Electroconductive Hydrogels for Tissue Engineering: Current Status and Future Perspectives

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Abstract

Over the past decade, electroconductive hydrogels, integrating both the biomimetic attributes of hydrogels and the electrochemical properties of conductive materials, have gained significant attention. Hydrogels, three-dimensional and swollen hydrophilic polymer networks, are an important class of tissue engineering scaffolds owing to their microstructural and mechanical properties, ability to mimic the native extracellular matrix, and promote tissue repair. However, hydrogels are intrinsically insulating and therefore unable to emulate the complex electrophysiological microenvironment of cardiac and neural tissues. To overcome this challenge, electroconductive materials, including carbon-based materials, nanoparticles and polymers, have been incorporated within non-conductive hydrogels to replicate the electrical and biological characteristics of biological tissues. This review gives a brief introduction on the rational design of electroconductive hydrogels and their current applications in tissue engineering, especially for neural and cardiac regeneration. The recent progress and development trends of electroconductive hydrogels, their challenges and clinical translatability, as well as their future perspectives, with a focus on advanced manufacturing technologies, are also discussed.

Keywords: hydrogels, scaffolds, conductive materials, tissue engineering, cardiac, neural.

Introduction

Tissue engineering (TE) is the application of materials science, engineering, chemistry, and biology to replace, repair or maintain biological tissues through the use of cells, scaffolds, and/or biochemical factors.[1] Ideal TE scaffolds mimic the biological, chemical, and physical properties of the native extracellular matrix (ECM), which influences cell adhesion, differentiation, migration, and proliferation.[2-4] Since many tissues in the body, including cardiac, cartilage, muscle, neural, and skin[5-12], require electrical synaptic interactions between cells to function, TE scaffolds for these specific tissues must also support bioelectrical signaling.[2] For instance, constructs for cardiac and neural TE should mediate electrochemical communications between cardiomyocytes (CMs) for a synchronous beating of the heart or neurons for a functioning nervous system.[13-15]

Hydrogels, three-dimensional (3D) cross-linked networks consisting of hydrophilic natural and/or synthetic polymers, are excellent TE scaffolds since they inherently mimic aspects of the native ECM. Furthermore, their biological, mass transport, mechanical, and topological properties can be fine-tuned to fit a specific TE application.[10, 16-30] For instance, hydrogels can be synthesized from ECM components (e.g., collagen, fibronectin, hyaluronic acid), providing cells with binding domains that dictate their behavior.[3, 31] Their architectures and porous structures can be adjusted to promote nutrient, waste and solute diffusion and cell organization, attachment, and migration.[2] Additionally, their mechanical properties (e.g., elasticity, compressibility, viscoelastic behavior) can regulate cell fate and function via mechanotransduction signaling.[2, 32] Lastly, hydrogel topology, controlled by the incorporation of nanomaterials or microfabrication techniques, can influence cell adhesion and orientation.[33] Although hydrogels are highly tunable, they typically have inadequate mechanical strength and are dielectric, limiting their widespread biomedical applications.[2, 33, 34]

Electroconductive hydrogels, biomaterials blended and/or hybridized with conductive materials, have recently been engineered to reinforce their mechanical and electroconductive properties while exhibiting some characteristics of biological tissues.[31, 34, 35] Several types of electroconductive dopants have been used, including carbon-based materials (nanotubes, graphene), metallic nanoparticles (gold, silver) and polymers (polyaniline (PANI), polypyrrole (PPy), polythiophene, and their derivatives).[34-36]

However, most of these conductive materials are usually brittle, non-biodegradable, cytotoxic, and need be integrated into hydrogels.[34] Electroconductive hydrogels are typically synthesized via the co-polymerization of various polymers, including electroconductive polymers, or the blending of conductive particles within a non-conductive hydrophilic polymer network.[37]

In this review, we first discuss the latest progress on the rational design in electroconductive hydrogels for cardiac and neural TE (Table 1). We emphasize how electroconductive hydrogels have the potential to treat diseases of the cardiovascular and nervous system, encompassing a number of major causes of deaths globally. In the subsequent sections, we critically evaluate the challenges that need to be addressed for this class of biomaterials to be clinically relevant. Lastly, we highlight a number of state-of-the-art manufacturing technologies applicable for the fabrication of advanced electroconductive hydrogels to further broaden their applications and expedite their bench-to-bedside translation.

Cardiac Tissue Engineering

Cardiovascular disease (CVD) is the number one cause of death globally, responsible for 17.9 million deaths in 2017 and costing over $32 billion per year in U.S. healthcare costs.[38, 39] The most common type of CVD, coronary artery disease, typically leads to a myocardial infarction (MI), in which blood flow to the heart is restricted. MI-induced ischemia leads to the permanent loss of nearly a billion of CMs, cells known to have limited regenerative capacities, in humans.[40] Furthermore, increased secretion of matrix metalloproteinases and inflammation result in non-functional scar tissue deposition, leading to decreased cardiac output, and often heart failure.[41] In fact, MI prognosis is poor with a 50% mortality rate within 5 years of diagnosis.[42] A heart transplantation is often required, a complex treatment that suffers from several post-surgery complications and a shortage of donor organs.[43, 44] A combination of global health policies and lack of therapies for MI have driven several treatments into the clinic, including cell therapy[45, 46] and tissue-engineered cardiac patches.[47, 48] These strategies aim to mitigate fibrosis and stiffening of the heart, restore contractile function, and enhance muscle regeneration.

However, the results have been modest at best due to several challenges such as (*i*) poor cell survival, retention, and function, (*ii*) invasive surgery, and (*iii*) inadequate electromechanical integration of transplanted stem cells.[49-53]

Electroconductive hydrogels have the potential to address a number of these limitations and ultimately succeed in the clinic. The incorporation of electroconductive materials within hydrogels, including conductive polymers[54-59], nanomaterials (e.g., carbon nanotubes, metallic nanoparticles)[60-66], graphene oxide (GO)[67-69], and bionic liquids[70], can enhance their electroconductivity and ability to restore the contractile function of the heart. For example, Shin and colleagues incorporated reduced graphene oxide sheets (rGO) into gelatin methacryloyl (GelMA) hydrogels.[69] rGO lowered the impedance values, and at a concentration of 1 mg/mL, rGO-containing hybrid hydrogels had a much lower impedance (~4 kΩ) than hydrogels made with non-reduced GO (~120 kΩ). Based on sarcomeric α-actinin (SAC) and connexin 43 (Cx43) immunofluorescent stainings in primary rat CMs, rGO-containing hydrogels exhibited more uniaxially aligned sarcomeric structures and enhanced cell-cell coupling. Additionally, GelMA hydrogels hybridized with 5 mg/mL of rGO induced a much higher beating rate of CMs than rGO-free GelMA hydrogels (~80 bpm *vs* ~10 bpm at day 6, respectively).[69] The incorporation of electroconductive nanomaterials into hydrogels not only influences the bulk electrical properties, but also the topography, which has been shown to influence cell retention and biology.[71] This was explored by Navaei and colleagues, who incorporated non-conductive silica nanomaterials and electroconductive gold nanorods into GelMA hydrogels.[64] Interestingly, unlike non-hybridized GelMA, both hybridized gel types had similar CM retention, expression of SAC, troponin I, and Cx43. This work indicated that non-conductive nanomaterials influenced gel topography and subsequently enhanced CM maturation and function.[64]

Arrythmias, irregular or abnormal heartbeats, resulting from cell therapies and other cardiac TE strategies are also a major concern.[72, 73] Several studies, both in vitro and in vivo, have demonstrated that electroconductive hydrogels support synchronous beating of non-contacting CMs.[57, 60, 66] For instance, Roshanbinfar and colleagues engineered electrically conductive hydrogels (eCA-gels) made with collagen, alginate and poly(3, 4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS).[57] The presence of PEDOT:PSS supported well-organized sand rose-like structures (Figure 1A). eCA-gels promoted rhythmic beating of neonatal rat CMs, whereas PEDOT:PSS-free gels (CA-gels) did not (Figure 1B). Interestingly, eCA-gels induced beating frequencies of approximately 220 per min after 11 days of culture, equivalent to the resting heartbeat of newborn rats. Immunofluorescent staining of CMs on both gel types revealed that eCA-gels promoted cellular alignment, elongation, and a unidirectional orientation (Figure 1C). Lastly, similar results were obtained with human induced pluripotent stem cell (hiPSC)-derived CMs, a more clinically relevant cell line.[57]

Injectable biomaterials, including injectable electroconductive hydrogels[54, 55, 58], obviate the need for open surgery and mitigate healthcare-associated infections and postsurgical complications.[24, 74] With respect to cardiac repair, injectable hydrogels may be deployed into the native myocardium, delivering cells with high viability, and improved local retention. For example, Dong and colleagues demonstrated that injecting C2C12 myoblasts encapsulated within a composite chitosan and polyethylene glycol (PEG)-based hydrogel did not alter cell viability.[55] Going even further, Liang and colleagues employed a two-step Michael addition reaction to synthesize composite hydrogels containing gelatin, polyethylene glycol diacrylate (PEGDA), and PPy (HPAE-Py/Gelatin). Their flowable biomaterial was directly painted onto the infarcted myocardium, circumventing potential damage from the injection or patch suturing (Figure 2).[59] The gelation time was approximately 8 seconds, such that they could be applied to the myocardium without leaking. When tested in an MI rat model, HPAE-Py/Gelatin or HPAE/Gelatin hydrogels were applied to the infarcted area using a brush attached to a syringe. After 4 weeks, both hydrogels were able to restore wave propagation, leading to a coordinated contraction and normal heartbeat. However, unlike non-conductive HPAE/Gelatin hydrogels, hearts treated with HPAE-Py/Gelatin hydrogels exhibited a lower infarct size, lower degree of fibrosis, higher left ventricle wall thickness, and avoided adverse cardiomegaly (e.g., enlarged heart).[59] In another example, Wang and colleagues injected hyaluronic acid (HA)/PEG/tetraniline-based hydrogels loaded with adipose-derived stem cells into the infarcted myocardium of rats.[54] This therapy restored cardiac output and decreased fibrosis in the infarcted area. Equally important, this study addressed another major challenge facing clinical implementation, the lack of vascularization of transplanted cells or tissues.[51, 53] The hydrogels were loaded with endothelial nitric oxide synthase (eNOs) encoding plasmid DNA to promote angiogenesis. When tested in mice, the gene therapy successfully increased expression of eNOs and four myocardium-related genes: vascular endothelial growth factor A (VEGF-A), Angiopoietin 1 (Ang-1), Cx43, and Cadherin 2 (Cdh-2).[55]

To be clinically relevant, cardiac patches must withstand the dynamic stress environment on the surface of the heart during a cardiac cycle.[75] The wall of a healthy heart can stiffen up to tenfold between diastole and systole due to the active mechanical properties of CMs. To this end, self-healing hydrogels have been engineered to recapitulate the ability of native tissues to regenerate through the formation of new chemical bonds.[76] Jing and colleagues synthesized chitosan-based hydrogels hybridized with GO and reported their self-healing properties.[68] When cut into two pieces, the hydrogel rapidly repaired itself and recovered its original pre-cut mechanical properties. The self-healing properties were attributed to covalent bonds, supramolecular interactions, hydrogen bonds and π-π stacking between chitosan and GO.[68] Dong and colleagues described similar properties with chitosan-grafted-aniline and PEG composite hydrogels.[55] Other studies have indicated that the incorporation of additional electroconductive materials into hydrogels can influence their elastic properties. For instance, Yang and colleagues engineered double network hydrogels based on gelatin and poly(thiophene-3-acetic acid).[56] When tested for their mechanical properties, the Young's moduli values ranged from approximately 20 to 500 kPa depending on the concentration of gelatin.[56] In another example, Noshadi and colleagues synthesized GelMA or PEG diacrylate hydrogels, both covalently conjugated with conductive choline-based Bio-Ionic Liquid (Bio-IL). Values for their Young's moduli ranged from approximately 5-101 kPa and 3-173 kPa, respectively.[70] The mechanical properties were dependent on the polymer concentration and ratio of polymer to Bio-IL.[70]

Neural Tissue Engineering

Electroconductive hydrogels can also be applied towards repairing or regenerating neural tissues, supporting endogenous cell signaling or delivery of exogenous electrical stimulation.[77] Neural injury and associated diseases affect almost 1 billion people around the world.[78] Unfortunately, current treatments for the central nervous system (CNS) and peripheral nervous system (PNS) are lacking.[79] CNS injuries include traumatic brain and spinal cord injury, stroke, tumors, and neurodegenerative diseases such as Huntington's, prions, and Alzheimer's.[80] Unlike the CNS, which has very limited regenerative capacity, the PNS is capable of self-regeneration although it has been associated with poor clinical outcomes.[79] Common PNS injuries include traumas such as falls, motor vehicle accidents, violence, or occupational hazards.[80] More rarely, PNS loss of function are associated with inflammatory or degenerative diseases such as Guillain-Barré, causing permanent damage to peripheral nerves.[81]

TE scaffolds have been proposed as a potential off-the-shelf method to facilitate neuroregeneration. For instance, soft hydrogels have been explored to promote nerve growth in vivo.[80] When hybridized with conductive polymers, hydrogels have the potential to drive stem cell differentiation into neurons and encourage nerve repair. Under electrical stimulation, electroconductive hydrogels (e.g., PPy-based hydrogels) have shown to differentiate human neural stem cells (hNSCs) into neurons with longer neurites.[82-85] Specifically, this approach increased gene expression of class III β-tubulin (Tuj1), a gene responsible for neuronal phenotype, over glial fibrillary acidic protein (GFAP), a gene associated with astrocyte phenotype. However, the differentiation phenomenon may have more complex dependencies. For example, another study showed that neurite outgrowth was dependent on the strength of the electric field applied, suggesting that neural differentiation is dependent upon the electroconductivity and external electrical stimulation.[84] Furthermore, the electrical stimulation was able to increase non-specific NSC differentiation towards neuronal and glial phenotypes.[83] However, the differentiation may not depend solely on one or the other. Recent studies have indicated that the influence of collagen-PPy hydrogels on electrically stimulated PC12 cells, a classical neuronal cell model, could selectively differentiate these cells into a specific phenotype. Differentiated PC12 cells exhibited high expressions of tubulin-β3 and L-VGCC, two proteins indicative of neurogenesis (Figure 3).[86] Additionally, the electrical stimulation can also be harnessed to control the delivery of relevant drugs without altering neuron cell differentiation. Zarrintaj and colleagues cultured PC12 cells on a gelatin-aniline hydrogel which released embedded dexamethasone (an anti-inflammatory/immune suppressor) upon application of electrical current.[87] Bagheri and colleagues built on this work using a chitosan-aniline gel which also demonstrated superior biocompatibility and tunable drug release properties under stimulation.[88] While these electroconductive hydrogels can reinforce neuronal cell differentiation and function in vitro, they present still a number of limitations when used in animal.

To promote nerve regeneration, given the challenge and time-consuming nature of administering electrical stimulation in vivo, stimulation-free conductive hydrogels are arguably more promising for clinical applications.[89] Unfortunately, only a few stimulation-free hydrogels exhibiting such properties have been reported.[90] Homaeigohar and colleagues showed that hydrogels functionalized with multi-wall carbon nanotubes (MWCNTs) promoted the total number of neurite-bearing cells.[91] Lee and colleagues indicated that 3D-printed scaffolds with similar MWCNTs enhanced neurite length.[85] Similarly, chitosan based-gels layered with PEDOT have also displayed a promising regenerative capacity.[92] Recently, Liu and colleagues reported that formulating electroconductive hydrogels with poly(2-(methacryloyloxy)ethyl)trimethylammonium chloride, a hydrogen bond donor, significantly increased the population of neurite-bearing cells.[93] These hydrogels can also upregulate secretion of neural growth factors, by virtue of their intrinsic conductive properties.[94]

Hydrogels, once blended with conductive polymers, tend to exhibit a higher mechanical strength. For instance, Xu and colleagues layered PEDOT on carboxymethyl chitosan to engineer a conductive scaffold with a Young's modulus reaching up to ~12.5 kPa. This value is close to the human brain tissues (~1.2 kPa)[95] and the hydrogels displayed higher cell adhesion when compared to their non-conductive counterparts.[92] Additionally, Yang and colleagues described that adding only 10 mM PPy to the alginate formulation resulted in a dramatic increase in gel stiffness, with a Young's modulus surging from 21 kPa to 178 kPa.[96] Interestingly, in another study, Homaeigohar and colleagues suggested that a scaffold with a higher Young's modulus than that of native neural tissues could enhance neurite extension. Their alginate-based hydrogels were reinforced when blended with graphite nanofilaments, resulting in a high Young's modulus (~56 MPa) and improved neurite growth.[91]

Advanced Manufacturing Technologies

State-of-the-art 3D printing and sophisticated bioreactors represent advanced manufacturing technologies that can accelerate the clinical translatability of electroconductive hydrogels. Three-dimensional printing provides unprecedented control over scaffold design, including geometry, anisotropy, pore size, and topography.[97, 98] In this context, various techniques have been explored to print electroconductive hydrogels in 3D, such as extrusion-based printing, light-based printing, and ink-jetting (Figure 4A).[37, 97, 98] Although, some of these technologies were employed for the fabrication of electroconductive hydrogels, such as those made with CNTs[63, 99-101] and conductive polymers[102-107], only a handful have been applied towards cardiac[63] and neural[99, 103] TE. For instance, using stereolithography, Lee and colleagues encapsulated MWCNTs within 3D printed PEGDA hydrogels, while finely controlling their porous microarchitecture.[99] They reported the beneficial effect of fine-tuning pore size on hNSC growth and length.[99]

Taking the technology one step forward, 3D printing has been leveraged to print biological materials (e.g., cells, bioinks). This technique, known as bioprinting, has been used to print cell-laden hydrogels while precisely controlling the spatial arrangement of growth factors and cells within the matrix.[97, 98] These attributes could address some of the challenges that electroconductive hydrogels are facing, including the homogenous distribution of cells within the gel constructs, the integration of various cell types in an anisotropic manner to better mimic native signal propagation, and the induction of neovascularization in vivo. For instance, blood vessel formation has been facilitated by fine-tuning pore size and by incorporating pro-angiogenic growth factors.[98, 108-110] Using a sacrificial ink strategy, Kolesky and colleagues vascularized a 3D perfusion chip using a temperature-sensitive tri-block copolymer-based bioink that was removed by cooling, leaving behind a macroporous network (Figure 4B).[108] The construct was subsequently cellularized with human umbilical vein endothelial cells (HUVECs), a step required for differentiating human mesenchymal stem cells.[108] A similar strategy could be used to create a vascularization network within electroconductive hydrogels. Although, to date, there is no report on bioprinted cell-laden electroconductive hydrogels, this technique has a tremendous potential to further advance the field.[37]

Bioreactors, another key manufacturing technology, represent a scalable, reproducible, automated, and sterile process to support the fabrication of new tissues in vitro (Figure 5A-B). This technique allows a dynamic culture condition while mimicking the native physiological environment of cells by controlling oxygen tension, carbon dioxide concentration, pH, and nutrient levels.[111, 112] Bioreactors have also been designed to electrically stimulate cell-laden scaffolds to drive functional maturation of cells to the desired phenotype[113-115]. For instance, Visone and colleagues designed an oscillating perfusion bioreactor (OPB) that simultaneously provided bidirectional perfusion of nutrients, electrical stimulation, and real-time monitoring of cell-laden constructs (Figure 5C).[115] When neonatal rat CMs were cultured in Matrigel-integrated OPB, cells exhibited high cell viability and differentiation as indicated by coordinated contraction, troponin I staining and a lowered excitation threshold.[115] Although, bioreactors are well suited to endure electrical stimulations, they have not been explored with electroconductive hydrogels yet. Increasing evidence suggests that leveraging electroconductive hydrogels with bioreactors may have a synergistic effect. In fact, combining both 3D bioprinting and bioreactor-assisted cell-laden scaffolds may hold the key to their translational therapeutic applications in the regeneration of cardiac and neural tissues.

Clinical Outlook and Future Perspectives

Over the past decade, tremendous progress has been achieved in the fields of cardiac and neural TE. One major milestone has been the utilization of electroconductive materials to recapitulate the conductive nature of myocardium and nervous tissues. Going one step further, the incorporation of conductive polymers and fillers into physiologically inspired hydrogels have addressed a number of their current limitations, particularly in alleviating biocompatibility concerns. For cardiac TE, electroconductivity promotes cell-cell coupling (Cx43)[57, 60, 62, 66, 69], cell elongation[57, 60, 62] and cell alignment[57, 60, 62], driving a mature CM phenotype. Interestingly, Navaei and colleagues found that non-conductive silica nanomaterials and conductive gold nanorods showed the same improvement with respect to CM cell-cell coupling, cell elongation, beating frequency and excitation threshold.[64] This finding suggests that the topographical effect of the substrates on CM fate needs to be further explored. For neural TE, it has been established that electroconductivity can substantially improve neuron elongation[86, 91, 93], but additional measures of efficacy and toxicology are required before implementing electroconductive biomaterials into clinical applications. Since inclusion of electroconductive components can not only change the electrical properties, but also the physical properties of hydrogels such as mechanics and topography, they provide a promising tool for tissue engineering and regeneration.

A number of reported electroconductive hydrogels for myocardium repair have performed remarkably well in a rat MI model[54, 59], indicating their readiness for clinical consideration. However, to date, electroconductive hydrogels have several challenges that must be overcome. In terms of their biocompatibility, short- and long-term biocompatibility of electroconductive hydrogels should be carefully studied in representative animal models, e.g., rat MI model. This is important, especially since some electroconductive materials are inherently cytotoxic. Additionally, in vivo studies are also required to evaluate their biodegradation, which may be significantly different from in vitro studies, due to our inability to emulate precisely the native microenvironment outside the human body. In fact, the biodistribution of hydrogels' degradation products need to be carefully explored to evaluate the extent of systemic toxicity and immunogenicity. Furthermore, the electrochemical stability of these gels needs to be explored as electroconductivity is dependent on the environment and may change over time. Lastly, prior to being introduced into the human body, electroconductive hydrogels must be properly sterilized according to the Food and Drug Administration (FDA) guidelines. Recently, our group has demonstrated that various standard hydrogels do not withstand the extreme conditions of high-pressure steam sterilization such as autoclaving[116, 117] a technique widely used in the field and FDA approved. As a result, other FDA-approved sterilization methods need to be investigated.[116-118]

With regards to cardiac TE, the amount of attention that has been placed towards electroconductivity needs to be equally given to hydrogel composition, stiffness, topography, and route of administration. Additionally, the cell type selected (e.g., cardiac progenitor, mesenchymal stem cells, bone marrow-derived stem cells) must be critically evaluated and considered on a case-by-case basis.[50, 119] Addressing one of the major challenges of TE, angiogenesis must be promoted to ensure blood supply and survival of transplanted cells.[53] As a proof of concept, Wang and colleagues reported an increased survival of transplanted cells with angiogenic gene therapy.[54] Yet, implementing this approach appends an additional degree of complexity to the current regulatory process.

For neural TE, a number of studies did not thoroughly evaluate the hydrogels' physical properties, which should be a prerequisite, given the importance of biomechanical cues on stem cell fate and function.[82, 84] Although, conductivity appears to have predominantly more effect on neural phenotypes than mechanics, further investigations are required to decouple these confounding factors. Additionally, future studies should examine more thoroughly not only neural cell differentiation, but also protein and RNA expression. Finally, it is recommended that follow-up in vivo studies are performed to confirm whether these electroconductive hydrogel candidates have the potential to promote nerve tissue regeneration in relevant medical conditions, such as for the treatment of PNS or CNS traumatic injuries. While outside the scope of this review, it is important to highlight that electroconductive hydrogels have great potential to improve the electrode-tissue integration and stability for brain-computer interface technologies.[120]

As Alzheimer's CNS and CVD remains among the major causes of death worldwide, the future of neural and cardiac TE holds great potential. Electroconductive hydrogels represent a very unique platform for treating these diseases and improving the quality of human life. The combination of several approaches such as 3D bioprinting, bioreactors and stem cell engineering with electroconductive hydrogels could further advance the development of cardiac and neural tissues or their corresponding organs in their entirety.

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Conflicts of Interest

The authors declare no conflict of interest.

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Table 1: Selected studies using electroconductive hydrogels for cardiac and neural TE.

Electroconductive Material	Hydrogel Composition	Findings	Refs
Cardiac Tissue Engineering			
Tetraaniline	Hyaluronic acid	Electroconductive gels loaded with plasmid DNA for eNOs nanocomplexes and adipose-derived stem cells (ADSCs) improved the electrical activity (QRS interval), decreased the fibrosis area and infarct size of the heart in an MI rat model. The treatment also promoted vascularization (increased ENOs and VEGF-A expression). This work takes a unique approach by combining cardiac TE and gene therapy.	54
Aniline	PEGDA	Injectable PEGDA-based gels improved adipose-derived mesenchymal stem cell retention when injected into mice subcutaneously compared to PBS injections. The hydrogels synthesized in this study spontaneously self-repair, an important feature for clinical translation.	55
Poly(thiophene-3-acetic acid) (PTAA)	Gelatin	Compared to gels without PTAA, electroconductive gels increased Cx-43 and cardiac troponin T expression of breast ADSCs in the presence of electrical stimulation.	56
PEDOT:PSS	Collagen and alginate	Electroconductive gels promoted the synchronous beating of neonatal rat CMs, whereas non-conductive gels did not. These gels also induced a high beating rate and promoted cellular alignment, elongation and a unidirectional orientation. Similar results were obtained with hiPSC-derived CMs. This study developed scaffolds that successfully differentiated hiPSCs into functional CMs.	57
Ppy	PEGDA, gelatin and pentaerythritol triacrylate (PETA)	Electroactive gels that were painted onto infarcted mice hearts restored electrocardiogram wave patterns after 4 weeks. Mice treated with gels without Ppy experienced an adverse cardiomegaly whereas mice with electroconductive gels did not. The authors takes a unique delivery approach by painting their material onto hearts, a technique less invasive than patching.	59
MWCNTs	Decellularized pericardial matrix	Gels with MWCNTs induced hiPSCs synchronous beating, a faster beating rate, greater unidirectional orientation, Cx43 expression and sarcomeric length compared to gels without MWCNTs and Matrigel.	60
MWCNTs	Decellularized pericardial matrix	Gels with MWCNTS increased Cx43 expression after 7 days of culture of CMs compared to gels without MWCNTs.	61

CNTs	GelMA	CMs cultured on a CNT-based network and encapsulated within a GelMA hydrogel increased CM uniaxial direction, Cx43 expression and sarcomere length. Layered CNT-based networks within a GelMA hydrogel-controlled distribution of CMs and endothelial cells within the scaffold. The scaffold in this study led to 3D cardiac anisotropy consisting of multiple cell types, which is highly relevant for clinical translation.	62
CNTs	Alginate and collagen	Electroconductive hydrogels improved human coronary artery endothelial cell attachment and elongation compared to non-conductive alginate gels.	63
Gold nanorods	GelMA	Gels with gold nanorods and non-conductive silica nanoparticles induced similar CM retention, expression of SAC, troponin I and Cx43 and excitation thresholds. This study concluded that scaffold stiffness and topography play a major role in cardiac tissue function.	64
Gold nanorods	GelMA	Electroconductive gels induced improved uniaxial alignment, increased Cx-43 expression compared to GelMA hydrogels.	65
Gold or silver nanoparticles	Collagen	Grafting gold nanoparticle and collagen patches onto the infarcted myocardium of rats stabilized electrical activity of the heart, increased vCM vasculogenesis (CD31+) and Cx43 expression after 7 days.	66
GO	Chitosan	Compared to chitosan gels, chitosan/GO gels increased H9C2 heart cell adhesion and Cx-43 expression.	67
GO	Chitosan	Electroconductive gels increased human embryonic stem cell-derived fibroblasts and CM viability, proliferation and beating rate compared to non-electroconductive gels.	68
rGO	GelMA	rGO-containing GelMA hydrogels greatly increased the beating rate of CMs compared to pristine GelMA. These gels also had higher CM cell retention compared to GelMA gels with GO. This study demonstrated that rGO may be more effective in promoting cardiac tissue function than GO.	69
Bio-IL	GelMA or PEGDA	CMs and cardiac fibroblasts encapsulated within electroconductive GelMA gels had higher cell viability and metabolic activity compared to cells within GelMA gels after 7 days of culture.	70
Neural Tissue Engineering			

PANI	PEGDA	Electroconductive gels supported proliferation and differentiation of NSCs but required electrical stimulation for increased β3 tubulin and PMP22 expression.	83
MWCNT	PEGDA	A 3D-printed gel promoted NSC differentiation. PCR showed that differentiation was dependent on ES.	85
PPy	Collagen	Neuronal phenotypes were up-regulated with or without ES when cultured on electroconductive collagen gels. The degree of differentiation depended on PPy content. This work demonstrated that neuronal differentiation may selectively be dependent on an exogenous electrical stimulation.	86
PEDOT:PSS	PEGDA	It was determined that PEDOT:PSS could be 3D printed and used as a scaffold for DRG cells encapsulated in GelMA. PEDOT structures showed increased expression of neural phenotype proteins, but only with ES.	90
Citric acid-functionalized graphite nanofibers (CAGNF)	Alginate	CAGNF-functionalized alginate gels increased the number of neurite-bearing cells as well as the neurite length, without ES. This study indicated that CAGNFs increase neurite proliferation with little-to-no inflammation response and do not require external stimulation, a promising result for clinical translation.	91
PEDOT	Chitosan	PEDOT-enhanced chitosan hydrogels increased spindle-shaped morphology and showed increased psuedopod presence on PC12 cells.	92
rGO and CNTs	PEG	Electroconductive PEG gels increased the number of neurite-bearing cells. This effect was enhanced by adding a positive charge with MTAC. This work demonstrated that in addition to electrical conductivity, neurite outgrowth and nerve cell function can be synergistically enhanced when neuronal cells are exposed to positive charges.	93
Aniline	Poly(glycerol sebacate)(PGS)	Aniline-containing hydrogels increased the expression of myelination genes (PMP22, NGF, BDNF and Krox20) in Schwann cells. In addition, a neuritogenic media from Schwann cell-laden aniline gels increased PC12 neurite length, a result attributed to increased growth factor secretion (e.g., neurotrophin) by stimulated Schwann cells.	94
PPy	Alginate	PPy-alginate hydrogels significantly increased expression of neural differentiation genes Tuj1 and MAP2. Subcutaneous implantation for 8 weeks resulted in mild inflammation.	96

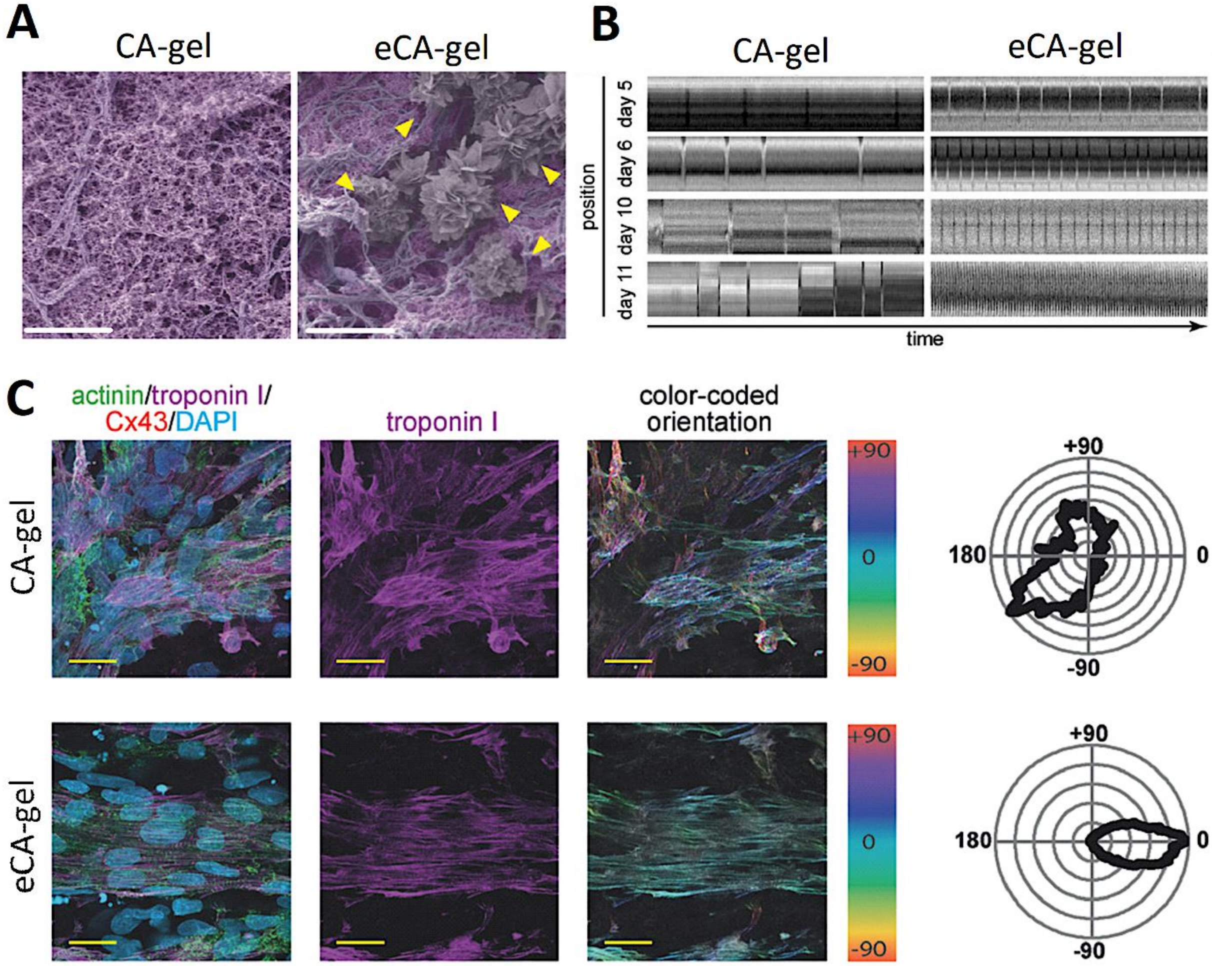

Figure 1: **Alginate, collagen and PEDOT:PSS-based hydrogels promote maturation of primary rat CMs**. **A**. Colored SEM images of collagen/alginate hydrogels (CA-gel) and PEDOT:PSS/collagen/alginate hydrogels (eCA-gel). Yellow arrows indicate sand rose-like structures induced by PEDOT:PSS. **B**. Kymograph analysis visualizing autonomous contractions (peaks) of neonatal rat CMs cultured on CA-gels and eCA-gels after 11 days. **C**. Representative confocal images of neonatal rat CMs cultured on CA-gels or eCA-gels. Immunofluorescent staining was used to determine cell orientation distribution. Actinin: green, Troponin I: purple, Cx43: red, and DAPI, turquoise. Scale bars: 5 μm (A) and 100 μm (C). Adapted from Roshinbanfar and colleagues[57] with permission from Wiley.

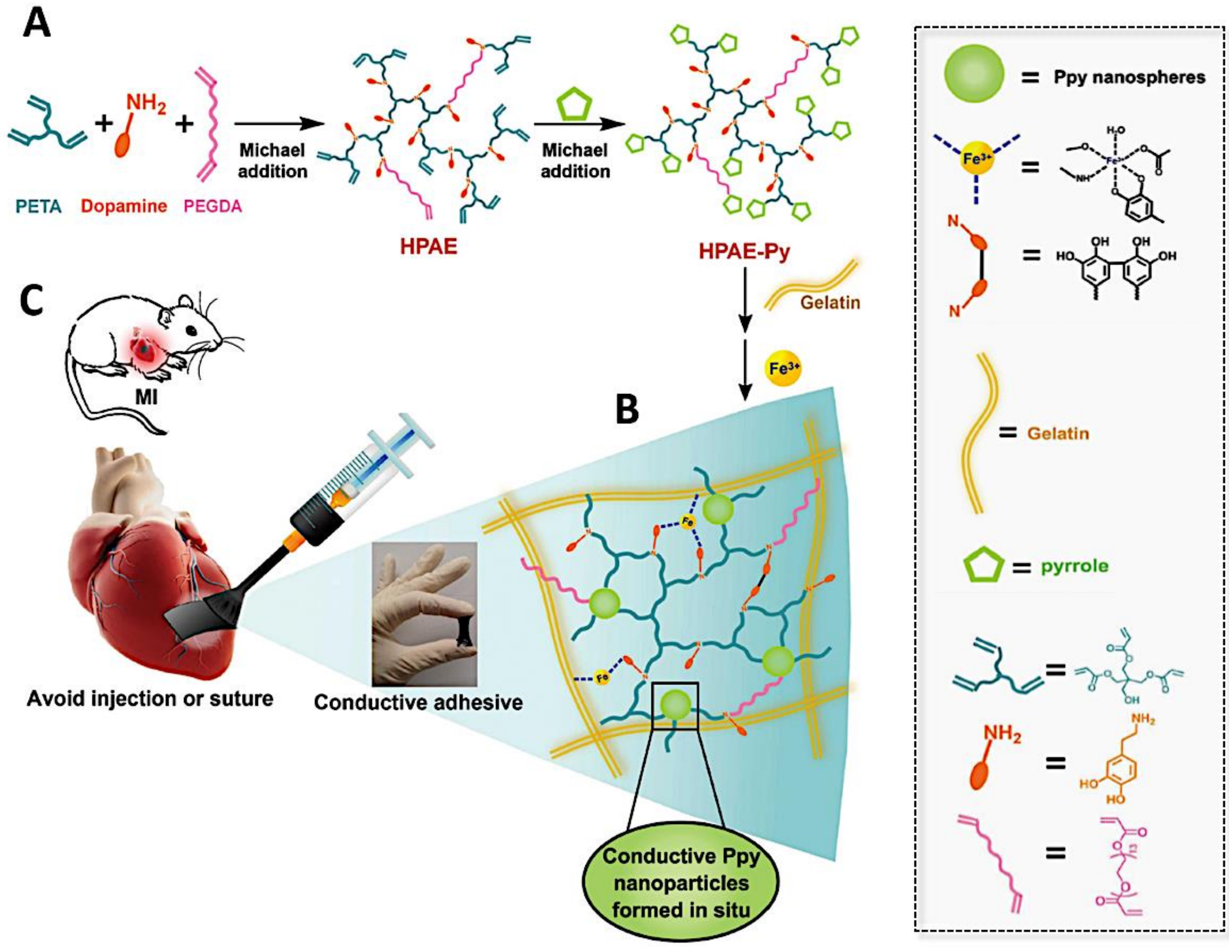

Figure 2: **Adhesive and electroconductive gelatin and PEGDA-based hydrogels can be directly painted onto infarcted myocardium**. **A**. Schematic depicting a two-step synthesis of HPAE-Py. First, dopamine hydrochloride, pentaerythritol triacrylate (PETA) and PEGDA are reacted together via Michael addition to form hyperbranched poly(amino ester) (HPAE). Next, pyrrole is reacted with HPAE via a second Michael addition reaction to form HPAE-Py. **B**. Schematic showing Fe^{3+}-mediated cross-linking of Gelatin with HPAE-Py, improving gel adhesion via catechol-Fe^{3+} complexation. **C**. HPAE-Py/Gelatin hydrogels can be directly applied to infarcted myocardium using a brush-syringe system. Once applied, the gel rapidly adheres to the myocardium without leaking. Adapted from Liang and colleagues[59] with permission from John Wiley and Sons.

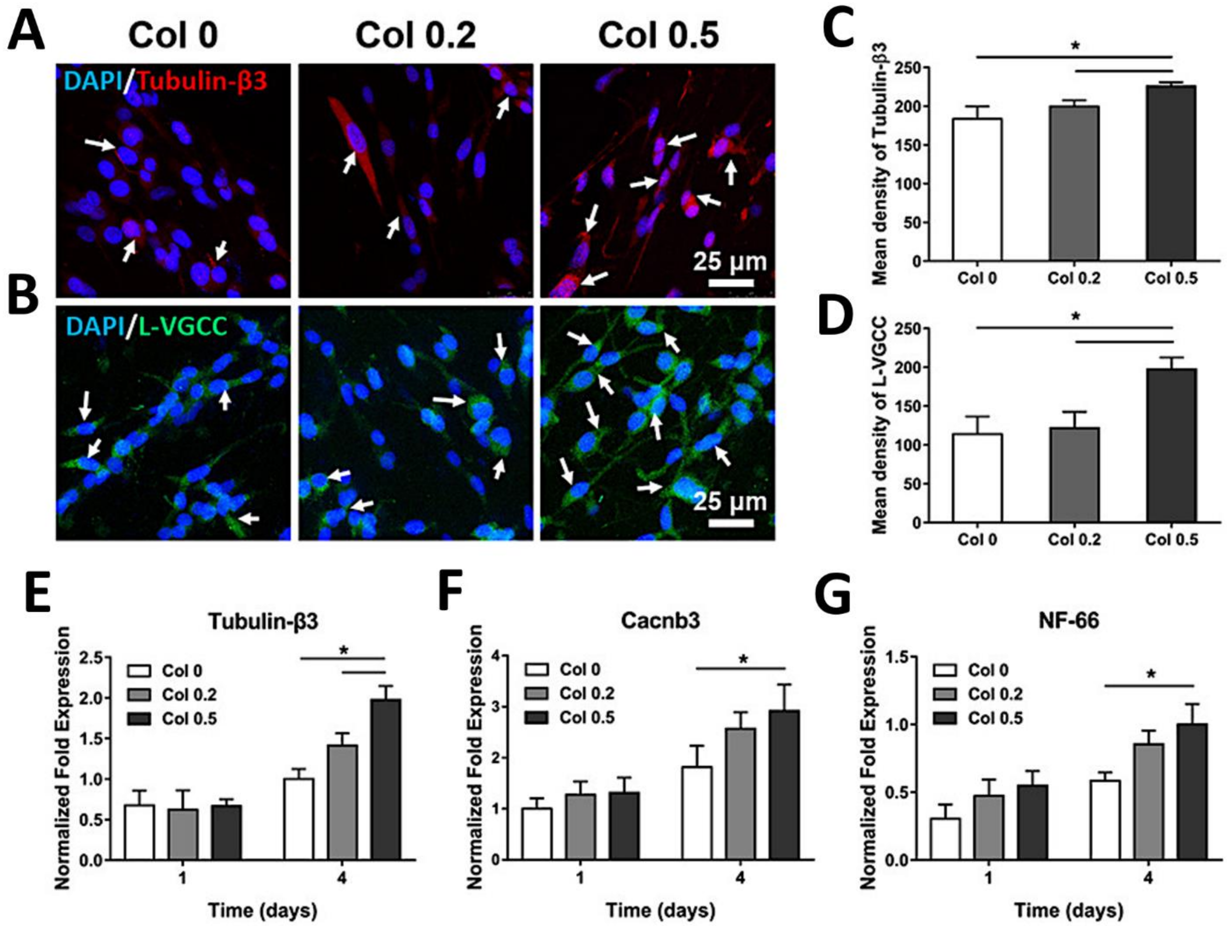

Figure 3: **Collagen-PPy hydrogel microfibers improve PC12 neurogenesis**. **A-B**. Immunofluorescent staining of Tubulin-β3 (red), L-VGCC (green) and DAPI (blue) of PC12 cells on several compositions of Collagen-PPy hydrogels. **C-D**. Semi-quantitation of Tubulin-β3 and L-VGCC expression. **E-G**. Normalized fold-changes of gene expression of Tubulin-β3, Cacnb3, and NF-66. * indicates significance at $p < 0.05$, $n = 3$. Adapted from Wu and colleagues[86] with permission from American Chemical Society.

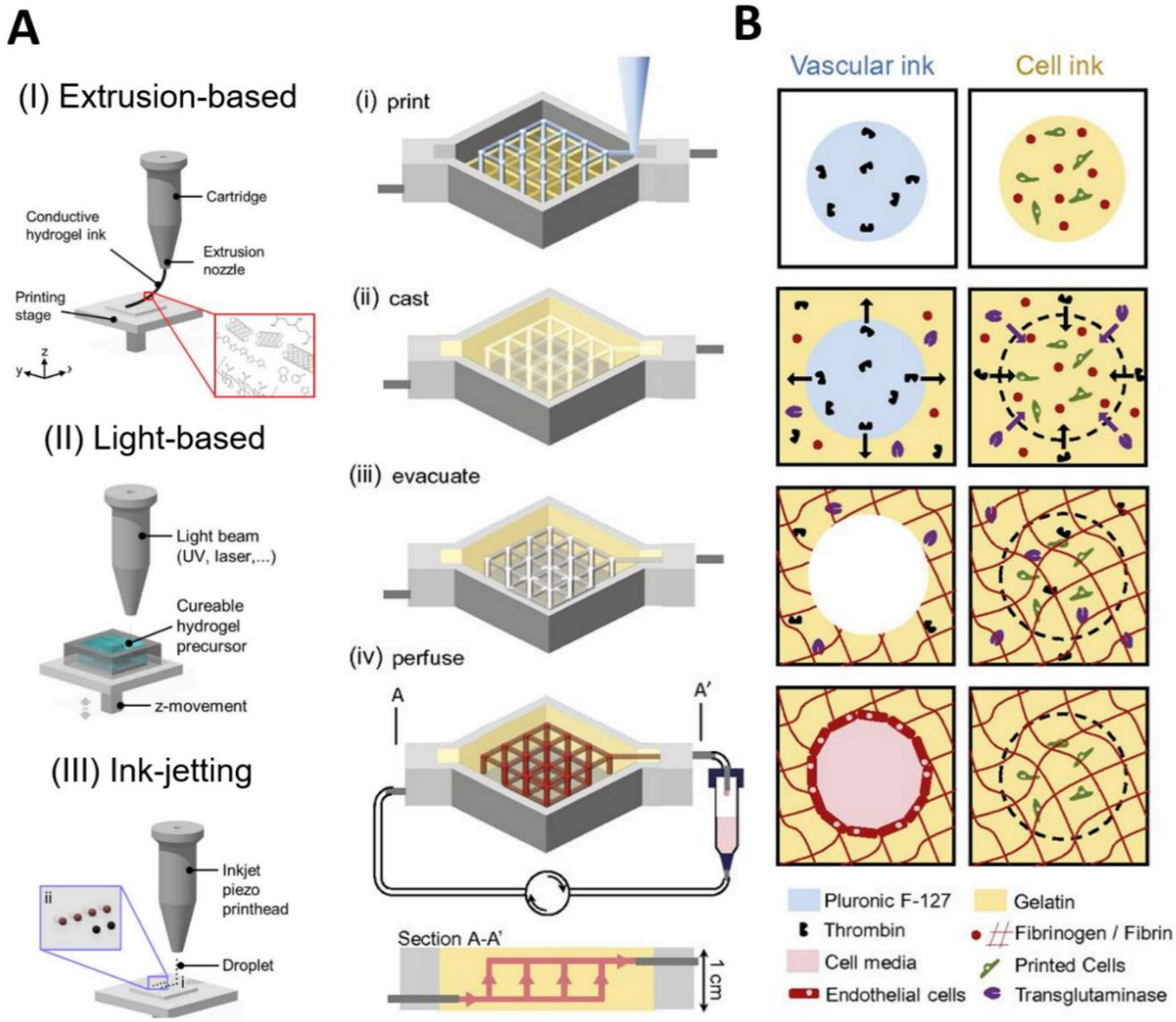

Figure 4: **3D printing technology for the fabrication of electroconductive hydrogels and vascularized networks**. **A**. Various technologies used for 3D printing electroconductive hydrogels. (I) Extrusion-based printing extrusion-based printing utilizes pneumatic or mechanical pressure to depose the gel or gel precursors. (II) Laser-based printing uses either a light or laser to cross-link gel precursors in a defined pattern. (III) Ink-jetting involves the deposition of gel or gel precursors into a predefined pattern using thermal or piezoelectric energy. **B**. Schematic describing the formation of a vascularized network within a 3D perfusion chip. (*i*) Print: Vascular ink, consisting of polyethylene oxide-polypropylene oxide-polyethylene oxide (Pluronic® F-127) and cell-laden ink (gelatin, fibrin and cells) are printed onto silicone and glass-based chips. (*ii*) Cast: Next, an ECM-mimetic, which is similar to the cell-laden ink but also contains thrombin and transglutaminase (TG), is cast onto the chip. Thrombin and TG induce fast polymerization of fibrinogen and slow polymerization of fibrinogen and gelatin, respectively, in both the ECM and cell ink. (*iii*) Evacuate: Chips are cooled down, causing a gel-fluid transition of the vascular ink, leaving behind a vascular network within the chip. (*iv*) Perfuse: The vascular network is then cellularized with endothelial cells using a perfusion system. A: reproduced from Distler and Boccacini[37] with permission from Elsevier. B: Reproduced from Kolesky and colleagues[108] with permission from National Academy of Sciences.

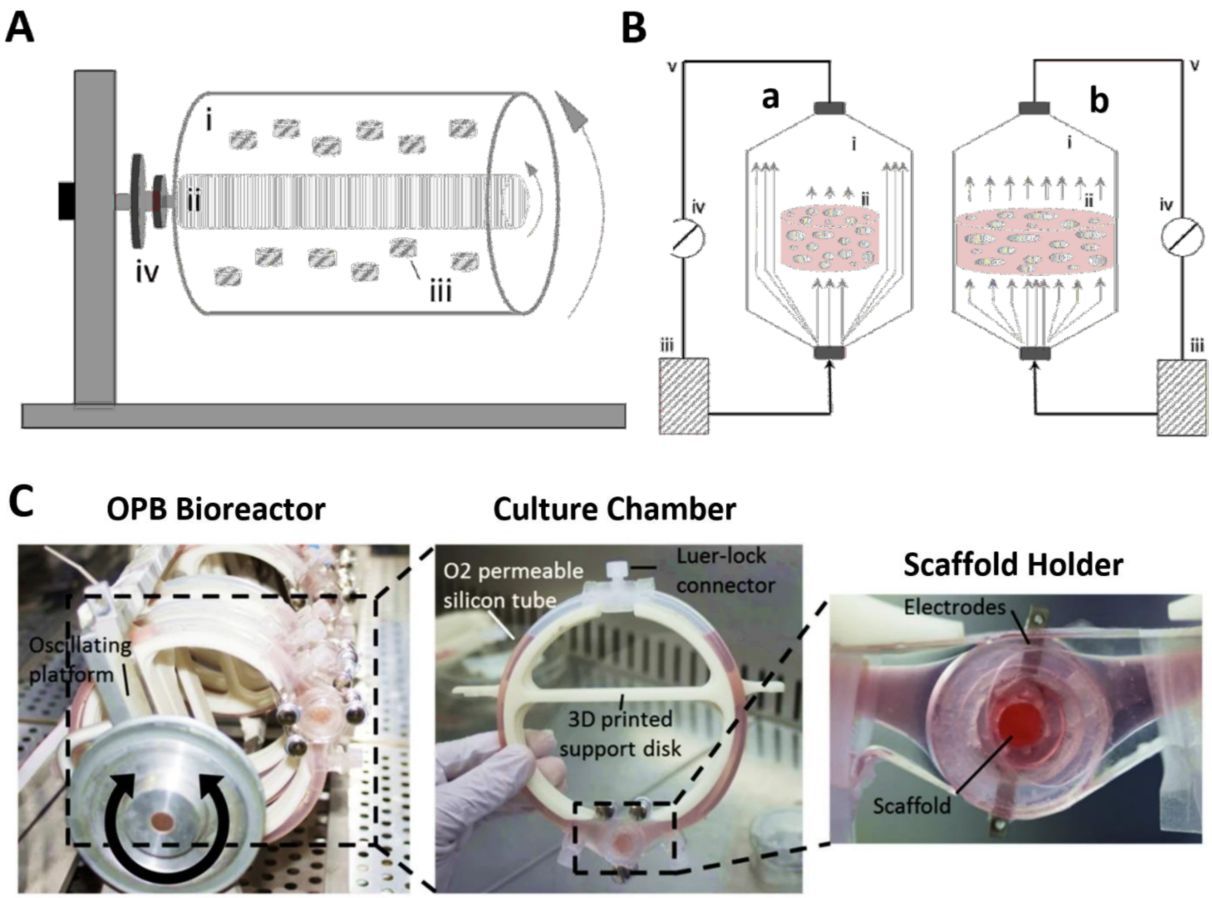

Figure 5: **TE bioreactors for dynamic cell culture integrating electroconductive hydrogels**. **A**. Schematic illustration of a rotary bioreactor consisting of outer (*i*) and inner (*ii*) cylinders, cell-laden scaffolds (*iii*), and the rotator support (*iv*). These systems are completely filled with liquid medium, in which gas is transferred via a silicon-rubber gas-transfer membrane. **B**. Schematic illustration of indirect (a) and direct (b) perfusion bioreactors, including the culture chambers (*i*), cell-laden constructs (*ii*), culture medium (*iii*), peristaltic pumps (*iv*), and tubing (*v*). In indirect perfusion, perfusing medium circumvents the constructs, whereas in direct perfusion, constructs are tightly fitted within the chamber such that medium perfuses through them. **C**. The oscillating perfusion bioreactor (OPB) can host up to 18 cell-laden scaffolds simultaneously. The culture chamber, consisting of 3D-printed polydimethylsiloxane (PDMS), promotes electrical stimulation, perfusion of media and real-time monitoring (digital microscopy) for each scaffold. A-B: Reproduced from Sladkova and colleagues under the terms and conditions of the Creative Commons Attribution License 3.0.[121] C: Reproduced from Visone and colleagues under the terms and conditions of the Creative Commons Attribution License 4.0.[115]

Glossary

Term	Acronym
Adipose-derived stem cell	ADSC
Angiopoietin 1	Ang-1
Bio-Ionic Liquid	Bio-IL
Cadherin 2	Cdh-2
Cardiomyocyte	CM
Cardiovascular disease	CVD
Central nervous system	CNS
Citric acid-functionalized graphite nanofibers	CAGNF
Carbon nanotube	CNT
Connexin 43	Cx43
Endothelial nitric oxide synthase	eNOs
Extracellular matrix	ECM
Food and Drug Administration	FDA
Gelatin methacryloyl	GelMA
Glial fibrillary acidic protein	GFAP
Graphene oxide	GO
Human induced pluripotent stem cell	hiPSC
Hyaluronic acid	HA
Human neural stem cell	HNSC
Human umbilical vein endothelial cell	HUVEC
Hyperbranched poly(amino ester)	HPAE
Multi-walled carbon nanotube	MWCNT
Myocardial infarction	MI
Oscillating perfusion bioreactor	OPB
Pentaerythritol triacrylate	PETA
Peripheral nervous system	PNS
Polyaniline	PANI
Polyethylene glycol	PEG
Polyethylene glycol diacrylate	PEGDA
Polypyrrole	PPy
Poly(glycerol sebacate)	PGS

Term	Acronym
Poly(thiophene-3-acetic acid)	PTAA
Poly(3, 4-ethylenedioxythiophene):polystyrene sulfonate	PEDOT:PSS
Reduced graphene oxide	rGO
Sarcomeric α-actinin	SAC
Three-dimensional	3D
Tissue engineering	TE
Transglutaminase	TG
Vascular endothelial growth factor A	VEGF-A