B.J.; Aschar-Sobbi, R.; Wang, E.Y.; Aggarwal, P.; Zhang, B.; Conant, G.; Ronaldson-Bouchard, K.; et al. A Platform for Generation of Chamber-Specific Cardiac Tissues and Disease Modeling. *Cell* **2019**, *176*, 913–927. [[CrossRef](#)] [[PubMed](#)]
- [25] Ding, M.; Andersson, H.; Martinsson, S.; Sabirsh, A.; Jonebring, A.; Wang, Q.D.; Plowright, A.T.; Drowley, L. Aligned Nanofiber Scaffolds Improve Func-

- tionality of Cardiomyocytes Differentiated from Human Induced Pluripotent Stem Cell-Derived Cardiac Progenitor Cells. *Sci. Rep.* **2020**, *10*, 13575. [[CrossRef](#)] [[PubMed](#)]
- [26] Zhao, G.; Zhang, X.; Lu, T.J.; Xu, F. Recent Advances in Electrospun Nanofibrous Scaffolds for Cardiac Tissue Engineering. *Adv. Funct. Mater.* **2015**, *25*, 5726–5738. [[CrossRef](#)]
- [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. [[CrossRef](#)] [[PubMed](#)]
- [28] Boffito, M.; Sartori, S.; Ciardelli, G. Polymeric Scaffolds for Cardiac Tissue Engineering: Requirements and Fabrication Technologies. *Polymers* **2018**, *10*, 544. [[CrossRef](#)] [[PubMed](#)]
- [29] Kharaziha, M.; Shin, S.R.; Nikkhah, M.; Topkaya, S.N.; Masoumi, N.; Annabi, N.; Dokmeci, M.R.; Khademhosseini, A. Tough and Flexible CNT-Polymeric Hybrid Scaffolds for Engineering Cardiac Constructs. *Biomaterials* **2014**, *35*, 7346–7354. [[CrossRef](#)] [[PubMed](#)]
- [30] Tandon, N.; Marsano, A.; Maidhof, R.; Numata, K.; Montouri-Sorrentino, C.; Cannizzaro, C.; Voldman, J.; Vunjak-Novakovic, G. Surface-Patterned Electrode Bioreactor for Electrical Stimulation. *Lab Chip* **2010**, *10*, 692–700. [[CrossRef](#)] [[PubMed](#)]
- [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*, e2303288. [[CrossRef](#)] [[PubMed](#)]
- [32] Cui, H.; Nowicki, M.; Fisher, J.P.; Zhang, L.G. 3D Bioprinting for Organ Regeneration. *Adv. Healthc. Mater.* **2017**, *6*, 1601118. [[CrossRef](#)] [[PubMed](#)]
- [33] Noor, N.; Shapira, A.; Edri, R.; Gal, I.; Wertheim, L.; Dvir, T. 3D Printing of Personalized Thick and Perfusible Cardiac Patches and Hearts. *Adv. Sci.* **2019**, *6*, 1900344. [[CrossRef](#)] [[PubMed](#)]
- [34] Kolesky, D.B.; Homan, K.A.; Skylar-Scott, M.A.; Lewis, J.A. Three-Dimensional Bioprinting of Thick Vascularized Tissues. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 3179–3184. [[CrossRef](#)] [[PubMed](#)]
- [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* **2024**, *16*, 2664. [[CrossRef](#)] [[PubMed](#)]
- [36] Hirt, M.N.; Boeddinghaus, J.; Mitchell, A.; Schaaf, S.; Bornchen, C.; Muller, C.; Schulz, H.; Hubner, N.; Stenzig, J.; Stoeck, A.; 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. [[CrossRef](#)] [[PubMed](#)]
- [37] Ruan, J.L.; Tulloch, N.L.; Razumova, M.V.; Saiget, M.; Muskheli, V.; Pabon, L.; Reinecke, H.; Regnier, M.; Murry, C.E. Mechanical Stress Conditioning and Electrical Stimulation Promote Contractility and Force Maturation of Induced Pluripotent Stem Cell-Derived Human Cardiac Tissue. *Circulation* **2016**, *134*, 1557–1567. [[CrossRef](#)] [[PubMed](#)]
- [38] Shadrin, I.Y.; Allen, B.W.; Qian, Y.; Jackman, C.P.; Carlson, A.L.; Juhas, M.E.; Bursac, N. Cardiopatch Platform Enables Maturation and Scale-Up of Human Pluripotent Stem Cell-Derived Engineered Heart Tissues. *Nat. Commun.* **2017**, *8*, 1825. [[CrossRef](#)] [[PubMed](#)]
- [39] Tulloch, N.L.; Muskheli, V.; Razumova, M.V.; Korte, F.S.; Regnier, M.; Hauch, K.D.; Pabon, L.; Reinecke, H.; Murry, C.E. Growth of Engineered Human Myocardium with Mechanical Loading and Vascular Coculture. *Circ. Res.* **2011**, *109*, 47–59. [[CrossRef](#)] [[PubMed](#)]
- [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. [[CrossRef](#)] [[PubMed](#)]
- [41] Sekine, H.; Shimizu, T.; Yang, J.; Kobayashi, E.; Okano, T. Pulsatile Myocardial Tubes Fabricated with Cell Sheet Engineering. *Circulation* **2006**, *114*, 187–193. [[CrossRef](#)] [[PubMed](#)]
- [42] Zhang, Y.S.; Arneri, A.; Bersini, S.; Shin, S.R.; Zhu, K.; Goli-Malekabadi, Z.; Aleman, J.; Colosi, C.; Busignani, F.; Dell'Erba, V.; et al. Bioprinting 3D Microfibrous Scaffolds for Engineering Endothelialized Myocardium and Heart-on-a-Chip. *Biomaterials* **2016**, *110*, 45–59. [[CrossRef](#)] [[PubMed](#)]
- [43] Hochleitner, G.; Jungst, T.; Brown, T.D.; Hahn, K.; Moseke, C.; Jakob, F.; Dalton, P.D.; Groll, J. Additive Manufacturing of Scaffolds with Sub-Micron Filaments via Melt Electrospinning Writing. *Biofabrication* **2015**, *7*, 035002. [[CrossRef](#)] [[PubMed](#)]
- [44] Marsano, A.; Maidhof, R.; Luo, J.; Fujikara, K.; Konofagou, E.E.; Banfi, A.; Vunjak-Novakovic, G. The Effect of Controlled Expression of VEGF by Transduced Myoblasts in a Cardiac Patch. *Biomaterials* **2013**, *34*, 393–401. [[CrossRef](#)] [[PubMed](#)]
- [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. [[CrossRef](#)]
- [46] Radisic, M.; Yang, L.; Boublik, J.; Cohen, R.J.; Langer, R.; Freed, L.E.; Vunjak-Novakovic, G. Medium Perfusion Enables Engineering of Compact and Contractile Cardiac Tissue. *Am. J. Physiol. Heart Circ. Physiol.* **2004**, *286*, H507–H516. [[CrossRef](#)] [[PubMed](#)]
- [47] Camelliti, P.; Borg, T.K.; Kohl, P. Structural and Functional Characterisation of Cardiac Fibroblasts. *Cardiovasc. Res.* **2005**, *65*, 40–51. [[CrossRef](#)] [[PubMed](#)]
- [48] Gerbin, K.A.; Yang, X.; Murry, C.E.; Coulombe, K.L.K. Enhanced Electrical Integration of Engineered Human Myocardium via Intramyocardial versus Epicardial Delivery in Infarcted Rat Hearts. *PLoS ONE* **2015**, *10*, e0131446. [[CrossRef](#)] [[PubMed](#)]
- [49] Xu, F.; Jin, H.; Liu, L.; Yang, Y.; Cen, J.; Wu, Y.; Chen, S.; Sun, D. Architecture Design and Advanced

- Manufacturing of Heart-on-a-Chip: Scaffolds, Stimulation and Sensors. *Microsyst. Nanoeng.* **2024**, *10*, 96. [[CrossRef](#)] [[PubMed](#)]
- [50] Broadwin, M.; Imarhia, F.; Oh, A.; Stone, C.R.; Selke, F.W.; Bhowmick, S.; Abid, M.R. Exploring Electrospun Scaffold Innovations in Cardiovascular Therapy: A Review of Electrospinning in Cardiovascular Disease. *Bioengineering* **2024**, *11*, 218. [[CrossRef](#)] [[PubMed](#)]
- [51] Bernava, G.; Iop, L. Advances in the Design, Generation, and Application of Tissue-Engineered Myocardial Equivalents. *Front. Bioeng. Biotechnol.* **2023**, *11*, 1247572. [[CrossRef](#)] [[PubMed](#)]

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