

# **Carbon-Based Nanoarchitectonics in Advancing Cardiac Tissue Bioprinting: A Review**

Mansi Dixit<sup>1</sup>, Lok Kumar Shrestha<sup>2,3</sup>, Katsuhiko Ariga<sup>2,4</sup>, Falguni Pati<sup>1\*</sup>

<sup>1</sup>Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, Kandi, Sangareddy, Telangana, 502284, India

<sup>2</sup> Research Center for Materials Nanoarchitectonics, National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba 305-0044, Japan.

<sup>3</sup> Department of Materials Science, Institute of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8573, Ibaraki, Japan

<sup>4</sup> Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa 277-8561 Chiba, Japan

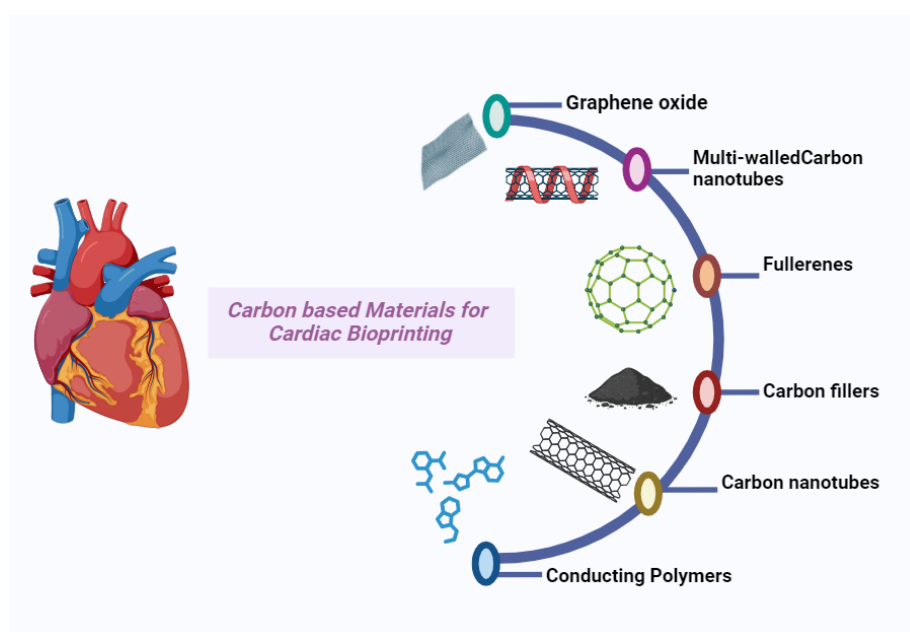
\*Author for correspondance: Falguni Pati ([falguni@bme.iith.ac.in](mailto:falguni@bme.iith.ac.in))

**Address Correspondence to:** Dr.Falguni Pati, Associate Professor, Department of Biomedical Engineering, Indian Institute of Technology (IIT) Hyderabad [falguni@bme.iith.ac.in](mailto:falguni@bme.iith.ac.in)

## Abstract:

Recent advancements in tissue engineering, particularly in cardiac tissue bioprinting, have been remarkable. A pivotal aspect of these advancements is the integration of electrically conductive biomaterials, which are essential for creating functional and viable substitutes for damaged cardiac tissue. Among these materials, carbon-based nanoarchitectonics such as graphene, carbon nanotubes (CNTs), and carbon nanofibers (CNFs) have garnered significant attention due to their exceptional electrical properties and biocompatibility. This review carefully explores the contemporary landscape of utilizing these carbon-based materials in cardiac tissue bioprinting, highlighting their unique properties and strong biocompatibility. Graphene, known for its single-layer carbon structure and exceptional electrical conductivity, plays a crucial role in enhancing cell communication and tissue functionality in engineered cardiac tissues. Similarly, carbon nanotubes (CNTs) and carbon nanofibers (CNFs) offer outstanding electrical conductivity and mechanical strength, making them ideal candidates for improving the structural integrity and electrical signaling within bioprinted cardiac constructs. The review emphasizes how these carbon-based materials seamlessly integrate into bioinks, facilitating three-dimensional bioprinting processes to create intricate cardiac tissue structures that closely mimic native tissues. This integration not only enhances the mechanical properties of bioinks but also supports cell adhesion, proliferation, and differentiation crucial for developing functional cardiac tissues. Overall, the transformative impact of carbon-based materials in regenerative medicine, particularly in cardiac regeneration, underscores an era of innovation. These materials hold immense promise for advancing treatment options for heart diseases, offering potential solutions for repairing and replacing damaged cardiac tissue effectively.

**Keywords:** Electrically conductive, Carbon-based materials, Cardiac bioprinting, Graphene, Carbon nanotubes, Carbon nanofibers, PANI, PPY.



**Graphical Abstract**

## **Introduction:**

Cardiovascular diseases, including myocardial infarction and heart failure, pose significant challenges to global public health, necessitating the development of innovative therapeutic approaches. Tissue engineering has emerged as a promising strategy for repairing and regenerating damaged cardiac tissue by combining cells, biomaterials, and biochemical cues to fabricate functional substitutes. Electrically conductive biomaterials play a crucial role in cardiac tissue engineering by providing a conducive microenvironment for cell proliferation, differentiation, and synchronized contraction(1).

In recent years, the field of tissue engineering has seen significant advancements, particularly in the development of Cardiac bioprinting. However, the current polymeric materials used in 3D bioprinting lack appropriate conductivity and mechanical strength compared to native cardiac tissue. Moreover, the degradability, biocompatibility, and cell interaction and migration in the scaffolds remain significant challenges for cardiac tissue engineers(2). Nonetheless, continued advancements in materials science and bioprinting technology hold promise for addressing these challenges and advancing the field toward effective cardiac tissue regeneration.

Carbon-based nanoarchitectonics, ranging from carbon nanotubes to conducting polymers, fullerenes and graphene, have emerged as promising conductive substrates for cardiac tissue engineering. Their unique biocompatibility, electrical conductivity, and mechanical strength position them as ideal candidates for integration into bioprinting processes. These materials offer structural support and provide a conducive environment for replicating the electrical signaling inherent in cardiac tissue functionality(3). The current landscape of cardiac tissue engineering reflects a dynamic amalgamation of interdisciplinary efforts. Researchers and clinicians actively exploring various approaches, including bioengineered scaffolds, cellular therapies, and bioprinting technologies. Despite notable advancements, challenges persist, particularly in achieving the intricate architecture and functionality of native cardiac tissue.

This comprehensive review explores the current advancements in cardiac tissue bioprinting, focusing on the utilization of carbon-based materials nanoarchitectonics, and conducting polymers. We meticulously analyze the benefits and drawbacks of these materials within the context of cardiac tissue engineering, placing a particular emphasis on their conductivity and their impact on cell regeneration. Additionally, we provide a detailed examination of the specific conductive polymers employed in tissue engineering, underscoring their pivotal role as promising biomaterials for cardiac repair. We aim to provide an understanding of the significant impact of carbon-based materials and conducting polymers on advancing the field of cardiac tissue engineering.

## **Cardiac Physiology and Microenvironment**

Replicating the human heart in vitro remains one of the most challenging tasks in physiology. This complex organ, with its four-chambered structure, is comprised of cardiac muscle, valves, and blood vessels. (4) The myocardium, the principal contractile component of

the heart wall, holds a pivotal role in its function. During embryonic development, the heart originates from a common structure known as the cardiac crescent, from which both endocardial and myocardial lineages emerge (5). The epicardium, the outer layer of the heart composed of mesoepithelial cells, plays a critical role in heart development and adult cardiac recovery. Epicardial cells undergo an endothelial-to-mesenchymal transition, giving rise to cardiac fibroblasts and coronary smooth muscle cells. Additionally, these epicardial-derived cells support ventricular cardiomyocyte proliferation during embryonic development. After birth, epicardial cells can migrate into the myocardium, particularly following injury, to generate endothelial cells and smooth muscle cells for new blood vessels (6). Endocardial cells, lining the atrial and ventricular chambers, maintain the heart's inner lining integrity and contribute to the formation of embryonic heart valves.

Cardiac neurons, integral components of the intrinsic neural system of the heart, operate autonomously to regulate heart rate, rhythm, and contractile force, thereby ensuring coordinated cardiac activity. The contraction of the heart is governed by the orientation of cardiomyocytes and a sophisticated built-in electrophysiological system that translates the microscopic movements of individual cardiomyocytes into the macroscopic contraction of the organ (7). Mechanical signals, such as changes in preload, afterload, or extracellular matrix stiffness, have the potential to modify cardiomyocyte contractile behavior, in accordance with the Frank-Starling Law of the heart and mechanosensing mechanisms. The unique biomechanical activity of cardiac muscle is intricately regulated by various cell types, including pacemaker cells located at the sinoatrial node, smooth muscle cells, and endothelial cells within the vascular lining, as well as cardiac fibroblasts within the myocardium. Therefore, an ideal in vitro cardiac model should facilitate the orientation of cardiomyocytes into anisotropic fibers and incorporate non-myocyte populations to contribute to cardiac development, myocardial structure, signaling, and electromechanical function (8).

Cardiomyocytes, also referred to as myocardiocytes or cardiac myocytes, serve as the primary contractile units of the heart, driving its rhythmic contractions and relaxations. These cells are smaller in size compared to skeletal myocytes and boast a rich sarcoplasm. Characterized by a central nucleus, cardiomyocytes possess an intricate network of contractile proteins and ion transporters that meticulously regulate their contractility through a spatially defined program of ion channels and exchangers. This program ensures precise control over calcium entry into and out of the cell and the sarcoplasmic reticulum, facilitating efficient blood pumping (9).

Identification of cardiomyocytes often relies on markers such as cardiac troponin-T (cTNT), sarcomeric  $\alpha$ -actinin, and myosin light chain 2, with MLC2a and MLC2v serving as atrial and ventricular-specific markers. Their distinctive shape and susceptibility to damage pose challenges in studying them. Notably, their surface striations represent a unique feature, with differentiated cardiomyocytes exhibiting a striated pattern akin to early-stage myocytes, along with indicators of gap junction development and electrophysiological coupling (10).

Contractile myofibrillar proteins, surface proteins, striations, shape, and length collectively contribute to the identification of cardiac cells. Among the noteworthy candidates for cell membrane markers are Popeye domain-containing 2 (POPDC2) and potassium voltage-gated channel subfamily A member 5, a calcium-dependent transmembrane adhesion protein, which play significant roles as protein markers of cardiomyocytes (11,12).

Cardiac fibroblasts constitute a vital cell population within the heart, accounting for approximately 15.5% to 24.3% of the total cell population. These cells express CD90, vimentin, and discoidin domain receptor 2 (DDR2), and establish physical connections with cardiomyocytes via connexin proteins (13). Maintaining a healthy balance of fibroblasts is crucial for facilitating the alignment of cardiomyocytes and the unidirectional propagation of electrical signals. However, an excessive abundance of fibroblasts can impede electrical signal propagation, potentially leading to arrhythmias (14). They have been said to contribute to monocyte to macrophage differentiation and indirectly affect T cell responses (15).

The primary function of cardiac fibroblasts lies in matrix deposition and maintenance, with significant implications in conditions such as myocardial infarction and chronic cardiac diseases where ventricular remodeling and fibrosis occur. Activated fibroblasts, distinguished by their expression of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), undergo proliferation and secrete collagen to facilitate wound healing, ultimately resulting in scar formation. This process diminishes heart tissue compliance, elevates overall matrix stiffness, and exacerbates impairment in cardiac output (16).

Additionally, cardiac fibroblasts contribute to myocardial regeneration by producing cytokines that activate proliferation in cultured cardiomyocytes. Furthermore, the structural proteins they produce may modulate cardiomyocyte cell-cycle activity by influencing the stiffness of the cardiac fibroblasts themselves. This intricate interplay underscores the multifaceted role of cardiac fibroblasts in both cardiac repair and pathological remodeling processes (17).

Endothelial cells, constituting approximately 7.8% to 12.2% of the heart's subpopulation, and mural cells, accounting for around 12.2% to 17.1%, are indispensable components of the vascular network. Common markers used to identify endothelial cells include CD144 (VE-cadherin), CD31, von Willebrand factor (vWF), TIE2, EPHB4, and ephrin B2 (18,19). These cells play a critical role in preserving the phenotype and viability of cardiomyocytes.

Endothelial cells secrete soluble factors that exert profound influences on various aspects of cardiac function, encompassing metabolism, growth, contractile performance, and the rhythmic beating of the heart. Their interactions with cardiomyocytes are pivotal for ensuring the proper functioning of the heart and maintaining overall cardiovascular health (19). This intricate crosstalk underscores the indispensable role of endothelial cells in orchestrating the physiological harmony within the cardiovascular system.

Among the diverse cardiac-resident immune cell populations, which constitute 5.3% to 10.4% of the total cell population, macrophages emerge as the most prevalent. These macrophages are interspersed among myocytes, fibroblasts, and endothelial cells within the heart (20). Through connexin-43, they establish physical connections with cardiomyocytes, exerting influence over their electrophysiological activity (Fig.1).

Macrophages play a pivotal role in the regulation of cardiac homeostasis and repair processes. However, in states characterized by inflammation and fibrosis, they can instigate excessive damage to the heart (20,21). This dual role underscores the complexity of macrophage-mediated responses within the cardiac microenvironment and highlights their significance in both physiological and pathological conditions of the heart.

The cardiac microenvironment actively participates in regulating T cell infiltration and exclusion within the heart and, hence, cardiac health and disease. This is supported by the evidence of a relatively low T cell number under physiological conditions in the heart, derived from multilevel mechanisms that confer on the heart a partial immune-privileged status (22). These are: tight endothelial barrier with low expression of adhesion molecules; production of immunosuppressive factors such as TGF- $\beta$  and IL-10; a limited lymphatic network; regulatory T cells; and absence of constitutive MHC class II expression on cardiomyocytes (23,24).

This well-regulated system disrupts various cardiac pathologies, leading to increased recruitment of T cells. It has been established that the cardiac microenvironment promotes T-cell infiltration in myocardial infarction, myocarditis, and heart failure. In MI, there is a dramatic recruitment of T cells, where CD4<sup>+</sup> T cells promote wound-healing processes of the myocardial infarct, and CD8<sup>+</sup> T cells are recruited into and activated within ischemic heart tissue, thereby contributing to adverse ventricular remodeling (25). Among these, some of the molecular mechanisms are the following: chemokines and their receptors, including CCL5-CCR5, CXCL10-CXCR3, enhanced expression of adhesion molecules on endothelial cells, and increased antigen presentation with expression of costimulatory molecules (26). Regarding the role of T cells within the heart, there is some duality. Therefore, regulatory T cells and some subsets of CD4<sup>+</sup> T cells can exert a role in tissue repair and also dampen excessive inflammation. However, cytotoxic CD8<sup>+</sup> T cells and pro-inflammatory Th1 and Th17 cells can contribute to cardiomyocyte death and fibrosis (27).

Interestingly, T cell dynamics has also been documented to be considerably different in CHF. There is a reduction of regulatory T cells in patients with chronic heart failure, and it does not get restored even after cardiac resynchronization therapy. Such lack of restoration of Tregs could attribute to the persistent inflammation underlying CHF (28). Besides, studies have documented global expansion and activation of CD4<sup>+</sup> T-lymphocytes in chronic ischemic heart failure, predominance of Th2 vs Th1 and Th17 vs Treg cells in failing hearts, and memory T-cells expansion in the spleen (25).

The interaction of these T cells with the cardiac microenvironment opens new therapeutic perspectives: enhancement of the function of Tregs, immune checkpoint modulation, targeting certain chemokine receptors, and antigen-specific therapies (26). Further research into this line of thought advanced approaches such as single-cell sequencing and spatial transcriptomics gone as far as to provide an unprecedented understanding of the diverse roles of T cell subsets in heart health and disease.

For instance, one recent single-cell atlas in cardiac myxoma underlined the heterogeneity of myxoma cells and their collaborative influence on immune cells, including T cells (29). Future studies are expected to determine specific mechanisms underlying T-cell exclusion and recruitment across diverse cardiac pathology while developing therapies targeted at the modulation of T cell responses in the heart.

T cell exclusion refers to the situation in which T cells, an immune cell subtype that attacks malformed or infected cells, are unable to penetrate or enter particular tissues or regions, like tumor or injured organs (such as in chronic heart failure) (26,28,30). While T cell exclusion is not as extensively studied in cardiac tissue as in tumors, several mechanisms can potentially limit T cell activity in the heart:

**Fibrosis and Extracellular Matrix:** Cardiac fibrosis is characterized by excessive deposition of extracellular matrix proteins and may act as a physical barrier, impairing T cell infiltration and movement in tissue (15).

**Immunosuppressive Factors:** Such cardiac microenvironment molecules may suppress T cell functions or their recruitment. For instance, during myocarditis, subsets of monocytes may support the immunosuppressive environment (15).

**Metabolic Regulation:** Metabolic states of the cardiac microenvironment could influence T-cell functions. Similar to tumor microenvironments, metabolic competition or nutrient availability may modulate T cell activity in cardiac tissue (30).

**Myocarditis:** The interplay between T cells and other immune cells within the cardiac microenvironment may importantly influence disease outcome in myocarditis, an inflammatory disease of the heart. For instance, it was identified that IL-3-producing CD4<sup>+</sup> T cells enhance inflammation by stimulating monocyte chemotaxis (31).

**Heart Failure:** Dysregulated T-cell responses may trigger chronic inflammation and adverse remodeling in heart failure. These T-cell function changes engendered by the cardiac microenvironment may prove a target for therapies (32).

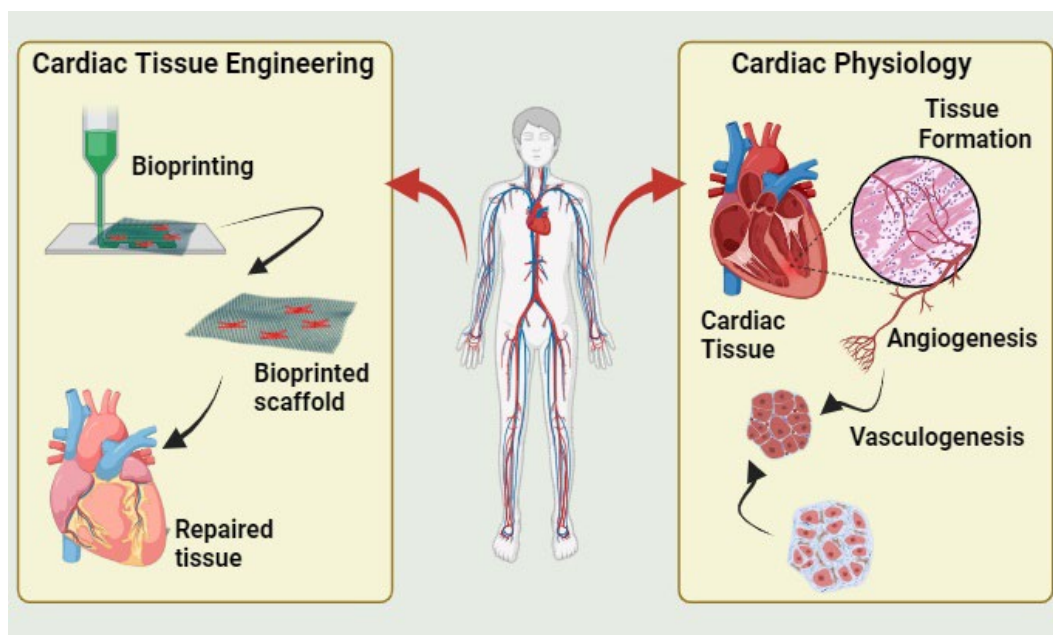


Figure 1: Overview of Cardiac Physiology and fabrication of scaffold for cardiac repair via 3D bioprinting

### Electrical system in Tissue engineering

Tissue engineering is a rapidly evolving field that combines biology and engineering to achieve tissue repair, replacement, and regeneration. The integration of stem cells, especially autologous ones, is pivotal for successful tissue regeneration (33). The status of cell growth directly impacts tissue regeneration effectiveness, and electrical stimulation plays a crucial role in promoting and regulating cell growth by activating intracellular signaling pathways and altering intracellular microenvironments. This stimulation has demonstrated enhanced therapeutic efficacy for ischemic cardiac diseases when applied to mesenchymal stem cells (MSCs) (34). Various tissue engineering techniques, such as scaffolds and biomaterials, enhance the viability and proliferative capacity of stem cells, thus addressing limitations in

stem cell therapy for human diseases (35). Controlled electrical stimulation can influence cell behavior by inducing alignment, migration, proliferation, and differentiation, thereby facilitating tissue regeneration. However, insufficient or excessive electrical stimulation yields no effect or cell death, respectively (36). Therefore, careful optimization of stimulation intensity and duration is essential to achieve the desired outcome. For instance, an electrical stimulation intensity below 10 V/cm promotes better cell alignment, while maintaining cell viability and phenotype for migration occurs between 0.1 V/cm and 12 V/cm. Furthermore, an intensity below 2 V/cm over an extended period, exceeding 7 days, specifically induces cell differentiation (37).

Kapeller et al. (26) demonstrated that modulating cardiomyocytes with microcurrent (MC) enhances proliferation without inducing morphological changes in vitro. They found that electrical stimulation has dual effects on MMP-2, MMP-9, TIMP-3, and TIMP-4 mRNA and protein expression in cardiomyocytes, with variations dependent on the age of the cells. In vivo experiments showed that MC treatment decreased MMP-2, MMP-9, and TIMP-4 levels while increasing TIMP-3 levels in young SHR (spontaneously hypertensive rats). However, in old SHR, MMP-2, MMP-9, and TIMP-4 were up-regulated, while TIMP-3 levels remained unaffected. Additionally, the application of a 1  $\mu$ A microcurrent (MC) for  $7.7 \pm 0.9$  hours per day was found to enhance cell proliferation and increase cell numbers in cardiomyocytes compared to cells without MC treatment (38).

## **2. Electrically conductive carbon-based materials**

Carbon-based materials have emerged as promising candidates for constructing bioinks in the bioprinting process, offering exceptional mechanical strength, electrical conductivity, and biocompatibility. These materials include graphene, carbon nanotubes, carbon nanofibers, fullerenes etc. which can enhance the structural integrity, mechanical strength and functionality of bioprinted cardiac tissues (Fig.2). For instance, graphene-based bioinks have demonstrated the ability to achieve a densely penetrating vasculature network, which is more prominent than GelMA bioinks. This enhanced angiogenic potential is crucial for developing functional cardiac tissues, as it ensures adequate nutrient supply and promotes synchronous contractile activity within the engineered tissues (39). The applications, advantage, and disadvantage of carbon-based material for cardiac tissue engineering are summarized in Table.1



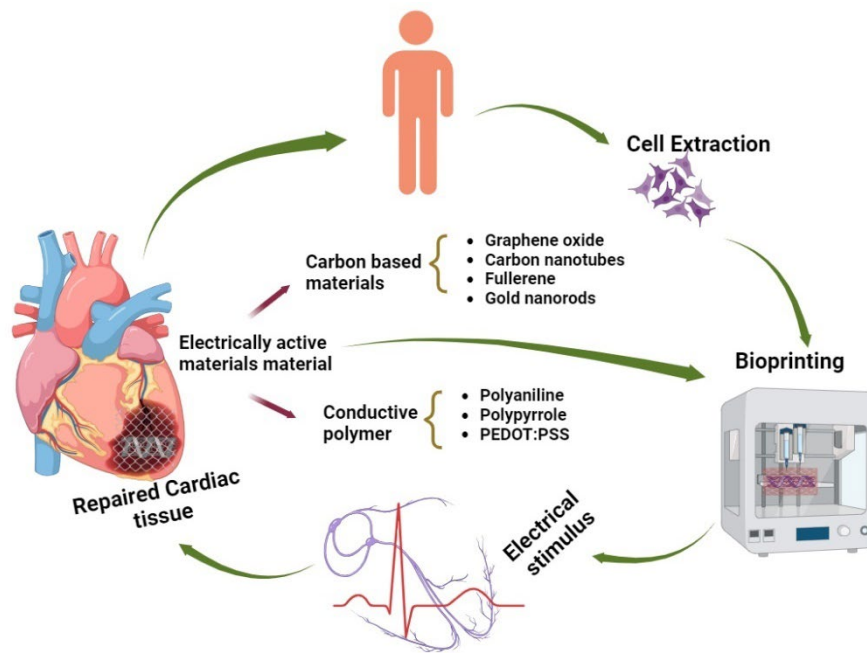


Figure 2: Electrically active materials suitable for 3D bioprinting of cardiac tissue

## 2.1 Graphene and its derivatives

Graphene-based materials (GBMs) are primarily composed of sheets of  $sp^2$ -hybridized carbon atoms, featuring a trigonal planar geometry within layers due to strong  $\sigma$ -bonds formed by an s orbital and two p orbitals. This structure imparts high mechanical stiffness, chemical, and thermal stability to GBMs (40). Moreover, their remarkable thermal and electrical conductivity arises from facile electron excitation between valence and conduction bands, facilitating electron conduction within the lattice. The electrical conductivity of GBMs is particularly advantageous in tissue engineering, especially in cardiac applications where electrical coupling with cells is crucial (41). GBMs, whether used alone or in combination with other materials, offer the potential for optimized electrical, physicochemical, and mechanical properties. The biosafety considerations, cytotoxicity profile, and safe dosage of carbon-based material for cardiac tissue engineering are summarized in Table. 2

The first investigations into the application of graphene nanosheets in cardiac tissue regeneration began in the early 2010s. The study by Kim et al. investigated the biocompatibility of graphene sheets with primary adult cardiomyocytes (CMs) for the first time [30]. Their study stated that graphene substrates did not disturb cell viability and significantly enhanced cell attachment compared to controls. Properly functional CMs, similar calcium transient activity, and sensitivity were also observed in the presence of graphene. Additionally, the study showed that a synthetic multipartite complex, such as the macrophage-targeting/polarizing graphene oxide (GO) complex, was capable of reducing reactive oxygen species (ROS) from macrophages. In another study, a mixture of the macrophage-GO complex and IL-4 plasmid DNA (pDNA) was applied to induce differentiation of M1 to M2 macrophages and secretion of regenerative cytokines for cardiac repair. Consequently, this approach reduced inflammation,

increased differentiation into M2 macrophages, and improved heart function in animal models (42).

A study by Wang et al. [31] confirmed the significant potential of partially reduced graphene oxide (rGO) for cardiac repair. They fabricated a 3D foam chip composed of partially reduced GO with cardiomyocytes (CMs) seeded, which supported the spontaneous beating of cells within 24 hours post cell seeding. Prolonged cultivation time also resulted in more CMs beating in a more synchronized manner. The electrical conductivity of the fabricated foam was measured to have an average value of 1.12 S/cm. These results suggest that partially reduced graphene oxide could be a promising material for cardiac repair and tissue engineering (43).

Bahrami et al. [32] fabricated 3D and 2D conductive graphene foam scaffolds and observed an over-expression of Connexin 43 (Conx43) and Transient Receptor Potential Cation Channel Subfamily V Member 2 (TrpT-2) in seeded cells in both experimental groups compared to tissue culture plates as control. The 3D graphene foam, with a reported conductivity value of 9 S/cm, notably exhibited the highest levels of Connexin 43 expression, highlighting the potential of graphene foam in promoting conductivity and providing a porous structure conducive to cellular growth and expression of specific markers (44).

Park et al. [33] evaluated the potency of graphene sheets for cardiomyogenic differentiation of mesenchymal stem cells (MSCs). The study first confirmed biocompatibility and then examined cardiomyogenic markers at the transcript level in graphene substrates without any exogenous chemical inducers. Enhanced levels of various markers, including cardiomyogenic transcriptional factors, cardiomyogenic contractile proteins, and gap junction proteins, were reported. The expression of genes related to extracellular matrix (ECM) proteins and cell signaling molecules was also observed to be enhanced. However, the study found that the enhanced electrical conductivity of the substrates within double-layer and triple-layer graphene did not significantly contribute to the differentiation process. Therefore, the upregulated gene expression of specific ECM proteins and cell signaling molecules was suggested to be the primary factor responsible for the observed effects (45).

Tsui et al. [34] fabricated electroconductive decellularized extracellular matrix (dECM) and reduced graphene oxide (rGO) hydrogels for engineering human cardiac microphysiological systems. The dECM-rGO hydrogels reported average conductivities ranging from  $0.93 \pm 0.12$  S/m to  $3.07 \pm 0.12$  S/m. Additionally, the expression of cardiac markers including TNNT2, TNNI3, TTN, and N2B was increased in the dECM-rGO hydrogel-based tissues. These results indicate the potential of dECM-rGO hydrogels in enhancing the mechanical properties and contractile function of engineered cardiac tissues (46).

Mousavi et al. [35] developed a ring-shaped cardiac tissue model using 3D bioprinting. They utilized photocrosslinkable natural biopolymers (AlgMA and GelMA) along with conductive nanomaterials such as reduced graphene oxide (rGO). Cellular assessments with encapsulated fibroblasts demonstrated excellent cell adhesion, viability, proliferation, and migration. Cardiac BioRings were created using various cardiac cell types, exhibiting robust cell growth, alignment, spreading, elongation, and interconnection, with distinct phenotypic

morphology and detectable cardiac structures. The reported conductivity value of the constructed BioRings was  $1.05 \pm 0.09$  mS/cm (47).

**Table 1: Applications, advantage, and disadvantage of carbon-based material for cardiac tissue engineering**

Material	Application	Advantage	Disadvantage	References
Graphene oxide	Enhance electrical conduction in the scaffold, promotes cardiomyocyte proliferation	Large surface area for cell growth, high mechanical strength	Risk of oxidative stress at high dose	(41,48–50)
Fullerene	Anti-oxidant for cardiac cells, protects from oxidative stress	High biocompatibility with functionalization, ROS scavenging property	Expensive and complex synthesis, poor water solubility	(51–53)
CNTs	Improves electrical and mechanical properties, supports cell alignment	High tensile strength,	Aggregation and dispersion issue	(54–57)
CNFs	Provides structural support in scaffolds, enhances electrical conductivity	Mechanical robustness and durability	Limited biodegradability, potential inflammatory response	(55,58)
Polypyrrole	Supports cardiomyocytes adhesion and growth, enhance electrical conductivity	Promotes cell proliferation, Tunable mechanical properties	Poor biodegradability requires careful synthesis to control toxicity	(59–63)
Polyaniline	Supports cell alignment and function, promotes cell adhesion	Stable under physiological conditions, cost-effective	Potential cytotoxicity without modifications, limited biodegradability	(59,61,64–67)
Polythiophene	Promotes cardiomyocyte alignment, enhance electrical properties of scaffold	High thermal stability, Good biocompatibility with functionalisation	Poor solubility and processibility	(61,65,68)
PEDOT	Supports cell proliferation and enhances mechanical	Highly stable, flexible and processable	High Production cost, limited degradation in the body	(59,65,68–70)

	property of scaffold			
--	----------------------	--	--	--

## 2.2 Carbon nanotubes (CNTs) and Carbon nanofibers (CNFs)

Carbon nanotubes (CNTs) are essentially graphene sheets rolled into one-dimensional hollow cylinders, typically a few nanometers in diameter and several micrometers in height, resulting in high aspect ratios (54). Single-walled carbon nanotubes (SWCNTs) consist of a single sheet, while multi-walled carbon nanotubes (MWCNTs) comprise 2 to 50 sheets, with an average diameter of 5 nm (71). Due to their catalyst content, CNTs are more contaminated compared to graphene, raising concerns about their biocompatibility and necessitating purification processes for biological applications.

Carbon nanofibers (CNFs) are bent graphene sheets forming concentric nanocones with diameters ranging from 50 to 500 nm (72). Both CNTs and CNFs are suitable for reinforcement in composites, although CNFs tend to have more defects, resulting in lower conductivity and weakened mechanical properties compared to CNTs (73). However, CNFs can be more toxic than CNTs, depending on synthesis and processing methods.

Martinelli et al. [62] conducted a study where they reported significant findings regarding cardiac marker expression profiles in CNF-chitosan scaffolds compared to a new chitosan control group. The study revealed a notable increase in gene expressions of Tnnc1, Cx43, Nkx2.5, Myh6, Myh7, GATA4, and Atp2a2 in the CNF-chitosan scaffolds. This indicates the potential of CNF-chitosan scaffolds in enhancing the expression of key genes associated with cardiac function and regeneration compared to traditional chitosan scaffolds (74).

Mehrabi et al. [46] performed subcutaneous implantation of fabricated patches to quantify angiogenic potency. Histological and immunohistochemistry observations revealed more remarkable cell migration and a greater number of capillaries were detected. This study highlights the potential of using conductive materials, such as carbon nanofibers (CNFs), to enhance angiogenesis and tissue repair. Other studies have also demonstrated the benefits of using conductive materials for cardiac tissue engineering, including improved electrical conductivity and the expression of cardiac genes (58).

Ahadian et al. [63] employed GelMA and synthesized GelMA-aligned CNT gels using the dielectrophoresis approach. Conductivity measurements revealed significantly higher and concentration-dependent values for GelMA-aligned CNT samples compared to GelMA-random CNT and pure GelMA samples. This increase in conductivity was attributed to the presence of CNTs and the parallel applied electrical field. Gene and protein expressions were characterized after 4 days of embryoid bodies (EBs) cultivation and under continuous 2 days of electrical stimulation (75).

The study by Roshanbinfar et al. (76) utilized functionalized multi-walled carbon nanotubes (CNTs) to enhance the electrical conductivity of a decellularized and enzymatically digested pericardial matrix of sheep (fig.3). The prepared gel showed a significant enhancement in electrical conductivity, reported to be  $1.42 \pm 1.2 \times 10^{-2}$  S/cm. The study

claimed the fabricated hydrogel to be a suitable substrate for human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (CMs), which exhibited autonomous synchronized contractions, more efficient contractions, improved calcium handling properties, elevated Connexin 43 (Cx43) expression, and increased sarcomeric length compared to cases without CNTs and the Matrigel groups. The study suggests the potential of functionalized CNTs in enhancing the electrical conductivity of biomaterials and their potential application in cardiac tissue engineering.

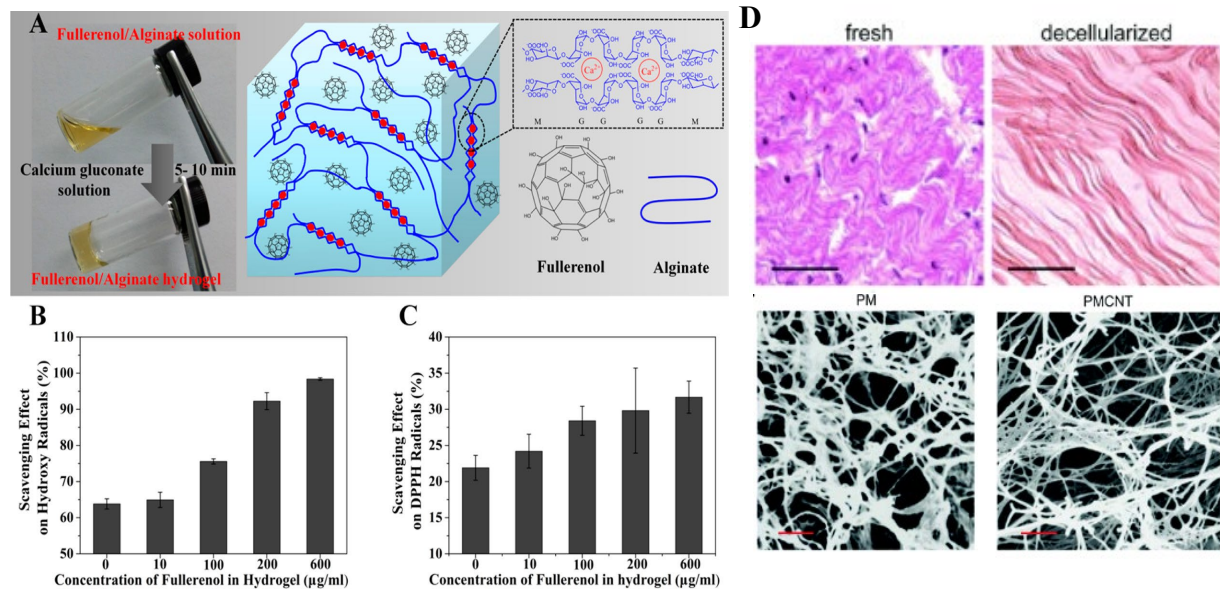


Figure 3: A) Formation and structure of fullerene/alginate hydrogel B) Scavenging effect of fullerene/alginate hydrogel on (B) hydroxyl radical and (C) DPPH radical reproduced from (77) D) Histological images stained with hematoxylin-eosin of fresh and decellularized pericardium, as well as SEM images of PM- and PMCNT-gels reproduced from (78)

## 2.3 Fullerenes and its derivatives

Fullerenes(C-60) are zero-dimensional spherical structures composed of various hexagonal and pentagonal carbon rings, exhibiting hybridizations between sp<sup>2</sup> and sp<sup>3</sup> orbitals. They offer superior biocompatibility and smaller diameters, allowing them to penetrate cell membranes and potentially manipulate cell behavior (53). Fullerene derivatives, such as fullereneol with higher water solubility, are preferred for biological applications. Fullerene-based materials (FBMs) have shown promising potential for cardiac tissue engineering applications due to their unique electrical, thermal and optical properties (52).

Hao et al. (77) developed an injectable alginate hydrogel conjugated with fullereneol nanoparticles for cardiac tissue engineering. The hydrogel exhibited superior mechanical properties and potent antioxidant activity, scavenging hydroxyl and superoxide radicals. When loaded with brown adipose-derived stem cells (BADSCs), the hydrogel enhanced cell survival and cardiomyogenic differentiation even under oxidative stress (fig.3). In a rat myocardial infarction model, the hydrogel reduced reactive oxygen species (ROS) levels, improved BADSC viability and proliferation, triggered angiogenesis, and enhanced cardiac functional recovery.

In a study C-60 fullerene particles were suspended in a cell culture medium containing brown adipose-derived stem cells (BADSCs). After seven days of culture, the presence of fullerenes was found to modulate the cardiomyogenic differentiation of BADSCs by improving the expression of MAPK signaling proteins. This enhancement of MAPK signaling by fullerenes led to several beneficial outcomes, including improved viability, increased proliferation, and enhanced cardiomyogenic differentiation of BADSCs. The improved cardiomyogenic differentiation was evidenced by the increased expression of cardiac-specific markers, such as cardiac troponin T2 (cTnT2) and  $\alpha$ -actinin, in the BADSCs cultured with fullerenes compared to the control group without fullerenes (79).

However, further research is necessary to thoroughly assess their biocompatibility and impact on cardiac cell behaviour, exclusively when using them as bioink for cardiac tissue bioprinting.

**Table 2: Biosafety considerations, cytotoxicity profile, and safe dosage of carbon-based material for cardiac tissue engineering**

Material	Biosafety Considerations	Cytotoxicity Profile	Safe Dosage	References
Graphene oxide	Increase oxidative stress and inflammation	High	$\leq 50 \mu\text{g/ml}$	(80–82)
CNTs	Impaired phagocytosis, Causes necrosis and degeneration of macrophages	High	$\leq 20\text{-}50 \mu\text{g/ml}$	(83–86)
CNFs	Causes inflammation and biodegradability	Moderately high	$\leq 50 \mu\text{g/ml}$	(83,85,87,88)
Fullerenes	Lowest cytotoxicity among tested carbon nanomaterials, impairs phagocytosis only at high doses	Low	$\leq 100 \mu\text{g/ml}$	(83,89–91)
PANI	Biocompatibility varies between salt and base form	Low to moderate high depending on the form	$\leq 10\text{-}50 \text{ug/ml}$	(92–95)
PPY	Considered more biocompatible than PANI	Low	$\leq 50\text{-}100\text{ug/ml}$	(96–98)

PEDOT	Good biocompatibility, stable in physiological condition	Low	0.1-1 % w/v	(99–101)
-------	--	-----	-------------	----------

## 2.4 Conducting Polymers

Conductive materials employed in tissue engineering can be broadly categorized into two groups: conductive polymers and conductive filler materials, with the latter encompassing carbon-based materials such as graphene, metal nanoparticles, and ionic liquids (65). Conductive polymers, characterized by diverse electrical properties, can be easily synthesized using various methods. Non-toxic and possessing excellent electrical characteristics, conductive polymers have found extensive applications in biomedical fields, including nerve conduits for neural tissue engineering, scaffolds supporting cell adhesion and proliferation, and drug delivery and release systems (102). Various fillers, such as carbon-based materials, metal nanoparticles, and ionic liquids, can be combined to augment conductivity. Additionally, the conductivity of conductive polymers can be significantly enhanced through chemical or electrochemical processes like p-doping (oxidation) and n-doping (reduction) (61). The use of different dopants and doping levels allows for highly flexible tunability of electrical properties. An overview of carbon-based nanoarchitectonics applied in cardiac tissue engineering is summarized in Table.3

Cardiac tissue engineering aims to fabricate scaffolds that not only support cell growth but also mimic the electrical properties of cardiac tissue. Conductive polymers such as polypyrrole, polythiophene, and particularly polyaniline (PANI) offer a straightforward solution (64). These polymers conduct electricity due to their unique chemical structure and can be adjusted to achieve varying levels of conductivity. This electrical conductivity, combined with their ability to influence cell behavior through electrical stimulation, makes them promising for promoting healthy cell growth and function within the scaffold (59).

However, conflicting reports on the biocompatibility of conductive polymers raise concerns about potential toxicity. Further research is crucial to ensure these materials are safe and effective for cardiac tissue regeneration (103). Advances in understanding their interaction with biological systems and refining their synthesis processes will be essential to harnessing their full potential in biomedical applications, particularly in cardiac tissue engineering.

Polyaniline (PANI) is a conducting polymer that has garnered considerable attention in the realm of cardiac tissue engineering. It can be synthesized through various methods, including chemical, electrochemical, and enzymatic polymerization (94). The electrical conductivity of PANI can be finely tuned by adjusting the doping level, rendering it a versatile material for biomedical applications (67,104). PANI has demonstrated capability in supporting the growth and differentiation of cardiac cells, underscoring its potential as a candidate for cardiac tissue engineering scaffolds (67). Its ability to mimic the electrical properties of native cardiac tissue

and its compatibility with electrical stimulation make PANI particularly promising for promoting healthy cell behavior within engineered cardiac constructs.

Polypyrrole (PPy) is another conducting polymer that has been extensively researched for its applications in cardiac tissue engineering. It can be synthesized using chemical or electrochemical methods, offering flexibility in fabrication. The electrical conductivity of PPy can be adjusted by controlling the doping level (105), which allows for fine-tuning its properties for specific biomedical applications. PPy has demonstrated the ability to support the growth and differentiation of cardiac cells. Its electrical conductivity can also be utilized to provide electrical stimulation to cells, which is beneficial for promoting healthy cell growth and enhancing tissue function within the scaffold environment (62). This capability makes PPy a promising material for cardiac tissue engineering scaffolds, where mimicking the electrical properties of native cardiac tissue is crucial for achieving proper cell integration and functional tissue development.

Polythiophene is being studied for its potential applications in cardiac tissue engineering. It can be synthesized using chemical or electrochemical methods, and its electrical conductivity can be tuned by adjusting the doping level (68,106). Studies have shown that polythiophene supports the growth and differentiation of cardiac cells (60). Furthermore, its electrical conductivity can be utilized to stimulate the cells, promoting healthy cell growth and function within the scaffolds.

Bidez et al. [55] evaluated the adhesion and proliferation of H9c2 cardiac myoblasts on non-conductive emeraldine base (PANI) and conductive salt (E-PANI) of PANI thin films. They found that H9c2 cells could adhere to both PANI and E-PANI similarly, but E-PANI showed a significantly extended lag phase of growth due to the leakage of residual acid dopants. However, after dopant dissipation, the doubling time of cells on E-PANI was shorter than that of cells growing on PANI and TCP controls. Physical parameters of the films, such as thickness and surface roughness, were also considered as key factors affecting cell-surface interaction. E-PANI retained a significant level of electrical conductivity for at least 100 hours in DMEM at 37°C. In a similar study, Bidez et al. covalently attached biologically active oligopeptides Tyr-Ile-Gly-Ser-Arg (YIGSR) and a scrambled control sequence Arg-Tyr-Ser-Gly-Ile (RYSGI) to PANI to enhance cell attachment, proliferation, and differentiation for neuronal and cardiac tissue engineering (67).

Dong et al. [74] synthesized a self-healing injectable conductive hydrogel based on tetra-aniline and polyethylene glycol (PEG), capable of carrying C2C12 and H9c2 cells. There in vitro and in vivo subcutaneous injection studies demonstrated that this platform could protect cells during and after injection, thereby enhancing myocardial infarction regeneration (107).

Spencer et al. (70) synthesized a gelatin poly(3,4-ethylenedioxythiophene) polystyrenesulfonate (PEDOT: PSS) hydrogel that supports C2C12 myoblasts. Their research indicates that to achieve optimal performance of the hydrogel, the percentage of PEDOT should be 0.1.



**Table 3: Overview of carbon-based nanoarchitectonics applied in cardiac tissue engineering**

<b>Paper Title</b>	<b>Key findings</b>	<b>Research gap</b>	<b>Explanation</b>	<b>References</b>
Electrically conductive carbon-based (bio)-nanomaterials for cardiac tissue engineering.	Review on carbon-based nanomaterials for cardiac repair, emphasizing conductivity	Long-term safety and biocompatibility	Limited long-term <i>in vivo</i> studies assessing safety and biocompatibility	(55)
Carbon nanotubes for cardiac tissue regeneration: State of the art and perspectives	Review of CNTs as promising electroactive components for heart tissue repair scaffolds	Optimal dosage and formulation	Lack of consequences on optimal concentrations for cardiac applications	(71)
Intrinsically Conductive Polymers for Striated Cardiac Muscle Repair	Review on conducting polymers for improving intercellular coupling and electrical signal, propagation	Standardization of methods for synthesis	Absence of methods for assessing efficacy and safety	(106)
Carbon Nanotube-Based Scaffolds for Cardiac Tissue Engineering-Systematic Review and Narrative Synthesis	Review of CNT-based scaffolds for cardiac tissue engineering	Translation to clinical applications	Most studies limited to <i>in vitro</i>	(57)
Cardiac tissue engineering: state-of-the-art methods and outlook	Review on various approaches in cardiac tissue engineering, including conductive polymers	Integration with host tissue	Need for improved seamless integration of engineered tissues with host myocardium	(108)
Carbon nanotube doped pericardial	Research on CNT-doped hydrogel providing a	Electrical stimulation protocol	Need for more research on optimal electrical	(76)

matrix derived electroconductive biohybrid hydrogel for cardiac tissue engineering	suitable environment for cardiomyocyte maturation		stimulation protocols	
Preparation of an Electrically Conductive Graphene Oxide/Chitosan Scaffold for Cardiac Tissue Engineering	Cardiac specific gene and protein expression involved in muscle conduction of electrical signals (Connexin-43)	Lack of <i>in-vivo</i> validation	Enhanced electrical signaling in vitro, but in-vivo testing is required to confirm efficacy	(50)
Electroactive graphene oxide-incorporated collagen assisting vascularization for cardiac tissue engineering	Upregulated expression of cardiac genes, including Cx43, Actin4, and Trpt-2	Limited cell types studied	Focused on HUVECs, but investigating other cell types may provide a broader understanding of material effects.	(49)
Tunable electroconductive decellularized extracellular matrix hydrogels for engineering human cardiac microphysiological systems,	Enhanced electrophysiological properties and regulated contractile function	Need for comprehensive biocompatibility tests	Electrophysiological properties improved, and more extensive biocompatibility and long-term stability studies are required.	(46)
Moldable elastomeric polyester-carbon nanotube scaffolds for cardiac tissue engineering,	Elastomeric, conductive, degradable, swellable	Effects on long-term mechanical stability	Long-term stability and performance in a biological environment need further investigation.	(109)
Injectable and thermoresponsive pericardial matrix derived conductive	Injectable, thermoresponsive	In-vivo efficacy and safety	Detailed in-vivo studies are needed to assess safety and effectiveness.	(78)

scaffold for cardiac tissue engineering				
Electrospun conductive nanofibrous scaffolds for engineering cardiac tissue and 3D bioactuators	Enhanced cell viability and proliferation	Material degradation	Understanding the degradation of the material and its impact on long-term tissue integration is needed.	(72)
Electrical coupling of isolated cardiomyocyte clusters grown on aligned conductive nanofibrous meshes for their synchronized beating	Enhanced cell adhesion	Biomechanical properties	The material enhances cell adhesion, but further research on its biomechanical properties and tissue integration is necessary.	(73)
Conductive silk–polypyrrole composite scaffolds with bioinspired nanotopographic cues for cardiac tissue engineering	Biocompatible, stable, electroconductive	Long-term stability	Biocompatible and electroconductive, but its long-term stability and performance in vivo require further research.	(63)

### 3. 3D Bioprinting with electrically conductive materials

Bioprinting is a technique where bioinks and biomaterials, mixed with cells, are 3D printed to construct functional tissue structures. This method has become pivotal in tissue engineering and regenerative medicine. Unlike traditional 3D printing methods, bioprinting requires considerations beyond rheological properties, including cell viability, printing resolution, speed, and accuracy (110).

The advantages of bioprinting include automation, precise control, and the ability to design complex geometries using a variety of materials. However, selecting the most suitable bioprinting technique involves evaluating critical parameters such as UV light intensity for light-based bioprinting and viscosity for inkjet bioprinting. In 2016, Anthony Atala's research

group developed a cardiac model using an integrated tissue organ printer (111). The preferred heart model was subsequently refined by Noor et al. in 2019 (112). Later in 2019, Lee et al. introduced the free-form reversible embedding of suspended hydrogel technique to bioprint human heart models across various size ranges. This technique promotes cell distribution and organization within the bioink, mimicking natural tissue. Additionally, the printed tissue can be exposed to specific stimuli to enhance cell growth, specialization, and overall functionality.

There are several bioprinting techniques, including extrusion-based printing, inkjet-based printing, and laser-assisted printing (Fig.4). Each of these techniques has its advantages, such as ensuring minimal cell loss during printing and offering high printing resolution. They are also efficient in addressing viscosity issues that arise from the immiscibility or high gelling capacity of the bioink.

To further enhance the mechanical and biological properties of scaffolds, researchers have turned to the development of nanocomposites. These are formed by incorporating nanoparticles (gold, silver), peptides, natural polymers (silk, chitosan, gelatin, sodium alginate), and carbon-based materials (carbon dots, carbon nanotubes, fullerenes, graphene, polyaniline, polypyrrole, PEDOT: PSS) into the base material. The inclusion of these materials can amplify the biochemical, mechanical, and biophysical responses of the resulting scaffolds.

Carbon-based materials, such as graphene and GO, carbon nanotubes, and carbon nanofibers, have been shown to enhance the biofunctionality of bioinks. For instance, graphene-based bioinks have demonstrated the ability to achieve a densely penetrating vasculature network, which is more prominent compared to GelMA bioinks. This enhanced angiogenic potential is crucial for the development of functional cardiac tissues, as it ensures adequate nutrient supply and fosters synchronous contractile activity within the engineered tissues. Overview of printing techniques and bioinks used for bioprinting of cardiac tissue is summarized in Table. 3

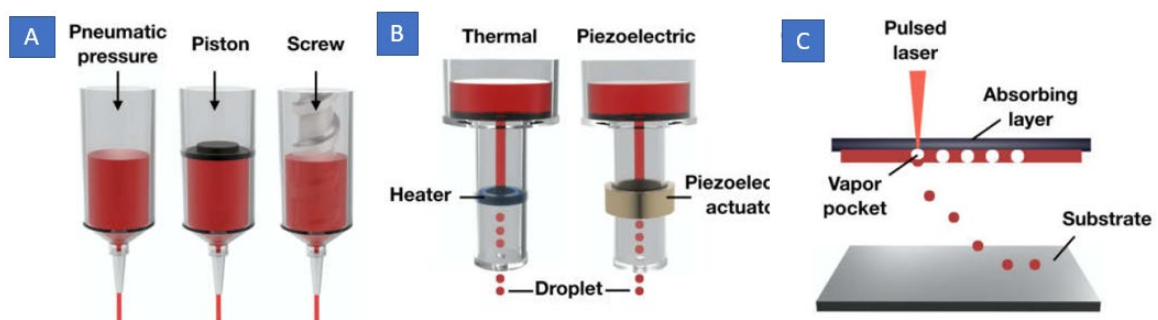


Figure 4: Representation of various 3D bioprinting techniques A) Extrusion based B) Inkjet based C) Laser based

### 3.1 Inkjet-based bioprinting (IBB):

Inkjet Bioprinting (IBB) employs various approaches including thermal, piezoelectric, electrostatic, and acoustic methods, with thermal and piezoelectric being the most commonly utilized (113–118). The thermal inkjet technique utilizes a heating element to elevate the bioink temperature above 300°C at the nozzle. This generates vapor bubbles that grow until the bioink

is expelled in droplet form (Figure 5B) (119). The temperature quickly rises within seconds to facilitate droplet formation (120), minimally impacting bioink temperature and biological components' viability (117). Thermal inkjet printing is widely adopted due to its cost-effectiveness (117,121). However, it can produce uneven, irregularly shaped droplets, and the thermal and shear stress can affect bioink and protein ink viability (122), limiting its application. Additional challenges include droplet reproducibility, shear forces within the nozzle, and cell sedimentation in the reservoir (123).

Piezoelectric inkjet printing operates by rapidly activating a piezoelectric actuator to eject bioink from the cartridge to the print bed. This method eliminates the need for heat, ensuring continuous, unidirectional flow and uniform droplet size to prevent orifice clogging (124,125). Prolonged use of piezoelectric printing may cause cell membrane damage or lysis (126). High cellular viability (>90%) has been reported for mammalian cells such as human fibroblasts, osteoblasts, and bovine chondrocytes (125). The inkjet method offers a wide range of bioink volumes, from nanoliters to picoliters, with printing resolution unaffected by volume variation. Droplet deposition rates can reach up to 10,000 droplets per second in 2D printing configurations (118). The viscosity range of bioink suitable for inkjet printing typically falls within 0.1 Pa, accommodating optimal cell densities for printing applications (123).

### **3.2 Extrusion-based bioprinting (EBB):**

Extrusion-Based Bioprinting (EBB) represents a modernized approach capable of generating predesigned 3D models using dispersions, solutions, and pastes. This technique employs screw, pneumatic, or plunger-based pressure to extrude bioinks through microneedles or microscale nozzles onto a stationary substrate, coordinating the movement of the extruder (127). Low-viscosity bioinks are utilized to prevent air bubble formation. Unlike Inkjet Bioprinting (IBB), EBB printers generate continuous filaments (128). EBB allows for printing biomaterials with multiple viscosities and varying cell concentrations (129), making it the preferred choice for researchers aiming to construct tissues with significant mechanical strength (130–133).

In EBB, bioink is extruded through a nozzle using pneumatic or mechanical extrusion principles, depositing continuous microfilaments on the receiving substrate to form a predetermined multilayered 3D structure (134). The receiving substrate can consist of solid materials (such as tissue culture plates, polymers), liquid (nutrient media), or gel (cell-encapsulated matrix) forms (127,135). Biocompatible polymers extensively used in this technique include PLGA, polylactide, agar, gelatin, GelMA, silk, sodium alginate, among others (Figure 5A) (119). During the bioprinting process, various cell types are employed, such as human mesenchymal stem cells (hMSCs), osteogenic progenitors, endothelial cells, and mouse pre-osteoblasts (136). EBB has demonstrated effectiveness in treating congenital ovine calvarial defects through cell bioprinting. While EBB is considered a facile, easy-to-program, and robust technique, challenges include selecting appropriate biomaterials and managing shear stress, as rapid gelation is essential for successful outcomes (123).

### **3.2 Laser-based bioprinting (LBB):**

Laser-guided direct writing (LGDW), or laser-induced forward transfer, is a bioprinting technique initiated by Bohandy et al. three decades ago (137). It is a nozzle-free method that achieves higher resolution in liquid deposition on solid substrates, especially suitable for the intricate structures of tissues (138). Using a low-energy laser, cell-laden bioink is transferred to the substrate layer employing the laser tweezers principle, minimizing cell viability loss (139,140). The system comprises a pulsed laser generator, a laser focusing instrument or lens, a laser-absorbing metallic ribbon, and a receiving substrate (141). The ribbon is a double-layered structure made of glass coated with micro gold or titanium film for efficient energy absorption.

Matrix-assisted pulsed laser evaporation direct writing (MAPLEDW) and Laser-induced forward transfer (LIFT) are extensively used techniques in laser-based bioprinting (119). MAPLEDW operates at lower pulsed laser energy compared to LIFT, allowing precise cell displacement in micro 3D structures (128). The laser pulse irradiates a predetermined area of the upper ribbon layer, causing evaporation. This generates high-pressure bubbles in the lower layer, which are subsequently deposited onto the receiving substrate as droplets of cell-laden hydrogel or biopolymer. During bioprinting, laser pulse energy and shear stress are critical factors influencing ink bubble dynamics (142–144).

Near-UV or nanosecond lasers are employed to print various materials including photoresists like SU-8, biopolymers, biomolecules (such as proteins), hydrogels, ceramics, and cell-laden hydrogels (122,145,146). Researchers have demonstrated the efficacy of LGDW with various cell types such as mouse C2C12 myoblasts, breast cancer (MCF-7) cells, human dermal fibroblasts, rat neural stem cells, and bovine pulmonary artery endothelial cells (BPAECs) (147–149). Factors influencing resolution, ranging from picometers to micrometers, include the rheological behavior of hydrogels, the thickness of biomaterial layers on the ribbon, substrate wettability, laser pulse speed, and structural arrangement during printing (141,147). LGDW offers superior attributes over other bioprinting techniques due to its nozzle-free approach, highly accurate printability, non-contact process, high resolution, and precise control over ink droplet deposition.

**Table 3: Printing techniques and bioinks used for bioprinting of cardiac tissue**

<b>Printing Technology</b>	<b>Bioink</b>	<b>Cells</b>	<b>References</b>
Extrusion based	dECM+rGO	Cardiomyocytes	(150)
Extrusion based	AlgMA+GelMA+rGO	H9c2	(47)
Extrusion	GelMA+alginate+Gold rods	cardiomyocytes	(151)
Extrusion	PEGDA+PANI	H9c2	(152)
UV assisted	CNT+Mecol+alginate	Cardiomyocytes	(153)
FDM	Gelatin+PCL+GO	H9c2	(154)

#### **4. Challenges and Future Perspectives**

Despite significant progress in utilizing electrically conductive carbon-based materials for cardiac tissue engineering, several challenges remain to be addressed. These include the scalability of fabrication methods, long-term biocompatibility, immunogenicity, and clinical translation. *In vitro* analyses conducted by the team observed cell proliferation and morphology; they found that carbon nanofibers were significantly more toxic than nanotubes. Much of the carbon nanotube and nanofiber cytotoxicity research has been performed using various lung models, as inhalation is a common exposure route. Cardiac cells seeded with these nanomaterials in scaffolds may exhibit different behaviors (56,66,69,155,156).

Additionally, modulating the size and length of the materials is essential to achieving appropriate biocompatibility. While the potential applications of carbon-based nanomaterials continue to expand, their biocompatibility remains a limiting factor. Several studies have shown mixed biological responses to these materials. Lung toxicity has been demonstrated to varying extents for both single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). Each study reported an inflammatory response to the CNTs, along with granulation around the particles (81). These inflammatory reactions are believed to be attributable to the material properties.

Future research directions may focus on developing scalable and cost-effective synthesis techniques for carbon-based nanomaterials, optimizing bioink formulations for enhanced electrical conductivity and cell viability, and conducting preclinical studies to evaluate the safety and efficacy of bioprinted cardiac constructs in animal models (2,39). As the nanomaterials community progresses towards clinical research consideration, several additional avenues for future research exist both *in vitro* and *in vivo*.

Greater mechanistic insights will be crucial for optimizing material design specifically tailored for cardiac regeneration. Incorporating nanomaterials into bulk materials often alters multiple properties simultaneously, including mechanical characteristics, electrical conductivity, cell interaction profiles, and surface roughness (105). These promising translational studies provide strong motivation for the clinical evaluation of these materials in the near future. Currently, organ transplantation and left-ventricular assist devices represent the primary treatments available for such patients. Cardiac tissue engineering using carbon-based materials offers the potential for a novel therapeutic option in the future.

#### **5. Conclusion**

Electrically conductive carbon-based materials, including graphene, carbon nanotubes, carbon nanofibers, rGO, GO, fullerenes, PANI, PPY, and PEDOT, hold immense potential for advancing cardiac tissue engineering applications. Their unique combination of electrical conductivity, mechanical strength, and biocompatibility makes them well-suited for fabricating functional cardiac tissue constructs using 3D bioprinting techniques. Despite existing

challenges, continued research efforts in this field offer a promising area for developing advanced therapies to address cardiovascular diseases and improve patient outcomes.

A wide range of biomaterials has been utilized so far to mimic the physiochemical properties of cardiac tissue. However, it is evident that all of them exhibit significant disadvantages despite their seemingly promising properties. One of the primary drawbacks is the lack of conductivity, which limits these materials from fully substituting myocardium. Consequently, the main challenges associated with the application of PANI-based materials in cardiac tissue engineering can be categorized as low cell adhesion, lesser biocompatibility, conductivity reduction over time, toxicity issues, and mechanical behavior. Addressing these challenges necessitates focused research efforts to enhance the performance and reliability of PANI-based materials in cardiac tissue engineering. Strategies such as surface modification to improve cell adhesion, optimizing polymerization conditions for better biocompatibility and sustained conductivity, and comprehensive biocompatibility assessments are essential steps toward overcoming these limitations. In conclusion, despite the challenges, ongoing research in utilizing conductive carbon-based materials for cardiac tissue engineering holds promise for developing advanced therapies that can effectively address cardiovascular diseases and significantly improve patient outcomes.

### **Authorship contribution**

Mansi Dixit- Conceptualization, writing original draft, editing, table and figure elucidation, Lok Kumar Shrestha- Suggestion and review, Katsuhiko Ariga- Suggestion and review, Falguni Pati-Supervision, formal analysis, review, and editing.

### **Conflict of interest**

Authors have no conflict of interest

### **Acknowledgment**

The authors would like to acknowledge the Department of Biomedical Engineering at IIT Hyderabad for their resource assistance and the Ministry of Education for financial support. Figure 1,3 was created with BioRender.com

### **References**

1. Islam M, Díaz Lantada A, Mager D, Korvink JG, Islam M, Mager D, et al. Carbon-Based Materials for Articular Tissue Engineering: From Innovative Scaffolding Materials toward Engineered Living Carbon. *Adv Healthc Mater* [Internet]. 2022 Jan 1 [cited 2024 Apr 16];11(1):2101834. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/adhm.202101834>
2. Chingale M, Cheng K, Huang K. 3D Bioprinting Technology – One Step Closer Towards Cardiac Tissue Regeneration. *Front Mater*. 2022 Feb 2;8:804134.
3. Qasim M, Haq F, Kang MH, Kim JH. 3D printing approaches for cardiac tissue engineering and role of immune modulation in tissue regeneration. *Int J Nanomedicine* [Internet]. 2019 [cited 2024 Apr 16];14:1311. Available from: [/pmc/articles/PMC6388753/](https://pubmed.ncbi.nlm.nih.gov/3488753/)



4. Human cardiovascular system - Heart Wall, Blood Flow, Circulation | Britannica. [cited 2024 Apr 23]. Available from: <https://www.britannica.com/science/human-cardiovascular-system/Wall-of-the-heart>
5. Jallerat Q, Feinberg AW. Extracellular Matrix Structure and Composition in the Early Four-Chambered Embryonic Heart. Available from: </pmc/articles/PMC7072588/>
6. Christoffels V, Jensen B. Cardiac Morphogenesis: Specification of the Four-Chambered Heart. Cold Spring Harb Perspect Biol [Internet]. 2020 Oct 1 [cited 2024 Apr 23];12(10):a037143. Available from: <http://cshperspectives.cshlp.org/content/12/10/a037143.full>
7. Cardiac Muscle and Electrical Activity | Anatomy and Physiology II [Internet]. [cited 2024 Apr 23]. Available from: <https://courses.lumenlearning.com/suny-ap2/chapter/cardiac-muscle-and-electrical-activity/>
8. OpenStaxCollege. Cardiac Muscle and Electrical Activity. 2013.
9. Keepers B, Liu J, Qian L. What's in a cardiomyocyte-And how do we make one through reprogramming? ☆. 2019 [cited 2024 Apr 23]; Available from: <https://doi.org/10.1016/j.bbamcr.2019.03.011>
10. Lauschke K, Volpini L, Liu Y, Vinggaard AM, Hall VJ. A Comparative Assessment of Marker Expression Between Cardiomyocyte Differentiation of Human Induced Pluripotent Stem Cells and the Developing Pig Heart. <https://home.liebertpub.com/scd> [Internet]. 2021 Mar 30 [cited 2024 Apr 23];30(7):374–85. Available from: <https://www.liebertpub.com/doi/10.1089/scd.2020.0184>
11. Cardiomyocyte Markers Research Area: R&D Systems [Internet]. [cited 2024 Apr 23]. Available from: <https://www.rndsystems.com/research-area/cardiomyocyte-markers>
12. Author C, Forough R, Scarcello C, Perkins M. Cardiac Biomarkers: a Focus on Cardiac Regeneration.
13. Ko T, Nomura S, Yamada S, Fujita K, Fujita T, Satoh M, et al. Cardiac fibroblasts regulate the development of heart failure via Htra3-TGF- $\beta$ -IGFBP7 axis. Nature Communications 2022 13:1 [Internet]. 2022 Jun 7 [cited 2024 Apr 23];13(1):1–17. Available from: <https://www.nature.com/articles/s41467-022-30630-y>
14. Harrington A, Moore-Morris T. Cardiac fibroblasts in heart failure and regeneration. Front Cell Dev Biol [Internet]. 2024 Apr 18 [cited 2024 Apr 23];12:1388378. Available from: <https://www.frontiersin.org/articles/10.3389/fcell.2024.1388378/full>
15. Xiao Z, Todd L, Huang L, Noguera-Ortega E, Lu Z, Huang L, et al. Desmoplastic stroma restricts T cell extravasation and mediates immune exclusion and immunosuppression in solid tumors. Nature Communications 2023 14:1 [Internet]. 2023 Aug 22 [cited 2024 Sep 24];14(1):1–21. Available from: <https://www.nature.com/articles/s41467-023-40850-5>
16. Chen W, Bian W, Zhou Y, Zhang J. Cardiac Fibroblasts and Myocardial Regeneration. Front Bioeng Biotechnol [Internet]. 2021 Mar 25 [cited 2024 Apr 23];9. Available from: </pmc/articles/PMC8026894/>
17. Sun H, Pratt RE, Dzau VJ, Hodgkinson CP. Neonatal and adult cardiac fibroblasts exhibit inherent differences in cardiac regenerative capacity. Journal of Biological Chemistry

- [Internet]. 2023 May 1 [cited 2024 Apr 23];299(5). Available from: <http://www.jbc.org/article/S0021925823003368/fulltext>
18. Lin A, Peiris NJ, Dhaliwal H, Hakim M, Li W, Ganesh S, et al. Mural Cells: Potential Therapeutic Targets to Bridge Cardiovascular Disease and Neurodegeneration. *Cells* 2021, Vol 10, Page 593 [Internet]. 2021 Mar 8 [cited 2024 Apr 23];10(3):593. Available from: <https://www.mdpi.com/2073-4409/10/3/593/htm>
  19. Chen Q, Zhang H, Liu Y, Adams S, Eilken H, Stehling M, et al. Endothelial cells are progenitors of cardiac pericytes and vascular smooth muscle cells. *Nature Communications* 2016 7:1 [Internet]. 2016 Aug 12 [cited 2024 Apr 23];7(1):1–13. Available from: <https://www.nature.com/articles/ncomms12422>
  20. Revelo XS, Parthiban P, Chen C, Barrow F, Fredrickson G, Wang H, et al. Cardiac Resident Macrophages Prevent Fibrosis and Stimulate Angiogenesis. *Circ Res* [Internet]. 2021 Dec 3 [cited 2024 Apr 23];129(12):1086–101. Available from: <https://www.ahajournals.org/doi/abs/10.1161/CIRCRESAHA.121.319737>
  21. Zaman R, Epelman S. Resident cardiac macrophages: Heterogeneity and function in health and disease. *Immunity*. 2022 Sep 13;55(9):1549–63.
  22. Broz MT, Ko EY, Ishaya K, Xiao J, De Simone M, Hoi XP, et al. Metabolic targeting of cancer associated fibroblasts overcomes T-cell exclusion and chemoresistance in soft-tissue sarcomas. *Nature Communications* 2024 15:1 [Internet]. 2024 Mar 20 [cited 2024 Sep 24];15(1):1–18. Available from: <https://www.nature.com/articles/s41467-024-46504-4>
  23. Heintzman DR, Fisher EL, Rathmell JC. Microenvironmental influences on T cell immunity in cancer and inflammation. *Cellular & Molecular Immunology* 2022 19:3 [Internet]. 2022 Jan 17 [cited 2024 Sep 24];19(3):316–26. Available from: <https://www.nature.com/articles/s41423-021-00833-2>
  24. Zhang Y, Guan XY, Jiang P. Cytokine and Chemokine Signals of T-Cell Exclusion in Tumors. *Front Immunol* [Internet]. 2020 Dec 14 [cited 2024 Sep 24];11. Available from: </pmc/articles/PMC7768018/>
  25. Bansal SS, Ismahil MA, Goel M, Patel B, Hamid T, Rokosh G, et al. Activated T-Lymphocytes are Essential Drivers of Pathological Remodeling in Ischemic Heart Failure. *Circ Heart Fail* [Internet]. 2017 Mar 1 [cited 2024 Sep 24];10(3):e003688. Available from: </pmc/articles/PMC5331621/>
  26. Blanton RM, Carrillo-Salinas FJ, Alcaide P. Adaptive Immunity in Cardiovascular Disease: T-cell recruitment to the heart: friendly guests or unwelcome visitors? *Am J Physiol Heart Circ Physiol* [Internet]. 2019 Jul 7 [cited 2024 Sep 24];317(1):H124. Available from: </pmc/articles/PMC6692732/>
  27. Feng Q, Li Q, Zhou H, Sun L, Lin C, Jin Y, et al. The role of major immune cells in myocardial infarction. *Front Immunol*. 2023 Jan 19;13:1084460.
  28. Martins S, António N, Carneiro T, Laranjeira P, Rodrigues R, Gonçalves L, et al. Reduced numbers of regulatory T cells in chronic heart failure seems not to be restored by cardiac resynchronization therapy. *BMC Cardiovasc Disord* [Internet]. 2023 Dec 1 [cited 2024 Sep 24];23(1):1–13. Available from: <https://bmccardiovascdisord.biomedcentral.com/articles/10.1186/s12872-023-03109-x>

29. Jiang Z, Kang Q, Qian H, Xu Z, Tong H, Yang J, et al. Revealing the crucial roles of suppressive immune microenvironment in cardiac myxoma progression. *Signal Transduction and Targeted Therapy* 2024 9:1 [Internet]. 2024 Aug 2 [cited 2024 Sep 24];9(1):1–16. Available from: <https://www.nature.com/articles/s41392-024-01912-2>
30. Scharping NE, Menk A V., Moreci RS, Whetstone RD, Dadey RE, Watkins SC, et al. The Tumor Microenvironment Represses T Cell Mitochondrial Biogenesis to Drive Intratumoral T Cell Metabolic Insufficiency and Dysfunction. *Immunity* [Internet]. 2016 Aug 16 [cited 2024 Sep 24];45(2):374–88. Available from: <http://www.cell.com/article/S1074761316302825/fulltext>
31. Hou X, Chen G, Bracamonte-Baran W, Choi HS, Diny NL, Sung J, et al. The Cardiac Microenvironment Instructs Divergent Monocyte Fates and Functions in Myocarditis. *Cell Rep* [Internet]. 2019 Jul 2 [cited 2024 Sep 24];28(1):172-189.e7. Available from: <http://www.cell.com/article/S221112471930765X/fulltext>
32. Zhang Y, Guan XY, Jiang P. Cytokine and Chemokine Signals of T-Cell Exclusion in Tumors. *Front Immunol* [Internet]. 2020 Dec 14 [cited 2024 Sep 24];11. Available from: </pmc/articles/PMC7768018/>
33. Kwon SG, Kwon YW, Lee TW, Park GT, Kim JH. Recent advances in stem cell therapeutics and tissue engineering strategies. *Biomater Res* [Internet]. 2018 Dec 19 [cited 2024 Apr 23];22(1). Available from: </pmc/articles/PMC6299977/>
34. Vapniarsky N, Arzi B, Hu JC, Nolta JA, Athanasiou KA. Concise Review: Human Dermis as an Autologous Source of Stem Cells for Tissue Engineering and Regenerative Medicine. *Stem Cells Transl Med* [Internet]. 2015 Oct 1 [cited 2024 Apr 23];4(10):1187–98. Available from: <https://dx.doi.org/10.5966/sctm.2015-0084>
35. Dionigi B, Fauza DO. Autologous Approaches to Tissue Engineering. *Stembook* [Internet]. 2012 Dec 10 [cited 2024 Apr 23]; Available from: <https://www.ncbi.nlm.nih.gov/books/NBK133256/>
36. Ahadian S, Ostrovidov S, Hosseini V, Kaji H, Ramalingam M, Bae H, et al. Electrical stimulation as a biomimicry tool for regulating muscle cell behavior. *Organogenesis* [Internet]. 2013 Apr [cited 2024 Apr 23];9(2):87–92. Available from: <https://www.tandfonline.com/doi/abs/10.4161/org.25121>
37. Chen C, Bai X, Ding Y, Lee IS. Electrical stimulation as a novel tool for regulating cell behavior in tissue engineering. *Biomaterials Research* 2019 23:1 [Internet]. 2019 Dec 5 [cited 2024 Apr 23];23(1):1–12. Available from: <https://biomaterialsres.biomedcentral.com/articles/10.1186/s40824-019-0176-8>
38. Kapeller B, Mueller J, Losert U, Podesser BK, Macfelda K. Microcurrent stimulation promotes reverse remodelling in cardiomyocytes. *ESC Heart Fail* [Internet]. 2016 Jun 1 [cited 2024 Apr 23];3(2):122–30. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/ehf2.12080>
39. Loukelis K, Helal ZA, Mikos AG, Chatzinikolaïdou M. Nanocomposite Bioprinting for Tissue Engineering Applications. *Gels* [Internet]. 2023 Feb 1 [cited 2024 Apr 18];9(2). Available from: </pmc/articles/PMC9956920/>
40. Soldano C, Mahmood A, Dujardin E. Production, properties and potential of graphene. *Carbon* N Y. 2010;48(8):2127–50.

41. Shin SR, Zihlmann C, Akbari M, Assawes P, Cheung L, Zhang K, et al. Reduced graphene oxide-gelMA hybrid hydrogels as scaffolds for cardiac tissue engineering. *Small*. 2016;12(27):3677–89.
42. Kim T, Ho Kahng Y, Lee T, Lee K, Kim H. Graphene Films Show Stable Cell Attachment and Biocompatibility with Electrogenic Primary Cardiac Cells. *Mol Cells* [Internet]. 2013 [cited 2024 Apr 23];36:577–82. Available from: <http://molcells.org>
43. Wang J, Cui C, Nan H, Yu Y, Xiao Y, Poon E, et al. Graphene Sheet-Induced Global Maturation of Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells. 2017 [cited 2024 Apr 23]; Available from: [www.acsami.org](http://www.acsami.org)
44. Bahrami S, Baheiraei N, Mohseni M, Razavi M, Ghaderi A, Azizi B, et al. Three-dimensional graphene foam as a conductive scaffold for cardiac tissue engineering. *J Biomater Appl*. 2019 Jul 1;34(1):74–85.
45. Park J, Park S, Ryu S, Bhang SH, Kim J, Yoon JK, et al. Graphene–Regulated Cardiomyogenic Differentiation Process of Mesenchymal Stem Cells by Enhancing the Expression of Extracellular Matrix Proteins and Cell Signaling Molecules. *Adv Healthc Mater* [Internet]. 2014 [cited 2024 Apr 23];3(2):176–81. Available from: <https://doi.org/10.1002/adhm.201300177>
46. Tsui JH, Leonard A, Camp ND, Long JT, Nawas ZY, Chavanachat R, et al. Tunable electroconductive decellularized extracellular matrix hydrogels for engineering human cardiac microphysiological systems. *Biomaterials* [Internet]. 2021 [cited 2024 Apr 23];272. Available from: <https://doi.org/10.1016/j.biomaterials.2021.120764>
47. Mousavi A, Hedayatnia A, Piet Van Vliet P, Dartora DR, Wong N, Rafatian N, et al. Development of photocrosslinkable bioinks with improved electromechanical properties for 3D bioprinting of cardiac BioRings. 2023 [cited 2024 Apr 23]; Available from: <https://doi.org/10.1016/j.apmt.2023.102035>
48. Sekuła-Stryjewska M, Noga S, Dźwigońska M, Adamczyk E, Karnas E, Jagiełło J, et al. Graphene-based materials enhance cardiomyogenic and angiogenic differentiation capacity of human mesenchymal stem cells in vitro – Focus on cardiac tissue regeneration. *Materials Science and Engineering: C*. 2021 Feb 1;119:111614.
49. Norahan MH, Amroon M, Ghahremanzadeh R, Mahmoodi M, Baheiraei N. Electroactive graphene oxide-incorporated collagen assisting vascularization for cardiac tissue engineering. *J Biomed Mater Res A* [Internet]. 2019 Jan 1 [cited 2024 Apr 22];107(1):204–19. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/jbm.a.36555>
50. Jiang L, Chen D, Wang Z, Zhang Z, Xia Y, Xue H, et al. Preparation of an Electrically Conductive Graphene Oxide/Chitosan Scaffold for Cardiac Tissue Engineering. *Appl Biochem Biotechnol* [Internet]. 2019;188(4):952–64. Available from: <https://doi.org/10.1007/s12010-019-02967-6>
51. Domingues Stocco T, Zhang T, Dimitrov E, Ghosh A, Marcio Hakme da Silva A, CMA Melo W, et al. Carbon Nanomaterial-Based Hydrogels as Scaffolds in Tissue Engineering: A Comprehensive Review. 2023 [cited 2024 Sep 17]; Available from: <https://doi.org/10.2147/IJN.S436867>
52. Kazemzadeh H, Mozafari M. Fullerene-based delivery systems. *Drug Discov Today*. 2019 Mar 1;24(3):898–905.

53. Sergio M, Behzadi H, Otto A, van der Spoel D. Fullerenes toxicity and electronic properties. *Environ Chem Lett*. 2013 Jun;11(2):105–18.
54. Barrejón M, Marchesan S, Alegret N, Prato M. Carbon nanotubes for cardiac tissue regeneration: State of the art and perspectives. *Carbon N Y*. 2021 Oct 30;184:641–50.
55. Jalilinejad N, Rabiee M, Baheiraei N, Ghahremanzadeh R, Salarian R, Rabiee N, et al. Electrically conductive carbon-based (bio)-nanomaterials for cardiac tissue engineering. *Bioeng Transl Med [Internet]*. 2023 Jan 1 [cited 2024 Sep 17];8(1):e10347. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/btm2.10347>
56. Sun H, Zhou J, Huang Z, Qu L, Lin N, Liang C, et al. Carbon nanotube-incorporated collagen hydrogels improve cell alignment and the performance of cardiac constructs. *Int J Nanomedicine*. 2017;3109–20.
57. Scott L, Jurewicz I, Jeevaratnam K, Lewis R. Carbon Nanotube-Based Scaffolds for Cardiac Tissue Engineering—Systematic Review and Narrative Synthesis. *Bioengineering 2021, Vol 8, Page 80 [Internet]*. 2021 Jun 9 [cited 2024 Sep 17];8(6):80. Available from: <https://www.mdpi.com/2306-5354/8/6/80/htm>
58. Mehrabi A, Baheiraei N, Adabi M, Amirkhani Z. Development of a novel electroactive cardiac patch based on carbon nanofibers and gelatin encouraging vascularization. *Appl Biochem Biotechnol*. 2020;190:931–48.
59. Haq AU, Carotenuto F, De Matteis F, Prosposito P, Francini R, Teodori L, et al. Intrinsically Conductive Polymers for Striated Cardiac Muscle Repair. *Int J Mol Sci [Internet]*. 2021 Aug 2 [cited 2024 Sep 17];22(16). Available from: <https://pubmed.ncbi.nlm.nih.gov/35236716/>
60. Puckert C, Gelmi A, Ljunggren MK, Rafat M, Jager EWH. Optimisation of conductive polymer biomaterials for cardiac progenitor cells. *RSC Adv [Internet]*. 2016 Jun 28 [cited 2024 Sep 17];6(67):62270–7. Available from: <https://pubs.rsc.org/en/content/articlehtml/2016/ra/c6ra11682e>
61. Li Y, Wei L, Lan L, Gao Y, Zhang Q, Dawit H, et al. Conductive biomaterials for cardiac repair: A review. *Acta Biomater*. 2022 Feb 1;139:157–78.
62. Parchehbaf-Kashani M, Ansari H, Mahmoudi E, Barekat M, Sepantafar M, Rajabi S, et al. Heart Repair Induced by Cardiac Progenitor Cell Delivery within Polypyrrole-Loaded Cardiolipid Post-ischemia. *ACS Appl Bio Mater [Internet]*. 2021 Jun 21 [cited 2024 Apr 18];4(6):4849–61. Available from: <https://doi.org/10.1021/acsabm.1c00133>
63. Tsui JH, Ostrovsky-Snyder NA, P Yama DM, Donohue JD, Seob Choi J, Chavanachat R, et al. Materials Chemistry B Materials for biology and medicine Conductive silk-polypyrrole composite scaffolds with bioinspired nanotopographic cues for cardiac tissue engineering Conductive silk-polypyrrole composite scaffolds with bioinspired nanotopographic cues for cardiac tissue engineering †. *J Mater Chem B*. 6:7185.
64. Shokrollahi P, Omid Y, Cubeddu LX, Omidian H. Conductive polymers for cardiac tissue engineering and regeneration. *J Biomed Mater Res B Appl Biomater [Internet]*. 2023 Nov 1 [cited 2024 Sep 17];111(11):1979–95. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/jbm.b.35293>

65. Liu W, Zhao L, Wang C, Zhou J. Conductive nanomaterials for cardiac tissues engineering. *Engineered Regeneration*. 2020 Jan 1;1:88–94.
66. Wu C, Zhang Y, Xu Y, Long L, Hu X, Zhang J, et al. Injectable polyaniline nanorods/alginate hydrogel with AAV9-mediated VEGF overexpression for myocardial infarction treatment. *Biomaterials*. 2023;296:122088.
67. Bidez PR, Li S, MacDiarmid AG, Venancio EC, Wei Y, Lelkes PI. Polyaniline, an electroactive polymer, supports adhesion and proliferation of cardiac myoblasts. *J Biomater Sci Polym Ed* [Internet]. 2006 Jan 1;17(1–2):199–212. Available from: <https://doi.org/10.1163/156856206774879180>
68. Shokrollahi P, Omid Y, Cubeddu LX, Omidian H. Conductive polymers for cardiac tissue engineering and regeneration. *J Biomed Mater Res B Appl Biomater* [Internet]. 2023 Nov 1 [cited 2024 Sep 17];111(11):1979–95. Available from: <https://pubmed.ncbi.nlm.nih.gov/37306139/>
69. Yu C, Yue Z, Zhang H, Shi M, Yao M, Yu Q, et al. Ultra-Histocompatible and Electrophysiological-Adapted PEDOT-Based Hydrogels Designed for Cardiac Repair. *Adv Funct Mater*. 2023;33(15):2211023.
70. Spencer AR, Primbetova A, Koppes AN, Koppes RA, Fenniri H, Annabi N. Electroconductive Gelatin Methacryloyl-PEDOT:PSS Composite Hydrogels: Design, Synthesis, and Properties. 2018 [cited 2024 Apr 22]; Available from: <https://pubs.acs.org/sharingguidelines>
71. Barrejón M, Marchesan S, Alegret N, Prato M. Carbon nanotubes for cardiac tissue regeneration: State of the art and perspectives. *Carbon N Y*. 2021 Oct 30;184:641–50.
72. Wang L, Wu Y, Hu T, Guo B, Ma PX. Electrospun conductive nanofibrous scaffolds for engineering cardiac tissue and 3D bioactuators. 2017 [cited 2024 Apr 22]; Available from: <http://dx.doi.org/10.1016/j.actbio.2017.06.036>
73. Hsiao CW, Bai MY, Chang Y, Chung MF, Lee TY, Wu CT, et al. Electrical coupling of isolated cardiomyocyte clusters grown on aligned conductive nanofibrous meshes for their synchronized beating. 2012 [cited 2024 Apr 22]; Available from: <http://dx.doi.org/10.1016/j.biomaterials.2012.10.065>
74. Martinelli V, Bosi S, Peñ B, Baj G, Long CS, Sbaizero O, et al. 3D Carbon-Nanotube-Based Composites for Cardiac Tissue Engineering. 2018 [cited 2024 Apr 23]; Available from: [www.acsabm.org](http://www.acsabm.org)
75. Ahadian S, Yamada S, Ramón-Azcón J, Estili M, Liang X, Nakajima K, et al. Hybrid hydrogel-aligned carbon nanotube scaffolds to enhance cardiac differentiation of embryoid bodies. [cited 2024 Apr 23]; Available from: <http://dx.doi.org/10.1016/j.actbio.2015.11.047>
76. Roshanbinfar K, Mohammadi Z, Sheikh-Mahdi Mesgar A, Mehdi Dehghan M, Oommen OP, Hilborn J, et al. Carbon nanotube doped pericardial matrix derived electroconductive biohybrid hydrogel for cardiac tissue engineering †. *Biomater Sci*. 2019;7:3906.
77. Hao T, Li J, Yao F, Dong D, Wang Y, Yang B, et al. Injectable Fullerenol/Alginate Hydrogel for Suppression of Oxidative Stress Damage in Brown Adipose-Derived Stem Cells and Cardiac Repair. *ACS Nano* [Internet]. 2017 Jun 27 [cited 2024 Sep 24];11(6):5474–88. Available from: <https://pubs.acs.org/doi/full/10.1021/acsnano.7b00221>

78. Roshanbinfar K, Ons Hilborn J, Varghese OP, Oommen OP. Injectable and thermoresponsive pericardial matrix derived conductive scaffold for cardiac tissue engineering †. 2017;
79. Hao T, Li J, Yao F, Dong D, Wang Y, Yang B, et al. Injectable Fullerenol/Alginate Hydrogel for Suppression of Oxidative Stress Damage in Brown Adipose-Derived Stem Cells and Cardiac Repair. *ACS Nano*. 2017 Jun 27;11(6):5474–88.
80. Vranic S, Rodrigues AF, Buggio M, Newman L, White MRH, Spiller DG, et al. Live Imaging of Label-Free Graphene Oxide Reveals Critical Factors Causing Oxidative-Stress-Mediated Cellular Responses. *ACS Nano* [Internet]. 2018 Feb 27 [cited 2024 Sep 17];12(2):1373–89. Available from: <https://pubs.acs.org/doi/full/10.1021/acsnano.7b07734>
81. de Luna LAV, Loret T, Fordham A, Arshad A, Drummond M, Dodd A, et al. Lung recovery from DNA damage induced by graphene oxide is dependent on size, dose and inflammation profile. *Part Fibre Toxicol* [Internet]. 2022 Dec 1 [cited 2024 Sep 17];19(1):1–21. Available from: <https://particleandfibretoxicology.biomedcentral.com/articles/10.1186/s12989-022-00502-w>
82. Malakhova AA, Rybin DK, Shtertser AA, Dudina D V. Nanoscale Detonation Carbon Demonstrates Biosafety in Human Cell Culture. *Micromachines (Basel)* [Internet]. 2022 Aug 1 [cited 2024 Sep 18];13(8). Available from: [/pmc/articles/PMC9414359/](https://pmc/articles/PMC9414359/)
83. Kang MS, Kwon M, Jang HJ, Jeong SJ, Han DW, Kim KS. Biosafety of inorganic nanomaterials for theranostic applications. Vol. 5, *Emergent Materials*. Institute for Ionics; 2022. p. 1995–2029.
84. Kyriakidou K, Brasinika D, Trompeta AFA, Bergamaschi E, Karoussis IK, Charitidis CA. In vitro cytotoxicity assessment of pristine and carboxyl-functionalized MWCNTs. *Food and Chemical Toxicology*. 2020 Jul 1;141:111374.
85. Fraser K, Kodali V, Yanamala N, Birch ME, Cena L, Casuccio G, et al. Physicochemical characterization and genotoxicity of the broad class of carbon nanotubes and nanofibers used or produced in U.S. facilities. *Particle and Fibre Toxicology* 2020 17:1 [Internet]. 2020 Dec 7 [cited 2024 Sep 18];17(1):1–26. Available from: <https://particleandfibretoxicology.biomedcentral.com/articles/10.1186/s12989-020-00392-w>
86. Shankar D, Jambagi SC, Gowda N, Lakshmi KS, Jayanthi KJ, Chaudhary VK. Effect of Surface Chemistry on Hemolysis, Thrombogenicity, and Toxicity of Carbon Nanotube Doped Thermally Sprayed Hydroxyapatite Implants. *ACS Biomater Sci Eng* [Internet]. 2024 Mar 11 [cited 2024 Sep 18];10(3):1403–17. Available from: <https://pubs.acs.org/doi/full/10.1021/acsbiomaterials.3c00912>
87. Li K, Ge P, Wu XL, Shen C. In vitro cytotoxicity assessment of carbonaceous gels for bone marrow mesenchymal stem cells. *Food and Chemical Toxicology*. 2024 Nov 1;193:114961.
88. Salesa B, Assis M, Andrés J, Serrano-Aroca Á. Carbon Nanofibers versus Silver Nanoparticles: Time-Dependent Cytotoxicity, Proliferation, and Gene Expression. *Biomedicines* [Internet]. 2021 Sep 1 [cited 2024 Sep 18];9(9). Available from: [/pmc/articles/PMC8467915/](https://pmc/articles/PMC8467915/)
89. Malhotra N, Audira G, Castillo AL, Siregar P, Ruallo JMS, Roldan MJ, et al. An Update Report on the Biosafety and Potential Toxicity of Fullerene-Based Nanomaterials toward Aquatic Animals. *Oxid Med Cell Longev* [Internet]. 2021 [cited 2024 Sep 18];2021. Available from: [/pmc/articles/PMC8313339/](https://pmc/articles/PMC8313339/)

90. Aschberger K, Johnston HJ, Stone V, Aitken RJ, Tran CL, Hankin SM, et al. Review of fullerene toxicity and exposure – Appraisal of a human health risk assessment, based on open literature. *Regulatory Toxicology and Pharmacology*. 2010 Dec 1;58(3):455–73.
91. Panteri E. Scientific Committee on Consumer Safety SCCS OPINION ON Fullerenes, Hydroxylated Fullerenes and hydrated forms of Hydroxylated Fullerenes (nano) Final Opinion Opinion on Fullerenes, Hydroxylated Fullerenes and hydrated forms of Hydroxylated Fullerenes (nano)
- 
- 

## 2 ACKNOWLEDGMENTS.

92. Humpolíček P, Kašpárková V, Pacherník J, Stejskal J, Bober P, Capáková Z, et al. The biocompatibility of polyaniline and polypyrrole: A comparative study of their cytotoxicity, embryotoxicity and impurity profile. *Mater Sci Eng C Mater Biol Appl* [Internet]. 2018 Oct 1 [cited 2024 Sep 17];91:303–10. Available from: <https://pubmed.ncbi.nlm.nih.gov/30033259/>
93. Li YS, Chen BF, Li XJ, Zhang WK, Tang H Bin. Cytotoxicity of Polyaniline Nanomaterial on Rat Celiac Macrophages In Vitro. *PLoS One* [Internet]. 2014 Sep 24 [cited 2024 Sep 18];9(9). Available from: </pmc/articles/PMC4175078/>
94. Karthikeyan L, Rithisa B, Min S, Hong H, Kang H, Thangam R, et al. Multimodal biomedical utility of polyaniline-based supramolecular nanomaterials. *Chemical Engineering Journal*. 2024 Aug 1;493:152530.
95. Kašpárková V, Humpolíček P, Stejskal J, Capáková Z, Bober P, Skopalová K, et al. Exploring the Critical Factors Limiting Polyaniline Biocompatibility. *Polymers (Basel)* [Internet]. 2019 [cited 2024 Sep 18];11(2). Available from: </pmc/articles/PMC6419196/>
96. De Souza NLGD, Cavallini GS, Alves TT, Pereira MM, Mello Brandão H de, Oliveira LFC de. Compatibility and cytotoxicity of poly( $\epsilon$ -caprolactone)/*polypyrrole-block-poly( $\epsilon$ -caprolactone)* blend films in fibroblast bovine cells. *Polímeros* [Internet]. 2024 Mar 1 [cited 2024 Sep 18];34(1):e20240007. Available from: <https://www.scielo.br/j/po/a/PN4rKsMJZDwn4chpfNWjgxH/>
97. Humpolíček P, Kašpárková V, Pacherník J, Stejskal J, Bober P, Capáková Z, et al. The biocompatibility of polyaniline and polypyrrole: A comparative study of their cytotoxicity, embryotoxicity and impurity profile. *Materials Science and Engineering: C*. 2018 Oct 1;91:303–10.
98. Vaitkuvienė A, Kaseta V, Voronovic J, Ramanauskaite G, Biziuleviciene G, Ramanaviciene A, et al. Evaluation of cytotoxicity of polypyrrole nanoparticles synthesized by oxidative polymerization. *J Hazard Mater* [Internet]. 2013 Apr 5 [cited 2024 Sep 18];250–251:167–74. Available from: <https://pubmed.ncbi.nlm.nih.gov/23454454/>
99. Tumová Š, Malečková R, Kubáč L, Akrman J, Enev V, Kalina L, et al. Novel highly stable conductive polymer composite PEDOT:DBSA for bioelectronic applications. *Polymer Journal* 2023 55:9 [Internet]. 2023 May 15 [cited 2024 Sep 18];55(9):983–95. Available from: <https://www.nature.com/articles/s41428-023-00784-7>



100. Miriani RM, Abidian MR, Kipke DR. Cytotoxic analysis of the conducting polymer PEDOT using myocytes. *Annu Int Conf IEEE Eng Med Biol Soc* [Internet]. 2008 [cited 2024 Sep 18];2008:1841–4. Available from: <https://pubmed.ncbi.nlm.nih.gov/19163041/>
101. Zhang S, Chen D, Gu Z, Luo H, Chen X, Fu Q. Ultrasound-triggered piezocatalytic conductive Guar gum/PEDOT: PSS/BTO composite hydrogels for bacterial-infected skin wound healing. *Nano TransMed*. 2024 Dec 1;3:100035.
102. Shokrollahi P, Omid Y, Cubeddu LX, Omidian H. Conductive polymers for cardiac tissue engineering and regeneration. *J Biomed Mater Res B Appl Biomater* [Internet]. 2023 Nov 1 [cited 2024 Apr 23];111(11):1979–95. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/jbm.b.35293>
103. Sahu D, Kannan GM, Tailang M, Vijayaraghavan R. In Vitro Cytotoxicity of Nanoparticles: A Comparison between Particle Size and Cell Type. *Journal of Nanoscience* [Internet]. 2016 Jan 1 [cited 2024 Sep 17];2016(1):4023852. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1155/2016/4023852>
104. Humpolíček P, Radaszkiewicz KA, Capáková Z, Pacherník J, Bober P, Kašpárková V, et al. Polyaniline cryogels: Biocompatibility of novel conducting macroporous material. *Scientific Reports* 2017 8:1 [Internet]. 2018 Jan 9 [cited 2024 Sep 17];8(1):1–12. Available from: <https://www.nature.com/articles/s41598-017-18290-1>
105. Hou R, Xie Y, Song R, Bao J, Shi Z, Xiong C, et al. Nanocellulose/polypyrrole hydrogel scaffolds with mechanical strength and electrical activity matching native cardiac tissue for myocardial tissue engineering. *Cellulose*. 2024;1–16.
106. Haq AU, Carotenuto F, De Matteis F, Proposito P, Francini R, Teodori L, et al. Intrinsically Conductive Polymers for Striated Cardiac Muscle Repair. *International Journal of Molecular Sciences* 2021, Vol 22, Page 8550 [Internet]. 2021 Aug 9 [cited 2024 Sep 17];22(16):8550. Available from: <https://www.mdpi.com/1422-0067/22/16/8550/htm>
107. Dong R, Zhao X, Guo B, Ma PX. Self-Healing Conductive Injectable Hydrogels with Antibacterial Activity as Cell Delivery Carrier for Cardiac Cell Therapy. 2016 [cited 2024 Apr 22]; Available from: [www.acsami.org](http://www.acsami.org)
108. Nguyen AH, Marsh P, Schmiess-Heine L, Burke PJ, Lee A, Lee J, et al. Cardiac tissue engineering: state-of-the-art methods and outlook. *Journal of Biological Engineering* 2019 13:1 [Internet]. 2019 Jun 28 [cited 2024 Sep 17];13(1):1–21. Available from: <https://link.springer.com/articles/10.1186/s13036-019-0185-0>
109. Ahadian S, Davenport Huyer L, Estili M, Yee B, Smith N, Xu Z, et al. Moldable elastomeric polyester-carbon nanotube scaffolds for cardiac tissue engineering. *Acta Biomater* [Internet]. 2017;52:81–91. Available from: <https://www.sciencedirect.com/science/article/pii/S174270611630678X>
110. Dixit M, Singh N, Das P, Datta P. Bioprinting in Pharmaceuticals. In: *Additive Manufacturing in Pharmaceuticals*. Springer; 2023. p. 293–325.
111. Kang HW, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nature Biotechnology* 2016 34:3 [Internet]. 2016 Feb 15 [cited 2023 Apr 12];34(3):312–9. Available from: <https://www.nature.com/articles/nbt.3413>

112. Noor N, Shapira A, Edri R, Gal I, Wertheim L, Dvir T. 3D Printing of Personalized Thick and Perfusable Cardiac Patches and Hearts. *Advanced Science* [Internet]. 2019 Jun 1 [cited 2023 Apr 12];6(11):1900344. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/advs.201900344>
113. Xu T, Zhao W, Zhu JM, Albanna MZ, Yoo JJ, Atala A. Complex heterogeneous tissue constructs containing multiple cell types prepared by inkjet printing technology. *Biomaterials*. 2013 Jan 1;34(1):130–9.
114. Ihalainen P, Määttä A, Sandler N. Printing technologies for biomolecule and cell-based applications. *Int J Pharm*. 2015 Oct 30;494(2):585–92.
115. Derby B. Bioprinting: inkjet printing proteins and hybrid cell-containing materials and structures. *J Mater Chem* [Internet]. 2008 Nov 25 [cited 2023 Apr 13];18(47):5717–21. Available from: <https://pubs.rsc.org/en/content/articlehtml/2008/jm/b807560c>
116. Xu C, Zhang M, Huang Y, Ogale A, Fu J, Markwald RR. Study of droplet formation process during drop-on-demand inkjetting of living cell-laden bioink. *Langmuir* [Internet]. 2014 Aug 5 [cited 2023 Apr 13];30(30):9130–8. Available from: <https://pubs.acs.org/doi/full/10.1021/la501430x>
117. Cui X, Boland T, D'D'Lima D, K. Lotz M. Thermal Inkjet Printing in Tissue Engineering and Regenerative Medicine. *Recent Pat Drug Deliv Formul*. 2012 May 31;6(2):149–55.
118. Saunders RE, Derby B. Inkjet printing biomaterials for tissue engineering: bioprinting. <http://dx.doi.org/10.1179/1743280414Y00000000040> [Internet]. 2014 Nov 1 [cited 2023 Apr 13];59(8):430–48. Available from: <https://www.tandfonline.com/doi/abs/10.1179/1743280414Y.00000000040>
119. Jeong HJ, Nam H, Jang J, Lee SJ. 3D Bioprinting Strategies for the Regeneration of Functional Tubular Tissues and Organs. *Bioengineering (Basel)* [Internet]. 2020 Jun 1 [cited 2023 May 4];7(2). Available from: <https://pubmed.ncbi.nlm.nih.gov/32244491/>
120. Cui X, Dean D, Ruggeri ZM, Boland T. Cell damage evaluation of thermal inkjet printed Chinese hamster ovary cells. *Biotechnol Bioeng* [Internet]. 2010 Aug 15 [cited 2023 Apr 13];106(6):963–9. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/bit.22762>
121. Murphy S V., Atala A. 3D bioprinting of tissues and organs. *Nature Biotechnology* 2014 32:8 [Internet]. 2014 Aug 5 [cited 2023 Apr 13];32(8):773–85. Available from: <https://www.nature.com/articles/nbt.2958>
122. Jana S, Lerman A. Bioprinting a cardiac valve. *Biotechnol Adv*. 2015 Dec 1;33(8):1503–21.
123. Dababneh AB, Ozbolat IT. Bioprinting Technology: A Current State-of-the-Art Review. *Journal of Manufacturing Science and Engineering, Transactions of the ASME* [Internet]. 2014 Dec 1 [cited 2023 Apr 12];136(6). Available from: <https://asmedigitalcollection.asme.org/manufacturingscience/article/136/6/061016/377608/Bioprinting-Technology-A-Current-State-of-the-Art>
124. Nakamura M, Kobayashi A, Takagi F, Watanabe A, Hiruma Y, Ohuchi K, et al. Biocompatible Inkjet Printing Technique for Designed Seeding of Individual Living Cells. <https://home.liebertpub.com/ten> [Internet]. 2006 Jan 13 [cited 2023 Apr 13];11(11–12):1658–66. Available from: <https://www.liebertpub.com/doi/10.1089/ten.2005.11.1658>

125. Saunders RE, Gough JE, Derby B. Delivery of human fibroblast cells by piezoelectric drop-on-demand inkjet printing. *Biomaterials*. 2008 Jan 1;29(2):193–203.
126. Seetharam R, Sharma SK. Purification and analysis of recombinant proteins. 1991;324.
127. Chia HN, Wu BM. Recent advances in 3D printing of biomaterials. *J Biol Eng [Internet]*. 2015 Mar 1 [cited 2023 Apr 13];9(1):1–14. Available from: <https://jbioleng.biomedcentral.com/articles/10.1186/s13036-015-0001-4>
128. Peng W, Datta P, Ayan B, Ozbolat V, Sosnoski D, Ozbolat IT. 3D bioprinting for drug discovery and development in pharmaceuticals. *Acta Biomater*. 2017 Jul 15;57:26–46.
129. Landers R, Hübner U, Schmelzeisen R, Mülhaupt R. Rapid prototyping of scaffolds derived from thermoreversible hydrogels and tailored for applications in tissue engineering. *Biomaterials*. 2002 Dec 1;23(23):4437–47.
130. Ozbolat IT, Hospodiuk M. Current advances and future perspectives in extrusion-based bioprinting. *Biomaterials*. 2016 Jan 1;76:321–43.
131. Colosi C, Shin SR, Manoharan V, Massa S, Costantini M, Barbetta A, et al. Microfluidic Bioprinting of Heterogeneous 3D Tissue Constructs Using Low-Viscosity Bioink. *Advanced Materials [Internet]*. 2016 Jan 1 [cited 2023 Apr 13];28(4):677–84. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/adma.201503310>
132. Trachtenberg JE, Placone JK, Smith BT, Piard CM, Santoro M, Scott DW, et al. Extrusion-Based 3D Printing of Poly(propylene fumarate) in a Full-Factorial Design. *ACS Biomater Sci Eng [Internet]*. 2016 Oct 10 [cited 2023 Apr 13];2(10):1771–80. Available from: <https://pubs.acs.org/doi/full/10.1021/acsbiomaterials.6b00026>
133. Faulkner-Jones A, Fyfe C, Cornelissen DJ, Gardner J, King J, Courtney A, et al. Bioprinting of human pluripotent stem cells and their directed differentiation into hepatocyte-like cells for the generation of mini-livers in 3D. *Biofabrication [Internet]*. 2015 Oct 21 [cited 2023 Apr 13];7(4). Available from: <https://pubmed.ncbi.nlm.nih.gov/26486521/>
134. Kumar A, Mandal S, Barui S, Vasireddi R, Gbureck U, Gelinsky M, et al. Low temperature additive manufacturing of three dimensional scaffolds for bone-tissue engineering applications: Processing related challenges and property assessment. *Materials Science and Engineering: R: Reports*. 2016 May 1;103:1–39.
135. Buyukhatipoglu K, Chang R, Sun W, Clyne AM. Bioprinted Nanoparticles for Tissue Engineering Applications. <https://home.liebertpub.com/tec> [Internet]. 2009 Nov 3 [cited 2023 Apr 13];16(4):631–42. Available from: <https://www.liebertpub.com/doi/10.1089/ten.tec.2009.0280>
136. Chien KB, Makridakis E, Shah RN. Three-Dimensional Printing of Soy Protein Scaffolds for Tissue Regeneration. <https://home.liebertpub.com/tec> [Internet]. 2012 Dec 4 [cited 2023 Apr 13];19(6):417–26. Available from: <https://www.liebertpub.com/doi/10.1089/ten.tec.2012.0383>
137. Bohandy J, Kim BF, Adrian FJ. Metal deposition from a supported metal film using an excimer laser. *J Appl Phys [Internet]*. 1998 Jun 4 [cited 2023 Apr 13];60(4):1538. Available from: <https://aip.scitation.org/doi/abs/10.1063/1.337287>

138. Gudapati H, Yan J, Huang Y, Chrisey DB. Alginate gelation-induced cell death during laser-assisted cell printing. *Biofabrication* [Internet]. 2014 [cited 2023 Apr 13];6(3). Available from: <https://pubmed.ncbi.nlm.nih.gov/25121715/>
139. Guillemot F, Guillotin B, Fontaine A, Ali M, Catros S, Kériquel V, et al. Laser-assisted bioprinting to deal with tissue complexity in regenerative medicine. *MRS Bull* [Internet]. 2011 Dec 1 [cited 2023 Apr 13];36(12):1015–9. Available from: <https://link.springer.com/article/10.1557/mrs.2011.272>
140. Zhu W, Ma X, Gou M, Mei D, Zhang K, Chen S. 3D printing of functional biomaterials for tissue engineering. *Curr Opin Biotechnol*. 2016 Aug 1;40:103–12.
141. Guillemot F, Souquet A, Catros S, Guillotin B. Laser-assisted cell printing: principle, physical parameters versus cell fate and perspectives in tissue engineering. <https://doi.org/10.2217/nnm1014> [Internet]. 2010 Apr 16 [cited 2023 Apr 13];5(3):507–15. Available from: <https://www.futuremedicine.com/doi/10.2217/nnm.10.14>
142. Serra P, Duocastella M, Fernández-Pradas JM, Morenza JL. Liquids microprinting through laser-induced forward transfer. *Appl Surf Sci*. 2009 Mar 1;255(10):5342–5.
143. Patrascioiu A, Fernández-Pradas JM, Palla-Papavlu A, Morenza JL, Serra P. Laser-generated liquid microjets: Correlation between bubble dynamics and liquid ejection. *Microfluid Nanofluidics* [Internet]. 2014 Jun 19 [cited 2023 Apr 13];16(1–2):55–63. Available from: <https://link.springer.com/article/10.1007/s10404-013-1218-5>
144. Ali M, Pages E, Ducom A, Fontaine A, Guillemot F. Controlling laser-induced jet formation for bioprinting mesenchymal stem cells with high viability and high resolution. *Biofabrication* [Internet]. 2014 Sep 12 [cited 2023 Apr 13];6(4):045001. Available from: <https://iopscience.iop.org/article/10.1088/1758-5082/6/4/045001>
145. Catros S, Fricain JC, Guillotin B, Pippenger B, Bareille R, Remy M, et al. Laser-assisted bioprinting for creating on-demand patterns of human osteoprogenitor cells and nano-hydroxyapatite. *Biofabrication* [Internet]. 2011 Apr 28 [cited 2023 Apr 13];3(2):025001. Available from: <https://iopscience.iop.org/article/10.1088/1758-5082/3/2/025001>
146. Trombetta R, Inzana JA, Schwarz EM, Kates SL, Awad HA. 3D Printing of Calcium Phosphate Ceramics for Bone Tissue Engineering and Drug Delivery. *Ann Biomed Eng* [Internet]. 2017 Jan 1 [cited 2023 Apr 13];45(1):23–44. Available from: <https://link.springer.com/article/10.1007/s10439-016-1678-3>
147. Guillemot F, Souquet A, Catros S, Guillotin B, Lopez J, Faucon M, et al. High-throughput laser printing of cells and biomaterials for tissue engineering. *Acta Biomater*. 2010 Jul 1;6(7):2494–500.
148. Barron JA, Wu P, Ladouceur HD, Ringeisen BR. Biological laser printing: A novel technique for creating heterogeneous 3-dimensional cell patterns. *Biomed Microdevices* [Internet]. 2004 Jun [cited 2023 Apr 13];6(2):139–47. Available from: <https://link.springer.com/article/10.1023/B:BMMD.0000031751.67267.9f>
149. Ringeisen BR, Kim H, Barron JA, Krizman DB, Chrisey DB, Jackman S, et al. Laser Printing of Pluripotent Embryonal Carcinoma Cells. <https://home.liebertpub.com/ten> [Internet]. 2004 Jul 9 [cited 2023 Apr 13];10(3–4):483–91. Available from: <https://www.liebertpub.com/doi/10.1089/107632704323061843>

150. Tsui JH, Leonard A, Camp ND, Long JT, Nawas ZY, Chavanachat R, et al. Tunable electroconductive decellularized extracellular matrix hydrogels for engineering human cardiac microphysiological systems. *Biomaterials* [Internet]. 2021 [cited 2024 Apr 22];272. Available from: <https://doi.org/10.1016/j.biomaterials.2021.120764>
151. Kieda J, Shakeri A, Landau S, Wang EY, Zhao Y, Lai BF, et al. Advances in cardiac tissue engineering and heart-on-a-chip. Vol. 112, *Journal of Biomedical Materials Research - Part A*. John Wiley and Sons Inc; 2024. p. 492–511.
152. Ul Haq A, Montaina L, Pescosolido F, Carotenuto F, Trovalusci F, De Matteis F, et al. Electrically conductive scaffolds mimicking the hierarchical structure of cardiac myofibers. *Sci Rep*. 2023 Dec 1;13(1).
153. Izadifar M, Chapman D, Babyn P, Chen X, Kelly ME. UV-Assisted 3D Bioprinting of Nanoreinforced Hybrid Cardiac Patch for Myocardial Tissue Engineering. *Tissue Eng Part C Methods* [Internet]. 2017 Oct 19;24(2):74–88. Available from: <https://doi.org/10.1089/ten.tec.2017.0346>
154. Shabankhah M, Moghaddaszadeh A, Najmoddin N. 3D printed conductive PCL/GO scaffold immobilized with gelatin/CuO accelerates H9C2 cells attachment and proliferation. *Prog Org Coat* [Internet]. 2024 [cited 2024 Apr 23];186:300–9440. Available from: <https://doi.org/10.1016/j.porgcoat.2023.108013>
155. Hou R, Xie Y, Song R, Bao J, Shi Z, Xiong C, et al. Nanocellulose/polypyrrole hydrogel scaffolds with mechanical strength and electrical activity matching native cardiac tissue for myocardial tissue engineering. *Cellulose*. 2024;1–16.
156. Mei T, Cao H, Zhang L, Cao Y, Ma T, Sun Z, et al. 3D Printed Conductive Hydrogel Patch Incorporated with MSC@ GO for Efficient Myocardial Infarction Repair. *ACS Biomater Sci Eng*. 2024;