

Cardiac Tissue Engineering Systems

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Cardiac tissue engineering is very much in a current focus of regenerative medicine research as it represents a promising strategy for cardiac disease modelling, cardiotoxicity testing and cardiovascular repair. Advances in this field over the last two decades have enabled the generation of human engineered cardiac tissue constructs with progressively increased functional capabilities. Numerous studies have demonstrated that the therapeutic benefits exerted by cells are mainly attributable to the release of complementary paracrine factors and the efficacy is limited as only a small percentage of transplanted cells engrafted in the infarcted tissue. Studies on animal models showed that combining cell therapy with tissue engineering techniques for the creation of cell sheets and patches, can increase stem cell survival and boost therapeutic action. Therefore, tissue engineering has been considered as a potential approach for cardiac regeneration after MI.

cardiac tissue engineering

human heart

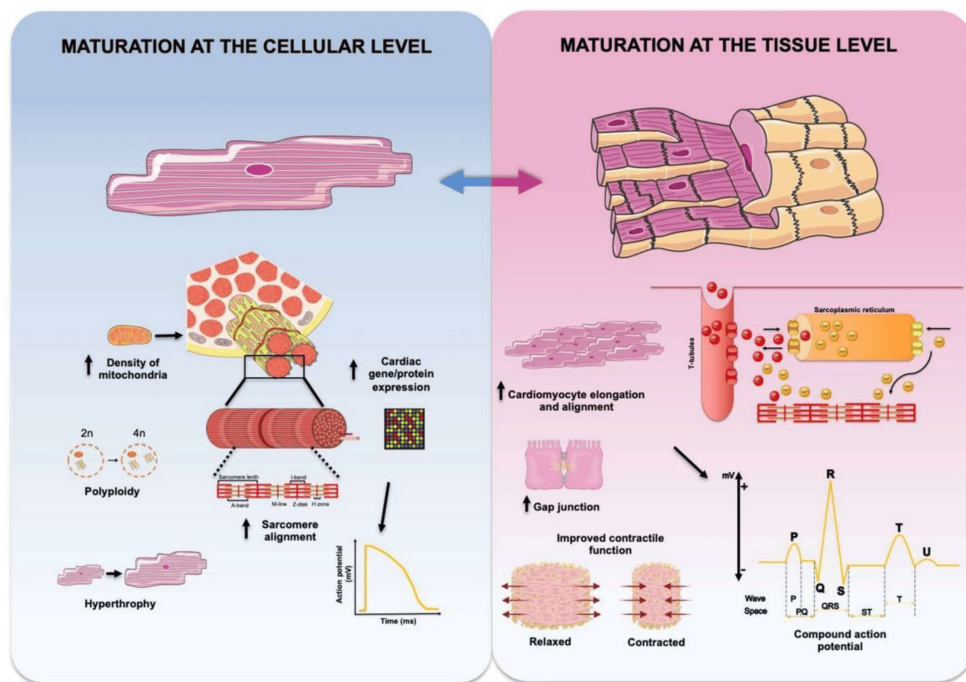
tissue maturation

1. Cell Sheets

The cell sheet technique was first reported by Shimizu et al. in 2002 for creating a transplantable 3D cell patch ^[1]. Cell sheet technology, also referred to as scaffold-free system or “Cell Sheet Engineering” ^{[1][2][3]}, is based on stacking monolayers (or sheets) of CMs cultured to confluency to form 3D tissue-like structures. By using cell culture surfaces coated with the temperature-responsive polymer poly(*N*-isopropylacrylamide) (PIPAAm), it is possible to readily detach intact cellular monolayers of CMs as cell sheets by lowering the temperature, without any enzymatic treatments. Overlaying these thin 2D monolayers then results in 3D cardiac constructs ^[4]. The benefits of this system have been analyzed in vivo in murine animal models of MI showing improvements in cell survival, cardiac function and tissue remodeling ^[5]. The use of cell sheets created new opportunities for in vitro tissue engineering and helped exploring new therapies and drugs for heart diseases ^{[6][7]}. Interestingly, Sekine et al. produced in vitro vascularized cardiac tissues with perfusable blood vessels by overlaying additional triple-layer cell sheets made by NRCMs cocultured with endothelial cells. Such sheets were then transplanted under the neck skin of nude rats and connected to the local vasculature. Constructs with vessel anastomoses survived and maintained their vascular structure up to two weeks after transplantation. However, the thickness of the constructs decreased over time indicating that uniform perfusion was insufficient for whole tissue survival. Moreover, no functional analyses were performed in the study to evaluate maturation at tissue level ^[8]. More recently, Kawatou et al. developed an in vitro drug-induced Torsade de Pointes arrhythmia model using 3D cardiac tissue sheets. Importantly, the authors showed the importance of using multi-layered 3D structures containing a hiPSC-derived heterogeneous cell mixture (CMs and non-myocytes) in order to recapitulate disease-related phenotypes in vitro ^[9]. In addition, phase II clinical trials have been performed by Japanese scientists to evaluate the efficacy and safety

of autologous skeletal myoblast sheet transplantation in patients with advanced heart failure. They demonstrated that the transplantation of engineered tissue promoted left ventricular remodeling, improved the heart failure symptoms and prevented cardiac death with a 2 year follow-up period [10]. Also, the potential use of cell-sheets that contain allogeneic hiPSC-CMs for clinical transplantation is under investigation [11].

A clear advantage of cell sheet technology for therapeutic applications is the absence of biomaterials, which reduces the risk of immune rejection that could arise from xenobiotic or non-autologous materials, and that no suture is needed to ligate the construct to the injured heart. Moreover, sheet technology enables direct cell-to-cell communications between cells in the transplanted sheets and the host tissue, facilitating electrical communication and vascular network formation within the cell sheet structure. On the downside, it has been argued that the fragility of these sheets makes them difficult to manipulate during implantation onto the heart [12]. Although cardiac tissue sheets have many advantages over other tissue engineering methods, these structures are not thick enough to reproduce the high complexity of the native myocardial tissue.



2. Scaffolds

ECT constructs made by repopulating cell-free scaffolds with suitable cells are usually referred to as cardiac patches. Scaffolds for CTE usually consist on a 3D polymeric porous structure that contributes to cell attachment and leads to the desirable cell interaction for further tissue formation [13][14][15][16][17][18][19][20][21]. Many different materials have been tested for the fabrication of scaffolds suitable for CTE, including natural and synthetic biomaterials. A commonly used synthetic material is polylactic acid (PLA), which is easily degradable forming lactic acid. PLA scaffolds were tested in some cardiovascular studies [22][23]. Another example is polyglycolic acid (PGA) and its copolymer with PLA poly(D,L-lactic-co-glycolic acid) (PLGA), an FDA approved biomaterial among the first tested for CTE due to its high porosity, biodegradability and processability [18][24][25]. However, it has been noted

that the high stiffness of PLGA may limit the capacity of CMs to remodel the scaffold and ultimately impinge on their maturation [12]. Collagen, being the most abundant protein in the cardiac extracellular matrix (ECM), has a fibrillar structure that facilitates CM scaffolding. In addition to good biocompatibility, biodegradability and permeability, collagen also elicits low immunogenicity and can be engineered in various formats including high porosity scaffolds, all of which make of collagen the most commonly used biomaterial in scaffold-based CTE [26][27][14][28][15][29][30]. Other natural biomaterials used in this context include alginate, a polysaccharide derived from algae used in ECT constructs [16][31] due its high biocompatibility and appropriate chemical and mechanical properties [32], and albumin fibers, which have been used to create biocompatible scaffolds of high porosity and elasticity [33][34].

The combination of different approaches has enabled the development of scaffold systems of increasing complexity, thus bringing them morphologically closer to heart tissue. For example, researchers have generated fibrous scaffold with spatially distributed cues that enabled CM alignment within the patch [33][35][36]. However, major limitations of CTE still remain the generation of thick constructs (over ~100 µm in thickness) and the lack of electromechanical coupling between the cardiac patch and the host myocardium [37][12]. The generation of ECT constructs with a clinically relevant size requires ensuring that appropriate levels of oxygen and nutrients are maintained within the construct to satisfy the metabolic demand of CMs. Perfusion bioreactor systems pioneered by the Vunjak-Novakovic laboratory have proven to be of great value for the generation of thick ECT constructs full of viable cells with aerobic metabolism. In this case, cells were seeded and cultured in porous collagen scaffolds (11 mm in diameter, 1.5 mm in thickness) under continuous perfusion for 7 to 14 days, which led to the formation of contractile thick cardiac tissues [19][38]. More complex bioreactor systems designed to perfuse ECT constructs while also delivering electrical signals mimicking those in the native heart have also been developed [28][29][34]. Maidhof et al. used NRCMs seeded under perfusion into porous poly(glycerol sebacate) (PGS) scaffolds (8 mm in diameter, 1 mm in thickness), which were maintained under continuous perfusion at a flow rate of 18 µL/min and electrically stimulated at a frequency of 3 Hz. After 8 days, the combination of perfusion and electrical stimulation resulted in cell elongation, structural organization and improved contractility of ECT constructs [17]. Recently, our laboratories have reported the generation of 3D engineered thick human cardiac macrotissues (CardioSlices). Human iPSC-CMs were seeded together with human FBs into large 3D porous collagen/elastin scaffolds and cultured under perfusion and electrical stimulation in a custom-made bioreactor. Two weeks after culture, stimulated ECT constructs exhibited contractile and electrophysiological properties close to those of working human myocardium [39].

In addition to scaffolds made from synthetic or natural biomaterials, the use of matrices obtained by decellularizing native tissues has gained popularity for CTE. The process of decellularization allows obtaining natural ECM that can be used to mimic the native tissue structure. In essence, decellularized ECM could be recellularized with CMs or mixtures of CMs and other cell types, or with PSCs that would be differentiated in situ toward the desired cell types [40]. Tissues from a wide variety of sources including human, animals and plants have been used for this purpose [12]. The porcine heart is a prime example of tissue source for animal-derived decellularized scaffolds, due to its large size and to it being a preferred experimental model for cardiovascular research. In this case, it has been reported that the decellularization procedure allows obtaining a cardiac scaffold with preserved vasculature,

mechanical integrity and biocompatibility [41]. Nevertheless, limitations noted with this approach include the extent of preservation of the ECM composition (which can be altered by the decellularization process), the difficulty in recellularizing the scaffold with clinically relevant numbers of CMs (in the order of billions), and the risk of eliciting immune rejection [12]. The issue of immune intolerance of animal-derived decellularized scaffolds has prompted research on plant-derived biomaterials as a source for ECT constructs [42]. Even though promising results have been reported using biomaterials derived from decellularized apple [43][44], spinach and parsley leaves [45], along with other cellulose-based scaffolds [46][47], further evaluation will be necessary to assess the usefulness of this type of materials for in vitro bioengineering and in vivo therapeutic applications.

Alternatively, human tissue might be a more appropriate source for decellularized ECM for therapeutic purposes, as it would address some of the limitations of animal- and/or plant-sourced materials described above [48][49][50][51]. In this respect, studies by Sanchez et al. demonstrated that the human acellular heart matrix can serve as a biocompatible scaffold for recellularization with parenchymal and vascular cells [52]. Moreover, Guyete et al. also used human decellularized heart tissue, which was in this case recellularized with iPSC-CMs and maintained in a custom-made bioreactor that provided coronary perfusion and mechanical stimulation. After 14 days in culture, the recellularized cardiac segment presented high metabolic activity and contractile function but exhibited low maturation state [53].

3. Hydrogels

Embedding suitable cells in hydrogels provide important 3D information cues and, in the context of CTE, the constructs generated in manner are typically known as cardiac grafts. Hydrogels are among the most widely studied types of biomaterials in CTE. In particular, hydrogel-based materials have been shown to provide structural/mechanical support to cells [54], promote vascularization [55] and cell migration, differentiation and proliferation [56], and to improve cardiac function after implantation in murine and porcine models of MI [57][58][59]. Hydrogels can be made from different biomaterials that are usually classified into three types: natural (type I collagen, fibrin, gelatin, alginate, keratin, among other), synthetic [polycaprolactone (PCL), polyethylene-glycol (PEG), PLA, PGA and their co-polymer PLGA], and hybrid hydrogels, which are made by combining natural and synthetic polymers [6][7]. Natural-based hydrogels are often preferred for generating ECT constructs because of their high bioactivity, biocompatibility and biodegradability [56].

Cardiac “bundles” are the most common structures generated when using hydrogel-based systems and are cylindrical ECT constructs in the form of cables, ribbons or rings [12]. These structures are usually formed by embedding CMs from various sources within hydrogels made up of fibrin, type I collagen or other biomaterials, and maintaining them in culture until constructs become spontaneously contractile. The formation of these bundles results in self-alignment and anisotropic organization of CMs, which is a hallmark of cell maturation. Moreover, these constructs provide an easy way to analyze the electrical and mechanical properties of CMs, thus enabling the readily evaluation of their maturation state and facilitating their use in drug screening and toxicity assessment platforms [60][61][62][63][64][65][66][67][68][69].

In a pioneering approach, Eschenhagen and Zimmermann generated cardiac bundles (which they termed engineered heart tissues, or EHTs) by casting a mixture of NRCMs and a blend of type I collagen type I and Matrigel into cylindrical molds. Under conditions of mechanical stretching, the resulting ring-shaped constructs exhibited improved contractile function and a high degree of cardiac myocyte differentiation [70]. In subsequent work, five of such rings were stacked on a custom-made structure creating multiloop tissue constructs that survived after implantation and improved the cardiac function of infarcted rats [59]. Using the same principle, Kensah et al. produced cardiac bundles by seeding NRCMs with FBs in a collagen/Matrigel hydrogel into a Teflon casting mold between two titanium rods and subjected to mechanical and/or chemical stimulation [71]. Similarly, human ECT constructs have also been generated by casting a cell/hydrogel suspension in different types of molds between or around flexible posts. Schaaf et al. used hESC-CMs in a fibrin hydrogel seeded into an agarose casting mold between 2 elastic silicone posts for 5 weeks [72]. Controlling the 3D microenvironment has been further reported to induce spatial organization and promote CM maturation in hydrogel-based systems. In a study by Thavandiran et al., hESC-CMs and hESC-derived cardiac FBs were seeded in a collagen/Matrigel hydrogel into polydimethylsiloxane (PDMS) microwells with integrated posts. The authors compared two-well designs side by side: an elongated microwell containing posts in both extremes (capable of inducing uniaxial mechanical stress) and a square well containing posts around the edges (thus effecting biaxial mechanical conditioning). These studies demonstrated that constructs on elongated microwells showed comparatively better aligned sarcomeres and more elongated and longitudinally oriented CMs [73]. In turn, the Bursac laboratory created thin (~70 µm in thickness) 3D sheet-like constructs of large surface dimensions (7 × 7 mm) by casting hESC-derived CMs in fibrin hydrogels into PDMS molds with hexagonal posts, resulting in improved maturation at the functional (conduction velocities of up to 25 cm/s and contractile forces and stresses of 3.0 mN and 11.8 mN/mm², respectively) and structural (increased sarcomeric organization and expression of cardiac genes) level [37]. Similarly, Turnbull et al. generated human ECT constructs with hESC-derived cells mixed in a collagen/Matrigel hydrogel in rectangular PDMS casting molds with integrated posts at each end and removable inserts. Forty-eight hours after casting, the inserts were removed from the mold, allowing the self-assembly of the tissues between the two flexible posts, which were used as force sensors. The resulting tissues exhibited typical features of human newborn myocardium tissue including contractile, structural and molecular characteristics [74].

Similar to hESC-CMs, iPSC-CMs also have been successfully cultured in hydrogel-based structures [13][62][75][76][77][64][65][78][66]. The Radisic laboratory pioneered the use of hiPSC-CMs to generate human ECT constructs by developing a platform in which cells in a collagen hydrogel organized around a surgical suture in a PDMS channel. The resulting 3D microstructures (3 mm²) were termed Biowires and contained aligned CMs that exhibited well-developed striations and showed improved cardiac tissue function after electrical stimulation [62]. A further improvement to this platform was the use of a polytetrafluoroethylene tube that allowed perfusion of the ECT microstructures and facilitated their use for drug toxicity testing [79]. More importantly, three independent studies reported in 2017 on the generation of clinical-size cardiac tissues by using hydrogel-based systems and hiPSC-CMs. Shadrin et al. generated human cardiac tissues of 36 × 36 mm that showed electromechanical properties close to those of working myocardium (conduction velocity of 30 cm/s and specific forces of 20 mN/mm²) by seeding hiPSC-CMs in a fibrin hydrogel into square PDMS molds [13]. Using a mixture of type I collagen and

Matrigel with hiPSC-derived CMs and endothelial and vascular cells (in a 3:1:1 ratio), Nakane et al. generated rectangular ECT constructs with different shapes (bundles and mesh junctions, parallel bundles, plain sheets) and sizes (from 15 × 15 mm to 30 × 30 mm). They analyzed the association of CM orientation and survival with construct architecture and found that bundles and mesh junctions resulted in the highest myofiber alignment and lowest percentage of dead cells. Moreover, functional integration was observed after 4 weeks of transplantation onto rat uninjured hearts [64]. Large ECT constructs (35 × 34 mm) were also generated in the Zimmermann laboratory by seeding hiPSC-CMs and FBs in a collagen hydrogel on a 3D-printed construct holder with flexible poles in a hexagonal casting mold [65]. In a further refinement of this approach, Gao et al. generated human ECT constructs of 4 × 2 cm comprising hiPSC-derived CMs, SMCs, and ECs (3:1:1 ratio) in a fibrin hydrogel and maintained them in culture on a rocking platform. After 7 days of culture, the constructs showed improved electromechanical coupling, calcium-handling, and force generation [78].

Synthetic hydrogels have received comparatively less attention for CTE than those of natural origin. Ma et al. used PEG to create cardiac microchambers (100 to 300 µm in height) that induced spatial organization of hESCs and hiPSCs and directed their cardiac differentiation [80][81]. In addition, hybrid hydrogels have a great potential for CTE as they can mimic biological properties of the ECM and, at the same time, be tuned to suit the mechanical properties expected or desired for cardiac constructs [82]. Despite their great potential, much research is still needed to ascertain the specific advantages that synthetic and hybrid hydrogels may have over commonly used natural hydrogels in the context of CTE. At any rate, irrespective of the type of hydrogel used, current 3D cardiac grafts are constrained in maximum thickness by the ~300 µm limit of oxygen diffusion in passive culture systems and, therefore, while ideal for miniature structures with some tissue-like functionalities, they may not be suited for applications that require fully capturing the high complexity of the native heart tissue structure.

4. Cardiac Spheroids (and‘Organoids’)

Spheroids are scaffold-free 3D cell constructs that rely on cell aggregation or self-organization and simulate aspects of the native cell microenvironment [83]. Cardiac spheroids can be constructed with CMs [84][85] and also include other cardiac resident cells such as FBs [86] or ECs [87]. Different percentages of various cell types have been tested for the generation of cardiac spheroids [86][88]. Spheroid-based systems are attractive to scientists for studying heterocellular interactions and drug effects because they only need low cell numbers to be formed. On the other hand, the absence of functional architecture limits the physiological analyses of the cells, like force generation and electrical conduction. Nevertheless, using the spheroid-based systems to deliver CMs into the damaged region of the heart has been reported. For instance, intramyocardial injection of cardiac spheroids in mice resulted in higher engraftment rates and improved electrical coupling with host myocardium, compared to single cell injection, which reveals potential for future clinical applications [89][90]. Moreover, several research groups are working on the generation of thicker functional structures using multicellular spheroids for further clinical testing. Noguchi et al. created a scaffold-free 3D tissue construct based on self-organization of 1×10^4 spheroids. The individual spheroids were, in turn, obtained by combining 3 cell sources: NRCMs, hECs and hFBs in a 7:1.5:1.5 ratio. ECT constructs generated in this way remained adherent and presented signs of vascularization seven

days after transplantation onto the heart of nude rats [\[91\]](#). In a related approach, scientists created a biomaterial-free cardiac tissue by 3D printing multicellular cardiac spheroids that displayed spontaneous beating and ventricular-like action potentials, which were engrafted as well into the rat heart tissue [\[92\]](#). A final example of this approach is the study by Kim et al., who generated elongated 3D heterocellular microtissues by fusing together multicellular cardiac spheroids containing CMs and cardiac FBs. The authors demonstrated that such microtissues formed an electrical syncytium after seven hours in culture [\[93\]](#).

Similar to spheroids, organoids are scaffold-free 3D cell constructs that simulate aspects of the native environmental conditions. However, a critically important characteristic of organoids that sets them apart from spheroids, is that organoids contain organ-specific cell types that self-organize in a way that is architecturally similar to that of the native organ [\[94\]\[95\]\[96\]](#). While some researchers may use the terms organoids and spheroids interchangeably, there are important differences between them. Both spheroids and organoids can be generated from PSCs, PSC derivatives, or tissue-specific stem/progenitor cells. When using PSCs, the technique of organoid formation is inspired in the embryoid body system [\[94\]](#), 3D aggregates of PSCs where cells undergo specification and differentiation into cell derivatives of the three main embryo germ layers [\[95\]](#). In contrast, spheroids are much simpler than organoids in terms of the cell types that conform them, do not self-organize into organ-like patterns or structures, and depend to a lesser extent on ECM properties and composition [\[96\]](#). Even though several published reports describe the generation of human “cardiac organoids”, these rely on direct cardiac differentiation of hiPSC-derived embryoid bodies [\[97\]](#) or aggregation of cardiac cell types (CMs, cardiac FBs and cardiac ECs) [\[98\]\[99\]\[100\]\[101\]\[102\]](#), which actually represent typical examples of spheroids [\[94\]\[95\]](#). We believe that the use of the term “cardiac organoids” in this context is misleading since the structures generated in those studies lacked the organ-like complexity characteristic of true organoids. Very recently, Lee et al. have described the generation of *bona fide* cardiac organoids in the mouse system that showed atrium- and ventricular-like structures highly reminiscent of the native embryonic heart. For this purpose, the authors induced sequential morphological changes in PSC-derived cells by including a laminin-entactin complex in the ECM and FGF-4 in serum-free medium [\[103\]](#). Perhaps the application of similar approaches to hiPSC derivatives could lead to the generation of true human cardiac organoids containing relevant organ-specific cell types with the capacity to self-organize in organ-like structures.

5. Heart-on-a-Chip

Microfluidic cell culture technologies enable researchers to create in vitro cell microenvironments that mimic organ-level physiology [\[104\]](#). The term ‘organ-on-a-chip’ is generally applied to a microphysiological system, including the slides or plates that are connected to microfluidic devices to control perfusion of culture medium and exposure of defined stimuli [\[105\]](#). Heart-on-a-chip technology refers specifically to microphysiological systems mimicking the function of heart tissue. In vivo-like cardiac cell culture systems could lead to a better understanding of (1) cardiac cell physiology; (2) cardiotoxicity of drugs intended for human use; (3) personalized treatments for CVD patients; and (3) mechanisms of heart regeneration [\[106\]\[107\]\[108\]](#). In physiological conditions, the heart tissue is in direct contact with body fluids such as blood and lymph that exert physical forces (shear stress) on the cells. This continuous flow stimulation determines the cardiac cells structure, phenotype, intra- and extracellular interactions

[109]. In vivo-like cardiac cell culture systems try to replicate these conditions in vitro. Thus, the heart-on-a-chip system provides suitable conditions to imitate biochemical, mechanical, and physical signals characteristic of heart tissue [110][111][112]. For example, it was shown that continuous perfusion enhances cell proliferation and parallel alignment of cells compared to static conditions [113]. In addition to perfusion, integrating mechanical and electrical stimulation into heart-on-a-chip devices also improves the maturation state of CMs [114][115][116][117], one of the key features for successful modeling of cardiac diseases [118]. Moreover, heart-on-a-chip systems are amenable to parallelization and thus to be used in high-throughput assays for drug screening and cardiotoxicity testing [108][119]. Particularly, the possibility of using hPSC-CMs brings an additional level of personalization to heart-on-a-chip systems [75][114][87][120][121][122][123]. For example, the Radisic laboratory developed a powerful platform, dubbed AngioChip, that integrated tissue engineering and organ-on-a-chip technologies to produce vascularized polymer-based microfluidic cardiac scaffolds. Such a platform can be used to generate both in vitro heart tissue models and in vivo implants for potential clinical application [122].

6. 3D Bioprinting

3D bioprinting is one of the latest additions to the tissue engineering toolbox, and one that could be used to create complex and large vascularized tissues [124][125]. Several methods of 3D bioprinting have been used in the context of CTE, including cell-laden hydrogel 3D structures [126], inkjet bioprinting [127], laser-assisted bioprinting [128], and extrusion-based bioprinting [129]. Biomaterials used in 3D bioprinting are based on piezo-resistive, high-conductance, and biocompatible soft materials. Gaetani et al. bioprinted a 2 × 2 cm ECT construct using human cardiac progenitor cells and alginate matrices, which was maintained for 2 weeks in vitro [31] or transplanted onto rat infarcted hearts where it led to cell engraftment [130]. Generation of 3D-bioprinted vascularized heart tissues using mouse iPSC-CMs with human ECs in a PEG/fibrin hydrogel has been recently reported showing improved connectivity to the host vasculature after subcutaneous transplantation in mice [131]. Despite the early stage of development, 3D bioprinting is a very promising technology for recapitulating the complex structure of heart tissue and already shows enormous potential in CTE. In a recent study, Noor et al. succeeded in bioprinting thick (2 mm) 3D vascularized and perfused ECT constructs that had high cell viability using an extrusion-based bioprinter. As bioinks, the authors used an ECM-based hydrogel derived from human decellularized omentum containing hiPSC-CMs, and gelatin containing iPSC-derived ECs and FBs. Computerized tomography of a patient's heart was used to reproduce in vitro the structure and orientation of the blood vessels into the tissue. Bioprinted ECT constructs were transplanted into the omentum of rats and analyzed after seven days, when elongated and well-aligned CMs with massive striations were observed [56]. This study demonstrated that the possibility of using fully personalized materials makes 3D bioprinting technology very promising for clinical application by reducing the risk of immune rejection after transplantation. However, the system is still limited and further analyses should be performed to evaluate if heart tissue bioprinted in this manner could sustain normal blood pressure levels after transplantation [132].

3D bioprinting can also be combined with microfluidic systems to provide superior organ-level response with greater prediction of drug-induced capability [133]. On the other hand, recent advances in nanomaterial technology

present an attractive platform for the creation of ECT constructs for biomedical applications. Electrospinning technology allows creating nanofibers with controlled dimensions and further development of 3D structures [134]. In addition, the nanofibrous structure provides appropriate conditions for pluripotent cells to anchor, migrate and differentiate [135]. Increasing attention is being given to these types of structures due to their distinct mechanical properties, high porosity and potential to induce formation of aligned tissues that can be successfully implanted to the heart [136].

7. Other Structures

So far, we have described the variety of approaches undertaken for the generation of ECT constructs that reproduce increasingly complex features of the human myocardial tissue. However, several groups have pursued approaches intended to model whole heart chambers. In an early attempt in 2007, scientists created a “pouch-like” single ventricle using a mixture of hydrogel composed of type I collagen and Matrigel and NRCMs, which was cultured in an agarose casting mold with the dimensions of a rat ventricle. After formation, it was transplanted onto the rat heart showing limited functional integration [137]. One year later, Lee et al. created a “cardiac organoid chamber” by seeding NRCMs mixed with a collagen/Matrigel hydrogel around a balloon-like shaped mold with a thickness of 0.65 mm and sizes of 4.5 to 9 mm for the unloaded outer diameter, equivalent to the sizes of embryonic rat hearts at 9.5–11 days of development. The authors succeeded in the creation of a heart ventricle but with a moderate contractile capacity [138]. In subsequent work, the same group used a similar method to produce a functional human cardiac chamber using hESC-CMs embedded in collagen-based hydrogel. They proved that such technology could facilitate drug discovery as it provides the capacity to measure clinically meaningful parameters of the heart like ejection fraction and developed pressure, as well as electrophysiological properties [139]. The Parker laboratory has also recently generated tissue-engineered ventricles by using nanofibrous scaffolds composed of PCL and gelatin seeded with either NRCMs or hiPSC-CMs on a rotating ellipsoidal collector. The authors showed that cells aligned following the fiber orientation, thus recapitulating the orientation of the native myocardium, although the contractile function was much smaller than those of the native rat/human ventricles most likely owing to the small thickness of the chamber [140]. Notably, the Feinberg group printed human cardiac ventricles in 2019 using the freeform embedding of suspended hydrogels (FRESH) method. In this case, type I collagen was used as a support material to create an ellipsoidal shell that was filled with hESC-CMs in a fibrinogen suspension. These engineered tissues displayed synchronized functional activity [141]. Finally, Kupfer et al. have very recently generated a 3D bioprinted chambered muscle pump using an optimized bioink formulation of ECM proteins that allowed hiPSC proliferation prior to differentiation. In this way, 3D bioprinted hiPSCs underwent cell expansion and differentiated into CMs in situ, yielding contiguous muscle walls of up to 500 µm in thickness. The resulting human chambered organoids showed macroscale contractions and continuous action potential propagation and were responsive to drugs and to external pacing [142].

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