

Cite this article as: Roche CD, Brereton RJL, Ashton AW, Jackson C, Gentile C. Current challenges in three-dimensional bioprinting heart tissues for cardiac surgery. *Eur J Cardiothorac Surg* 2020;58:500–10.

Current challenges in three-dimensional bioprinting heart tissues for cardiac surgery

Christopher D. Roche ^{a,b,c,d}, Russell J.L. Brereton^b, Anthony W. Ashton^a, Christopher Jackson^a and Carmine Gentile ^{a,c,*}

^a Northern Clinical School of Medicine, University of Sydney, Kolling Institute, St Leonards, Sydney, NSW, Australia

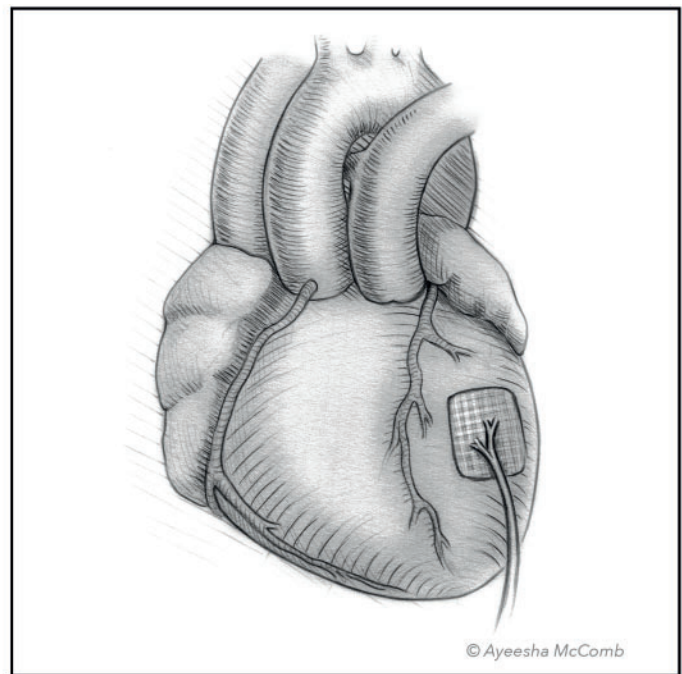
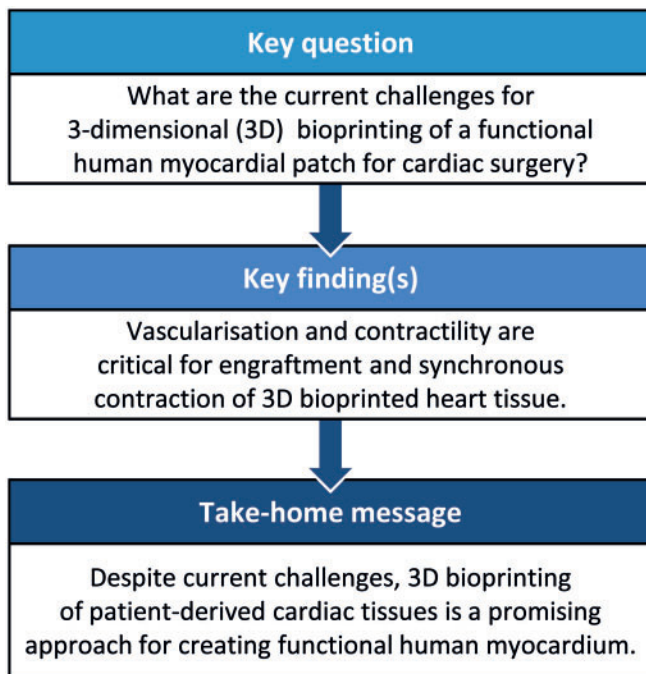
^b Department of Cardiothoracic Surgery, Royal North Shore Hospital, St Leonards, Sydney, NSW, Australia

^c Department of Biomedical Engineering, Faculty of Engineering and IT, University of Technology Sydney (UTS), Ultimo, Sydney, NSW, Australia

^d Department of Cardiothoracic Surgery, University Hospital of Wales, Cardiff, UK

* Corresponding author. Department of Biomedical Engineering, Faculty of Engineering and IT, Building 11, 81 Broadway, Ultimo, NSW 2007, Australia. Tel: +61-2-95144502; e-mail: carmine.gentile@uts.edu.au (C. Gentile).

Received 29 November 2019; received in revised form 27 January 2020; accepted 18 February 2020



Summary: Previous attempts in cardiac bioengineering have failed to provide tissues for cardiac regeneration. Recent advances in 3-dimensional bioprinting technology using prevascularized myocardial microtissues as ‘bioink’ have provided a promising way forward. This review guides the reader to understand why myocardial tissue engineering is difficult to achieve and how revascularization and contractile function could be restored in 3-dimensional bioprinted heart tissue using patient-derived stem cells.

Keywords: Bioprinting • Revascularization • Transplantation • Regenerative medicine • Cardiac tissues • Stem cells

ABBREVIATIONS

CFs	Cardiac fibroblasts
CMs	Cardiomyocytes
3D	3-Dimensional
ECM	Extracellular matrix
ECs	Endothelial cells
ESCs	Embryonic stem cells
iPSCs	Induced pluripotent stem cells

INTRODUCTION

With recent advances in the technological triad of induced pluripotent stem cells (iPSCs), 3-dimensional (3D) cardiac microtissues (referred to as 'cardiospheres', 'cardiac spheroids', 'cardiac organoids' [1]) and 3D bioprinting, the enticing prospect has arisen of fabricating cardiac tissues to replace diseased native tissues and to regenerate damaged hearts [2–10].

The incentive for this is strong: in the UK, 30–40% of patients diagnosed with heart failure will die within 1 year of diagnosis with an additional 10% annually thereafter [11]. Moreover, the period before death is associated with a severe disease burden and reduced quality of life [12]. For end-stage patients, the gold standard treatment is a heart transplant [13]. However, this is not suitable for all, carrying significant morbidity and a global mortality rate of ~15% and 25% at 1 and 5 years, respectively [14]. In addition, there is significant urgency to find a treatment, which is not waiting list dependent, for those patients awaiting a donor. Last year, patients on the Australian heart transplant list had a 56% chance of being transplanted by the end of the year, a 31% chance of being carried over to next year and a 3.5% chance of dying whilst waiting [15]. For the UK, the equivalent figures were 29% transplanted, 55% carried over and 3% dying [16]. The median waiting time for an adult categorized as non-urgent on the UK heart transplant list approaches 3 years [16].

In this context, several new approaches have been developed for regenerating the myocardium [4–7, 9, 10, 17–23]. Recent studies have demonstrated the feasibility for 3D bioprinting of myocardial tissues from patient-derived stem cells; however,

limitations of such approaches still remain, including full vascularization and synchronous contractile activity [5, 9, 10]. This review explores why this is difficult and why it is worth pursuing.

CHALLENGES: OVERVIEW

General challenges limiting engineered cardiac tissues include (i) limited tissue survival following transplantation; (ii) limited ability to generate tissue of adequate size; (iii) optimizing the mix of cardiac cell types; (iv) cell sourcing from patients; (v) the immature phenotype of stem cell-derived cardiac cells; (vi) safety concerns for potentially undifferentiated stem cells; and (vii) immunogenicity requiring immunosuppression (Table 1).

Other considerations particularly pertaining to myocardial generation are the need for: (i) a vascular network spanning many orders of magnitude from arterial to capillary level and (ii) a synchronously contractile, electrically conductive tissue.

STEM CELLS AND THEIR NICHE

It is not feasible to use mature, differentiated, adult myocardial cells for the therapeutic regeneration of myocardial tissue, as these cells exhibit reduced viability and proliferation when engrafted [17]. Instead, current approaches prefer stem cells that can be coaxed into desired, fully differentiated forms in response to microenvironmental cues, which can be controlled [4–6]. With the exception of bone marrow transplant, stem cells have not fulfilled their expected clinical potential in regenerative medicine [24]. However, the advent of iPSCs provides a promising cell source for regeneration, including 3D bioprinting of myocardial tissue [5, 9, 25, 26].

It has long been understood that the stem cell microenvironment, called the 'niche', provides cues that determine how tissues develop and function [27–29]. These cues include extracellular matrix (ECM) proteins, temperature and oxygen, which are known to influence tissue growth and stem cell behaviour [28]. Cells in a suboptimal niche may not survive and will perform poorly [28]. Cells implanted directly into diseased host myocardium exhibit poor survival and ability to organize into functional

Table 1: Challenges for the development of bioengineered cardiac tissues

General	Vascularization	Contractility
Cell survival postimplantation in diseased environment	Observation of appropriate vascular tissue maturation without cell destruction or alteration	Integration with host depolarization-repolarization wave without arrhythmogenicity
Host immune response to implanted material	Functional validation of the bioprinted vascular tree	Engineering the correct electrochemical environment for cardiomyocyte maturation
Possible biological scaffold needed with its own biofabrication challenges	Surgical integration with pre-existing host vasculature	Coculture with other cell types for appropriate maturation of stem cell-derived cardiomyocytes
Correct cell mix mimicking mature myocardial tissue	Vascular lumen generation and integration of hierarchical arteriovenous tree segments	Optimization of 3D culture techniques required for formation of cell–cell connections
Expanding cells to an appropriately high number	Stem cell selection for clinically useable vascular cell source	
Reproducibility and scalability to meet demand	Time requirement for vascular tissue maturation <i>in vitro</i> (pre) and <i>in vivo</i> (post) implantation	
Cost-effectiveness		
Ethics/safety		

3D: 3-dimensional.

tissues, as they lack the appropriate microenvironment [17, 30]. The native heart contains ~2 billion cardiomyocytes (CMs) and many more of other cell types [31]. The exact cardiac cell ratio is not universally agreed upon; however, fully differentiated adult human ventricular myocardium contains 33% CMs, 24% endothelial cells (ECs) and 43% other cells (including fibroblasts) [32]. To provide a 3D microenvironment for cells to grow before their transplantation, they have been cultured as 3D patches [4, 6, 22]. To further improve cell patch survival, these have been delivered either under host pericardium [33] or matured on vascular host omental tissue before transfer to the epicardium [34].

In addition to the niche, it is important to consider that different stem cell types present their own unique challenges (Table 2) [29].

'Embryonic stem cells' (ESCs) are pluripotent (i.e. they may differentiate into mature cells of any of the 3 original germ cell layers), do not undergo senescence in culture and can self-renew indefinitely [29]. However, whilst harvesting from an embryo raises ethical concerns, the host would also require immunosuppression from their implantation [29, 33] and there is a risk of developing malignancy [29, 35].

Table 2: Limitations of using stem cells for 3D bioprinting myocardial tissue

Stem cell type	Known limitations
Embryonic	Immunosuppression required Ethical concerns Teratoma formation
Adult	Finite capacity for self-renewal Limited mature cell types Senescence in culture
Induced pluripotent	Teratoma formation

3D: 3-dimensional.

'Multipotent adult stem cells', such as mesenchymal stem cells from either bone marrow-mesenchymal stem cells or adipose tissue-mesenchymal stem cells, could be readily isolated from the host [29]. This eliminates ethical concerns, and as autografts, there is no need for immunosuppression [29]. However, unlike ESCs, adult stem cells have a limited differentiation repertoire, a finite ability to self-renew and undergo senescence during *in vitro* expansion [29].

Alternatively, somatic cells (skin/blood/urine) can be reprogrammed into iPSCs by transfecting them with a variety of transcription factors [25, 26]. Like adult stem cells, autologous iPSCs are patient specific and not isolated from an embryo [29]. Yet, like ESCs, they self-renew without undergoing senescence and can be coaxed to differentiate into almost any mature cell phenotype in culture [26, 29, 36]. However, iPSCs present the risk of teratoma formation when transplanted if not fully differentiated [29]. An additional risk comes from using a virus to transfect the stem cell factors, which may place the patient at the risk of malignancy [25]. As an alternative, they can be reprogrammed using a non-integrating RNA vector and without the use of a viral vector [25].

3-DIMENSIONAL BIOPRINTING AND BIOINK CHALLENGES

The definition of biofabrication for regenerative medicine is: 'the automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates such as microtissues, or hybrid cell-material constructs, through bioprinting or bioassembly and subsequent tissue maturation processes' [37].

In this process, the 3D bioprinter is used to deposit bioink(s) in hydrogels to generate viable and functional tissues, with the end product controlled by the 3D bioprinting platform used [38]. For cardiac tissue, some of the most common systems use extrusion-based bioprinters with pneumatic or screw-driven

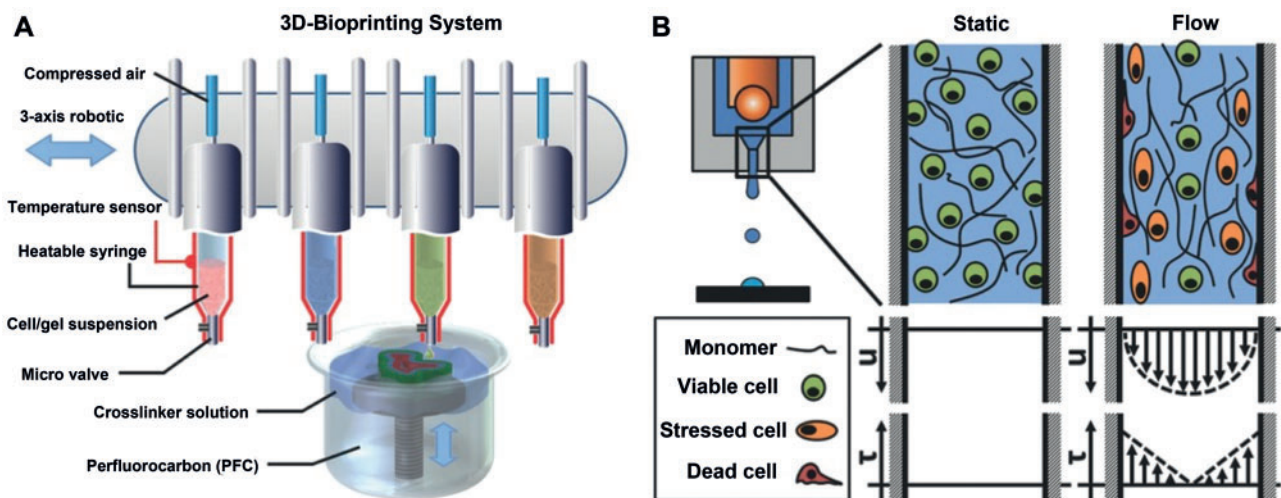


Figure 1: Extrusion-based 3D bioprinting and cell viability. **(A)** Four-nozzle extrusion 3D bioprinting system using pneumatic force to extrude 4 different bioinks. In this example, bioinks are extruded directly into a crosslinker solution, which acts on the bioink to create bonds within the bioprinted structure to retain its shape. **(B)** Downward arrows with greater size indicate greater velocity centrally in the bioink and lower velocity at the periphery, which is in contact with the chamber wall. Upward arrows show resulting shear stress on cells in bioink during 3D bioprinting. Greater shear at the periphery results in stressed cells (orange) and some dead cells (red). Shear stress can be reduced by slowing down the velocity of extrusion, which is readily controlled in 3D bioprinting (reproduced with permission from Blaeser *et al.* [18]). 3D: 3-dimensional.

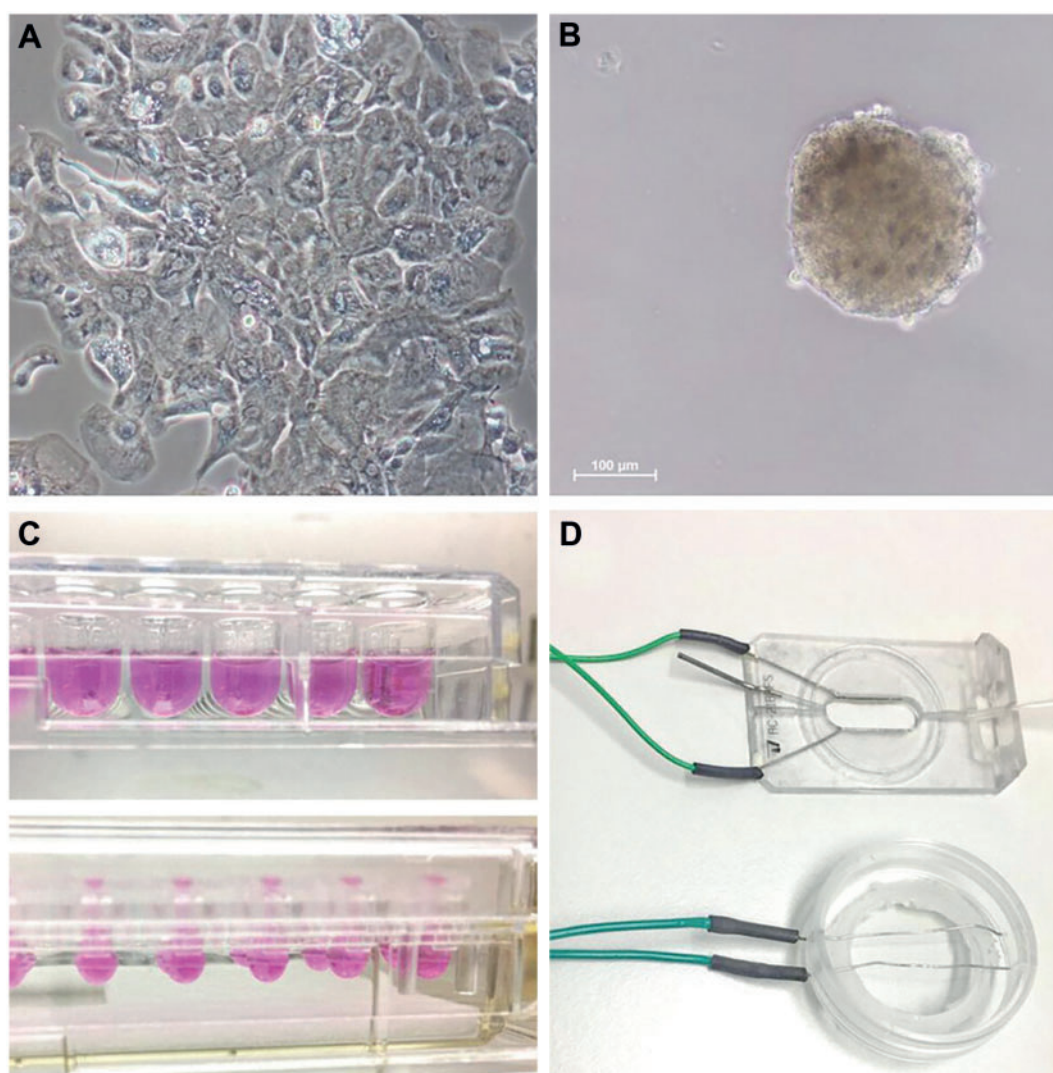


Figure 2: Three-dimensional cardiac microtissue generation and physiology. **(A)** Standard 2-dimensional cell culture of human-induced pluripotent stem cell-derived cardiomyocytes. **(B)** Single (~200 μm diameter) cardiac spheroid microtissue—a 3-dimensional aggregate of cardiac cells with ability to control spheroid size and cell number and optimize cell–cell interactions, 3-dimensional mechanical signals and extracellular support. **(C)** Two methods for generating cardiac spheroids: U-shaped non-adhesive wells (top panel) and hanging drop cultures (bottom panel). **(D)** Two types of field pacing chambers for providing electrical stimulation to spheroids for the optimal maturation of cardiomyocyte phenotype: a perfusion chamber (above) and culture dish (below) with electrodes to allow for field potential stimulation across cardiac spheroid culture area (modified with permission from Zuppinger [1]).

rotational force to move cellular bioink through a nozzle [3]. The bioprinting method chosen is critical to meet the challenge of delivering cells with minimal shear stress and avoid damaging them in the process (Fig. 1) [18]. A variety of approaches, bioprinters, bioinks and hydrogels to print cells into 3D myocardium have been explored [3, 5].

Ideal bioinks for cardiac regeneration should mimic the native 3D myocardial microenvironment [6, 30]. Bioinks based on cardiac tissue spheroids (which are scaffold free, self-sustainable cell aggregates with a defined diameter and cell number) have been used as building blocks in this process (Fig. 2) [20, 30, 39]. Optimizing cell-specific challenges should be considered when creating cardiac spheroids in bioinks for heart tissue. This includes a clinically relevant scale-up of cell numbers for transplantable bioprinted heart tissues [3, 40]. In addition, mature adult CMs cannot be used for spheroid-based bioinks as they have low adhesive properties and do not form spheroid cultures [41], whereas proliferating neonatal and iPSC-derived CMs (iPSC-

CMs) form spheroids within a few days [30]. Furthermore, addition of more adhesive cells to the cellular mix within a spheroid, such as cardiac fibroblasts (CFs), reduces the time for spheroid formation [42]. ECs are a third important cell type found in the human heart, and *in vitro* cardiac spheroids have been generated using a ratio of primary adult CMs:ECs:CFs of 1:3:6, which was optimal for adult CM viability, whereas for CMs derived from iPSCs, a ratio of 2:1:1 was optimal for iPSC-CMs, ECs and CFs, respectively [36, 42].

The use of preformed vascularized microtissues such as spheroids for 3D bioprinting offers the prospect of overcoming many bioink cell-related challenges for tissue engineering of viable and functional myocardium [6, 43]. This includes, but is not limited to, generating a vascular system within the bioink and better integration of physiological contractile function, including speed of contraction, contraction amplitude and calcium transients [1, 44].

In addition to the cellular component, the hydrogel used to support cells in bioink plays a major role in overall patch

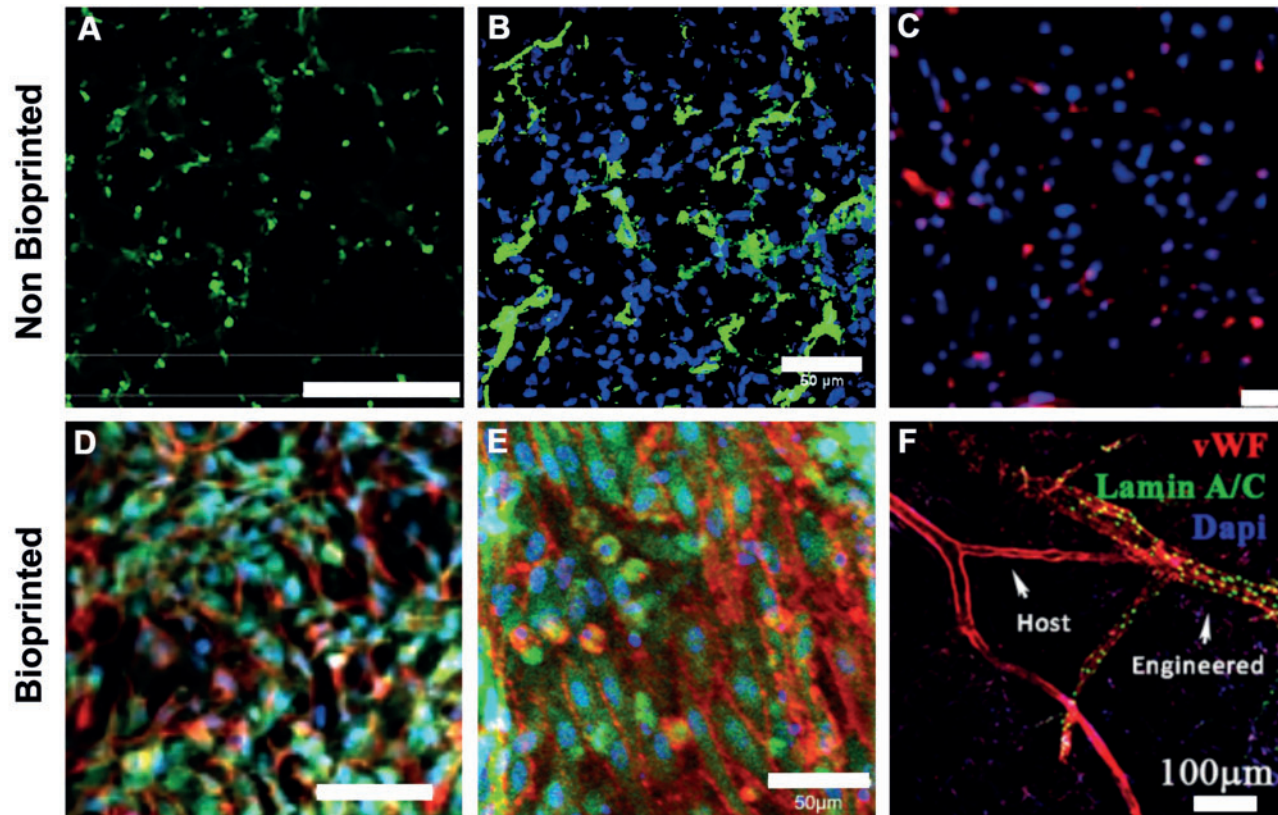


Figure 3: Vascularization in tissue-engineered cardiac patches. (A) CD31 expression (green) in a cardiac patch generated by a fibrinogen moulding method and engrafted in pigs (modified with permission from Gao *et al.* [4]). (B) CD31 expression (green) in a cardiac patch generated by a net moulding method and engrafted in rats (modified with permission from Yang *et al.* [19]). (C) CD31 expression (red) in a fibrin-based cardiac patch generated by a spheroid fusion method and engrafted in mice (modified with permission from Mattapally *et al.* [6]). (D) CD31 expression (green) in a cardiac patch generated by a 3-dimensional bioprinting method (modified with permission from Noor *et al.* [5]). (E) CD31 expression (red) in a cardiac patch generated by a 3-dimensional bioprinting method (modified with permission from Zhang *et al.* [10]). (F) vWF (red) and lamin A/C (green) expressions in a cardiac patch generated by a 3-dimensional bioprinting method and engrafted in mice with the evidence of host-patch anastomosis (modified with permission from Maiullari *et al.* [9]). Scale bar appearances are due to source data. vWF: von Willebrand factor.

geometry, size, survival and function by better mimicking the mechanical properties of the native ECM [45]. Hydrogel/bioink composition determines important characteristics such as biocompatibility, biodegradability, paracrine signalling, non-immunogenicity and stiffness, all of which present optimization challenges [46–48]. To date, the formulation of cardiac bioinks with optimal chemical–mechanical properties to better control the cardiac niche has not been defined [46, 48]. The requirement for a cell-friendly, flowing bioink limits other factors such as the precision and resolution of the print, although this may be improved by applying microfluidic devices to the extrusion nozzles of bioprinters [9, 49]. Whilst the bioink must flow well during the print, it should also be mechanically robust and yet still allow for the tissue to remodel and the cells to interact in the post-printing phase [46].

Several hydrogels have been tested for cardiac tissues, either using natural materials (such as decellularized cardiac ECM [46] and gelatin-based hydrogels combined with fibrinogen, alginate or hyaluronan [47]) or synthetic-origin materials (such as polyurethane) [50]. Decellularized cardiac ECM promotes angiogenesis and cell proliferation but cannot be easily isolated from the same donor heart [46]. Gelatin has excellent rheological properties and biodegradability, but its use is highly temperature dependent [47]. Electrically conductive polymeric hydrogels such as

poly(ethylenedioxythiophene) allow for improved cell electroactivity, whilst they present undesired properties such as increased hydrophobicity and reduced elasticity [45, 51]. Other hydrogels may present cell-specific paracrine signalling factors [48]. Therefore, the challenges associated with engineering the optimal hydrogel are of paramount importance and should address several chemical and biomechanical parameters.

VASCULARIZATION

Another major challenge for creating a cardiac patch is engineering a hierarchical vascular tree (Table 1) [10, 39]. The maximum tissue diameter without the development of a necrotic core varies since it depends on cell-specific oxygen consumption, but it generally is common in any cardiac tissue thicker than $\sim 200\mu\text{m}$ in diameter [9, 30]. Neovascularization has been attempted by culturing cardiac tissues in highly vascular areas *in vivo*, such as the omentum [34]. Others have used hydrogels from dissolved omentum, with potential use for 3D bioprinted cardiac tissues [5, 44]. However, to date, only capillary-sized, disorganized vasculature has been generated and recreating a fully branched vascular network for cardiac tissue engineering remains a challenge (Fig. 3) [4–6, 9, 10, 19, 49].

Further progress is being made in engineering vascularized tissues by co-culturing CMs with ECs. For example, a microvascular network was achieved in spheroid cultures containing rat ECs, CMs and fibroblasts before their implantation in nude rats [52]. It has been suggested that a better branched vascular network could be achieved by fusing prevascularized spheroids [20, 39, 43]. Vascular network anastomosis between graft and host *in vivo* is critical for new blood flow to promote the engraftment and function of transplanted patches [7, 9, 20, 39, 53]. At a cellular level, such graft–host anastomoses may form directly, for instance by a ‘wrap and tap’ mechanism whereby graft lumen-forming ECs anastomose with host vasculature by wrapping around host vessels and ‘tapping’ through the vessel walls, facilitated by matrix metalloproteases [54]. Paracrine signalling including a ‘secretome’ of cytokines, exosomes and growth factors such as vascular endothelial growth factor is a major regulator of tissue angiogenesis and potentially of cardiac regeneration [4, 9, 23, 48, 55].

The emerging evidence that regenerative transplanted tissue may act by mechanisms other than direct replenishment of lost cells would explain why functional improvement has been seen for patches containing only 8 million cells [4, 33]. It would be advantageous if paracrine mechanisms turned out to be more important than numerical replenishment, as another major challenge is how to expand replacement cells, including non-myocytes (such as ECs and CFs) and myocytes, to a high enough number [3, 40].

An alternative method to bioprinted cardiac tissues is to pre-form a vascular scaffold. This may be bioprinted in patterns such as networks by depositing cellular bioink [8, 10, 49]. For example, Zhang *et al.* [49] used microfluidic printheads to bioprint ECs within hydrogels. These were fixed in position by ionic and ultraviolet (UV) light crosslinking. The ionic crosslinker (calcium chloride) was added to the alginate scaffold in real time during the print, followed by UV crosslinking of gelatin methacryloyl. Following formation of a vascularized scaffold, CMs were added to the construct to generate cardiac tissue.

CONTRACTILE FUNCTION

For myocardial tissue to first engraft in the host and eventually improve cardiac function, it should not only synchronously contract by itself but also together as a functional syncytium with the host [40]. Electrical properties of cells and tissues, such as cardiac excitation–contraction coupling, calcium transients and cell–cell interactions, are critical [1, 40, 51]. iPSC-CMs do not present the fully mature contractile behaviour typical of adult cells, unless they are cultured with additional cellular and extracellular stimuli, such as electromechanical conditioning, electrical field stimulation and continuous pacing (Fig. 4 and Videos 1 and 2) [21, 56]. Cardiac spheroids from iPSC-CMs spontaneously develop synchronous contractile function, which is linked with improved speed and amplitude of contraction, and calcium transients compared to single-cell cultures [1, 36, 42]. Adding conductive polymers propagates the electrical signal between different areas in a cell culture, providing assistance with current passage in the absence of fully developed cell–cell electrochemical pathways [51, 57]. Overall, physiological contractile function and integration of engineered tissue with the host remain some of the main challenges to overcome (Table 1) [1, 21, 56].

PRECLINICAL SUCCESS

In animal models of myocardial infarction, functional improvement after the engraftment of heart patches generated from cells in moulding devices has already been demonstrated in mice [6], rats [19] and pigs [4]. Direct injection of hydrogel laden with paracrine signalling factors has shown a significant reduction in infarct size from 38% to 16% in a rat model of myocardial infarction [48]. Furthermore, a small rat-sized cardiac ventricle (1/250th the size of a human ventricle) has been tissue-engineered by MacQueen *et al.* [58]. However, this only approximated 100 millionth (-10^8) of the contractile work of a native human ventricle. Indeed, at $\sim 1\%$, the ejection fraction of this model was $\sim 1/50$ th of a normal rat’s ejection fraction and the cell density was 10-fold less than normal for a rat. Other studies that used 3D bioprinting reported improved cardiac patch vascularization, automation and precision *in vitro* and *in vivo* in mice [5, 9, 10]. These studies provide examples demonstrating significant pre-clinical progress and pave the way for human trials and scaled up models.

SURGICAL PERSPECTIVES

Whilst surgical treatment of aortic, coronary and valve disease can now be undertaken with excellent results, repair or reconstruction of the contractile pump element, the myocardium, is currently limited [2]. Excluding infrequently performed procedures such as the Dor (pericardial-lined Dacron endoventricular circular plasty) [59] and Batista (reduction ventriculoplasty) [60] procedures, the only option beyond resynchronization is transplantation [7]. Whether this is human or genetically modified xenotransplantation, the loss of the recipient’s heart is obligatory and absolute dependence on the function of the new heart follows. Cellular cardiomyoplasty has been evaluated to allow the augmentation of existing cardiac function but, despite showing some functional merit, has largely been disappointing overall [23].

Early clinical trials using ESC-derived cardiac cells in patients have shown promising results by culturing cells in a patch before transplantation [33, 61]. Following the first case report of a cellular patch applied to a human heart in 2015 [61], the same group reported that transplantation of these patches in 6 patients with heart failure is safe (the ESCORT trial) [33]. Cardiac patches of $\sim 20\text{ cm}^2$ were surgically applied to the epicardium of patients undergoing coronary artery bypass surgery and secured under a pericardial flap (Fig. 5). As the patches were not 3D bioprinted, the advantages of a scalable manufacturing process were not present; nonetheless, the trial paved the way for more extensive trials testing efficacy.

With prevascularized patches, the study by Maiullari *et al.* [9] showed that host–patch anastomosis in mice is feasible using 3D bioprinted poly(ethylene glycol)-fibrinogen hydrogels embedded with iPSC-CMs and human umbilical vein ECs (Fig. 3F). An augmentative approach may be the use of pro-angiogenic factors to promote vascular fusion between graft and host, similar to that which is observed during vascular development [43].

To progress with the transplantation of thick bioprinted heart tissues with potentially low-calibre vasculature in patients, the surgeon may be tasked with creating a macroscopic anastomosis of the graft to an existing blood supply [53]. This would especially



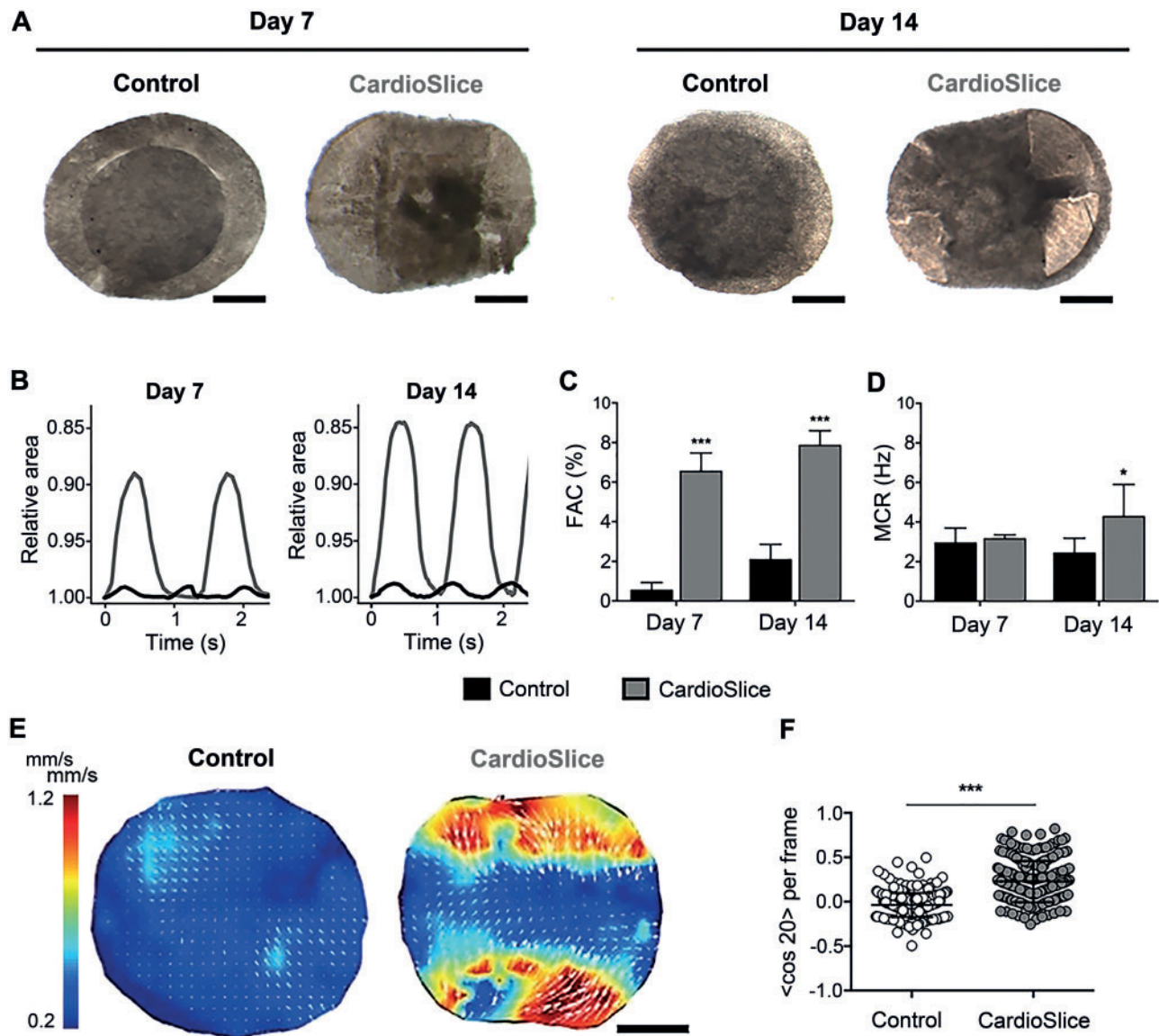


Figure 4: Electrical stimulation of cell culture leads to greater contractility in engineered cardiac tissue. Beating macro-tissues were generated by seeding induced pluripotent stem cell-derived cardiomyocytes and human fibroblasts in a 3-dimensional porous scaffold and culturing for 14 days as either control (no electrical stimulation) or 'CardioSlice' (electrical stimulation applied whilst culturing). **(A)** Human cardiac macro-tissues after 7 or 14 days of culture, either without (control) or with (CardioSlice) electrical stimulation (scale bars 2.5 mm) (see also Videos 1 and 2). **(B)** Contraction amplitude of control versus CardioSlice bioengineered cardiac tissues. Fractional contraction area (compared to area of the tissue at rest) is represented over time, and control tissue remains close to 1.0 when contracting whereas CardioSlice patches contract to 0.85 (85% of size of tissue at rest). **(C)** The percentage of FAC for control (unstimulated) versus electrically stimulated CardioSlice cardiac macro-tissues. **(D)** MCR for control compared to electrically stimulated CardioSlice macro-tissues when paced. At 14 days, CardioSlice macro-tissues were able to be paced at over 4 Hz, approximately double the MCR of control tissues. **(E)** Beating cardiac macro-tissue velocity maps after 14 days of culture. Blue colours and shorter white mini arrows represent lower velocities. Higher velocities (redder areas and longer mini arrows) were observed in CardioSlice macro-tissues versus controls (scale bar 2.5 mm). **(F)** Alignment analysis comparing direction of the electrical field vector and subsequent beating direction of bioengineered cardiac tissues. The order parameter $\cos 2\theta$ was used: random distribution gives values close to 0 whereas parallel alignment gives values close to 1 (modified with permission from Valls-Margarit *et al.* [21]). FAC: fractional area change; MCR: maximum capture rate.

be true if naturally forming low-calibre anastomoses to the diseased native myocardial tissue underneath the graft were insufficient. Foreseeably, microsurgical anastomoses may have to be made initially to allow time for naturally occurring graft-host connections to form [53].

One approach to promote host-patch vascular anastomosis could be to use non-diseased, larger-calibre source vessels such as the gastroepiploic or thoracodorsal artery with the tissue cultivated on the omentum or the latissimus dorsi muscle, respectively. Bioengineered tissue could be allowed time to form

anastomoses with the underlying tissue, and capillaries formed from ECs within a tissue-engineered cardiac patch can retain patency [53]. Blood flow through the graft could be assessed in advance whilst it lay on its incubating native tissue. Once blood flow and viability of the patch are demonstrated, it should be surgically feasible to then rotate a flap to the myocardium within the pericardium without loss of blood supply (Fig. 6). Conversely, minimally invasive procedures could be used to transplant bioprinted cardiac tissues in innovative ways for cardiac surgical patients [62].



Video 1: Bioengineered human cardiac tissue showing spontaneous beating after 14 days in culture—non-stimulated control tissue shown (reproduced with permission from Valls-Margarit *et al.* [21]).



Video 2: Bioengineered human cardiac tissue showing spontaneous beating after 14 days in culture—electrically stimulated (CardioSlice) tissue shown (reproduced with permission from Valls-Margarit *et al.* [21]).

TRANSLATIONAL PERSPECTIVES

As is expected when multiple emerging technologies/techniques (iPSCs, 3D culture, bioprinting) make rapid preclinical gains, translation of the preclinical potential into a useful clinical therapeutic presents specific challenges [40, 63, 64]. Laboratory workflows for autologous iPSCs are appealing in offering a fully patient-specific supply of tissue; however, they can be relatively difficult and resource intensive to achieve for 1 patient per workflow [23]. For translation, workflows need to conform to good manufacturing practice standards, usually making them more challenging, for example, by replacing commonly used xenogenic laboratory products such as foetal bovine serum with human serum albumin or carrying out the work in a fully certified good manufacturing practice facility [63, 64]. An alternative, using allogeneic iPSCs from donors, comes with the advantage of potentially being able to cryobank multiple haplotyped iPSCs and iPSC-derived cells or tissues [23, 40]. Having the cells banked in cold storage could allow for a more technically, logistically and economically feasible solution: an 'off-the-shelf' selection of a best-matched tissue at the time of need [23, 40]. However, this would be accompanied with immunosuppression, difficulties acquiring matched tissue (especially for diverse ethnic groups) and a likely need for international coordination for a large enough tissue repository [23, 40].

DISCUSSION

Whilst 3D bioprinting of a fully functional human myocardial patch has not yet been achieved, there are several areas where 3D bioprinting of other tissues has already reached clinical trials. For example, 3D bioprinting of ears of 5 children showed promising results up to 2.5 years of follow-up [65]. However, 3D bioprinting of heart patches presents a different level of complexity.

Important questions remain regarding the application of bioprinted tissue for human cardiac surgery and the patient population it will most benefit [2, 3]. Whether advances in other technologies, such as ventricular assist devices, or even a

refined surgical plication technique, could be combined with bioprinted heart tissue in innovative ways remains an open question.

Throughout this review, there has been an assumption that the optimal microenvironment of cardiac tissue should be achieved, but this may not be required and good results may be obtained even in the absence of cells [55, 62]. Recently, a study showed that macrophages may play a major role in cardiac regeneration by inducing inflammation following stem cell injection [66]. It is possible that mechanisms such as this could be implicated in the surprising finding that even acellular patches or hydrogels applied to the heart may have a positive effect on cardiac regeneration via the immune response [55, 62]. Nevertheless, any foreign material or cells within a patch will lead to an immune response with a rim of fibrosis, potentially isolating the graft from the host [40]. Therefore, a better understanding of these mechanisms may be beneficial to developing novel approaches to better couple the graft with the host. These may include the use of either conductive polymers (to allow electroactivity to bypass the fibrotic rim) [57], sacrificial hydrogels disintegrating over time [8, 53] or adjunctive anti-inflammatory and/or 'pro-survival' compounds [67, 68].

It would be critical to compare the cost-effectiveness of a stem cell-derived 3D bioprinted cellular patch to other techniques for long-term treatment but with the price of bioprinters falling rapidly [38], cells derived from patients themselves and workflows utilizing basic laboratory materials [1, 47], there is reason to speculate that 3D bioprinted patient-specific multicellular patches could be a cost-effective surgical therapeutic.

For end-stage cardiac pathology, this is one of the few emerging technologies that provides hope of a cure. It is unique in that it may offer a paradigm-shifting solution for patients with cardiac failure who could otherwise only be 'cured' with a heart transplant. This potential generates considerable media attention which raises specific ethical considerations. These are only recently being elucidated in the bioethical literature, for example, how media hype can inflate expectations for desperate patients, which may also create an environment for unscrupulous health providers, or how public misinformation may impact patients' decision-making significantly [63, 69]. Safe translation of animal

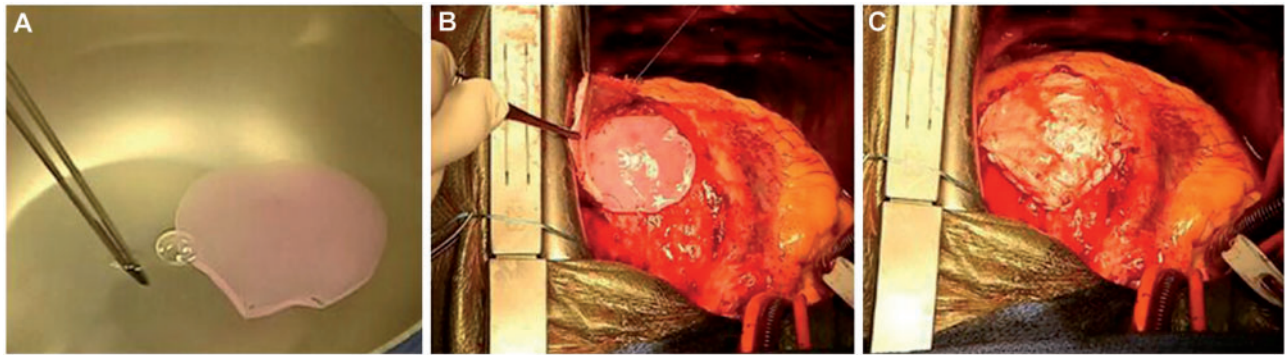


Figure 5: Embryonic stem cell-derived cardiac patches for patients with heart failure undergoing coronary artery bypass grafting (CABG). (A–C) A fibrin-based patch infused with allogenic cardiac progenitor cells derived from embryonic stem cells was applied to the epicardial surface of human cardiac failure patients undergoing CABG and shown to be safe in a phase I clinical safety trial (modified with permission from Menasché *et al.* [33]).

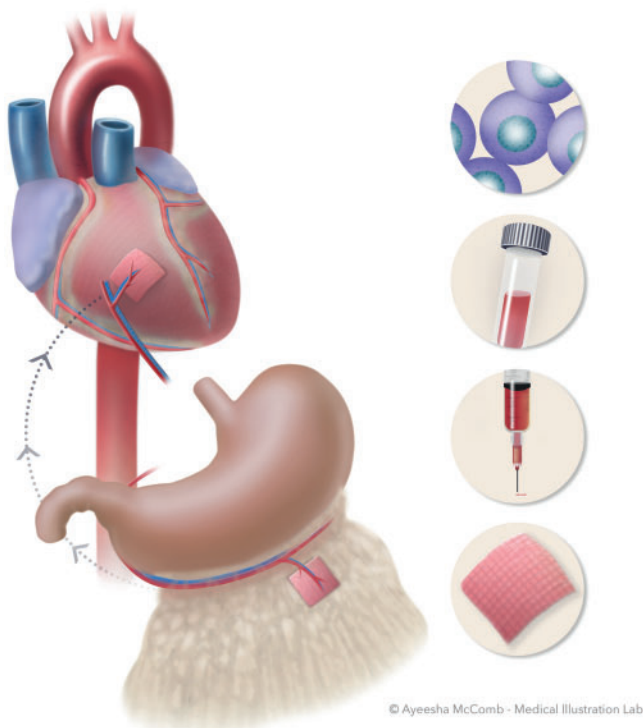


Figure 6: The human body as a natural bioreactor for bioprinted cardiac patch vascularization. Patient-specific induced pluripotent stem cell-derived bioinks obtained by reprogramming cells taken from patient blood could be used to 3-dimensional bioprint cardiac tissue patches. This tissue could be matured on the patient's omentum, anastomosed to the gastroepiploic artery and subsequently rotated onto the epicardial surface to regenerate the myocardium—a waiting list-free and surgically feasible alternative to donor heart allotransplantation.

to human trials may require new 3D bioprinting-specific regulation [69].

CONCLUSION

For patients with non-functional areas of myocardium, 3D bioprinting of personalized heart tissues presents several challenges but also the potential to develop a clinically available approach in the coming years. If successful, this technology has the potential to re-shape the cardiac therapeutic environment,

resolve an unmet need for surgical practice and actualize a long-standing desire for surgeons to promote cardiac regeneration in patients.

ACKNOWLEDGEMENTS

The authors thank Ayeesha Clare McComb, Chief Medical Illustrator at Medical Illustrations laboratory, for her work in the design and generation of illustrations for this manuscript.

Funding

Christopher D. Roche was supported by a Sir John Loewenthal Scholarship 2019 (University of Sydney), the Le Gros Legacy Fund New Zealand (PhD012019) and a Heart Research Australia PhD Scholarship (2019-02). Carmine Gentile was supported by a University of Sydney Kick-Start Grant, University of Sydney Chancellor's Doctoral Incentive Programme Grant and a Sydney Medical School Foundation Cardiothoracic Surgery Research Grant.

Conflict of interest: none declared.

Author contributions

Christopher D. Roche: Conceptualization; Data curation; Formal analysis; Funding acquisition; Project administration; Visualization; Writing—original draft; Writing—review & editing. **Russell J.L. Breerton:** Conceptualization; Funding acquisition; Validation; Writing—original draft; Writing—review & editing. **Anthony W. Ashton:** Writing—review & editing. **Christopher Jackson:** Writing—review & editing. **Carmine Gentile:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Project administration; Supervision; Writing—original draft; Writing—review & editing.

REFERENCES

- [1] Zuppinger C. Measurement of contractility and calcium release in cardiac spheroids. *Methods Mol Biol* 2019;1929:41–52.
- [2] Beyersdorf F. Three-dimensional bioprinting: new horizon for cardiac surgery. *Eur J Cardiothorac Surg* 2014;46:339–41.
- [3] Puluca N, Lee S, Doppler S, Münsterer A, Dreßen M, Krane M *et al.* Bioprinting approaches to engineering vascularized 3D cardiac tissues. *Curr Cardiol Rep* 2019;21:90.

- [4] Gao L, Gregorich ZR, Zhu W, Mattapally S, Oduk Y, Lou X *et al.* Large cardiac muscle patches engineered from human induced-pluripotent stem cell-derived cardiac cells improve recovery from myocardial infarction in swine. *Circulation* 2018;137:1712–30.
- [5] Noor N, Shapira A, Edri R, Gal I, Wertheim L, Dvir T. 3D printing of personalized thick and perfusable cardiac patches and hearts. *Adv Sci* 2019; 6:1900344.
- [6] Mattapally S, Zhu W, Fast VG, Gao L, Worley C, Kannappan R *et al.* Spheroids of cardiomyocytes derived from human-induced pluripotent stem cells improve recovery from myocardial injury in mice. *Am J Physiol Heart Circ Physiol* 2018;315:H327–39.
- [7] Zhang J, Zhu W, Radisic M, Vunjak-Novakovic G. Can we engineer a human cardiac patch for therapy? *Circ Res* 2018;123:244–65.
- [8] Miller JS, Stevens KR, Yang MT, Baker BM, Nguyen DH, Cohen DM *et al.* Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat Mater* 2012;11:768–74.
- [9] Maiullari F, Costantini M, Milan M, Pace V, Chirivi M, Maiullari S *et al.* A multi-cellular 3D bioprinting approach for vascularized heart tissue engineering based on HUVECs and iPSC-derived cardiomyocytes. *Sci Rep* 2018;8:13532.
- [10] Zhang YS, Arneri A, Bersini S, Shin SR, Zhu K, Goli-Malekabadi Z *et al.* Bioprinting 3D microfibrillar scaffolds for engineering endothelialized myocardium and heart-on-a-chip. *Biomaterials* 2016;110:45–59.
- [11] Hobbs FD, Roalfe AK, Davis RC, Davies MK, Hare R; the Midlands Research Practices Consortium (MidReC). Prognosis of all-cause heart failure and borderline left ventricular systolic dysfunction: 5 year mortality follow-up of the Echocardiographic Heart of England Screening Study (ECHOES). *Eur Heart J* 2007;28:1128–34.
- [12] National Institute for Health and Care Excellence (NICE). Chronic Heart Failure in Adults: Diagnosis and Management. NICE Guideline [NG106], 2018. <https://www.nice.org.uk/guidance/ng106> (29 November 2019, date last accessed).
- [13] MacGowan GA, Crossland DS, Hasan A, Schueler S. Considerations for patients awaiting heart transplantation—insights from the UK experience. *J Thorac Dis* 2015;7:527–31.
- [14] Lund LH, Khush KK, Cheriak WS, Goldfarb S, Kucheryavaya AY, Levvey BJ *et al.* The registry of the International Society for Heart and Lung Transplantation: thirty-fourth adult heart transplantation report-2017; focus theme: allograft ischemic time. *J Heart Lung Transplant* 2017;36:1037–46.
- [15] Australian and New Zealand Cardiothoracic Organ Transplant Registry (ANZCOTR). 2018 Report. http://www.anzcotr.org.au/pub/e0cc941a/PDFS/ANZCOTR2018_text.pdf (29 November 2019, date last accessed).
- [16] UK NHS Blood and Transplant. Organ Donation and Transplantation Annual Activity Report. 2018/2019. <https://nhsbt.dbe.blob.core.windows.net/umbraco-assets-corp/16537/organ-donation-and-transplantation-activity-report-2018-2019.pdf> (29 November 2019, date last accessed).
- [17] Reinecke H, Zhang M, Bartosek T, Murry CE. Survival, integration, and differentiation of cardiomyocyte grafts: a study in normal and injured rat hearts. *Circulation* 1999;100:193–202.
- [18] Blaeser A, Duarte Campos DF, Puster U, Richtering W, Stevens MM, Fischer H. Controlling shear stress in 3D bioprinting is a key factor to balance printing resolution and stem cell integrity. *Adv Healthcare Mater* 2016;5:326–33.
- [19] Yang B, Lui C, Yeung E, Matsushita H, Jeyaram A, Pitakong I *et al.* A net mold-based method of biomaterial-free three-dimensional cardiac tissue creation. *Tissue Eng Part C Methods* 2019;25:243–52.
- [20] Visconti RP, Kasyanov V, Gentile C, Zhang J, Markwald RR, Mironov V. Towards organ printing: engineering an intra-organ branched vascular tree. *Expert Opin Biol Ther* 2010;10:409–20.
- [21] Valls-Margarit M, Iglesias-García O, Di Guglielmo C, Sarlabous L, Tadevosyan K, Paoli R *et al.* Engineered macroscale cardiac constructs elicit human myocardial tissue-like functionality. *Stem Cell Reports* 2019;13:207–20.
- [22] Zhang D, Shadrin IY, Lam J, Xian HQ, Snodgrass HR, Bursac N. Tissue-engineered cardiac patch for advanced functional maturation of human ESC-derived cardiomyocytes. *Biomaterials* 2013;34:5813–20.
- [23] Hatzistergos KE, Selem S, Balkan W, Hare JM. Chapter 17—Cardiac stem cells: biology and therapeutic applications. In: Atala A, Lanza R, Mikos AG, Nerem R (eds). *Principles of Regenerative Medicine*, 3rd edn. Cambridge, MA, USA: Academic Press, 2019, 247–72.
- [24] Curfman G. Stem cell therapy for heart failure: an unfulfilled promise? *JAMA* 2019;321:1186–7.
- [25] Yoshioka N, Dowdy SF. Enhanced generation of iPSCs from older adult human cells by a synthetic five-factor self-replicative RNA. *PLoS One* 2017;12:e0182018.
- [26] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663–76.
- [27] Mawad D, Figtree G, Gentile C. Current technologies based on the knowledge of the stem cells Microenvironments. *Adv Exp Med Biol* 2017;1041:245–62.
- [28] Kimura W, Sadek HA. The cardiac hypoxic niche: emerging role of hypoxic microenvironment in cardiac progenitors. *Cardiovasc Diagn Ther* 2012;2:278–89.
- [29] Campbell M, Surija L, Peceros K, Sharma P, Figtree G, Gentile C. Stem cell spheroids. In: Reis RL (ed). *Encyclopedia of Tissue Engineering and Regenerative Medicine*. Cambridge, MA, USA: Academic Press, 2019, 387–93.
- [30] Gentile C. Filling the gaps between the *in vivo* and *in vitro* microenvironment: engineering of spheroids for stem cell technology. *Curr Stem Cell Res Ther* 2016;11:652–65.
- [31] Adler CP, Costabel U. Cell number in human heart in atrophy, hypertrophy, and under the influence of cytostatics. *Recent Adv Stud Cardiac Struct Metab* 1975;6:343–55.
- [32] Bergmann O, Zdunek S, Felker A, Salehpour M, Alkass K, Bernard S *et al.* Dynamics of cell generation and turnover in the human heart. *Cell* 2015; 161:1566–75.
- [33] Menasché P, Vanneau V, Hagege A, Bel A, Cholley B, Parouchev A *et al.* Transplantation of human embryonic stem cell-derived cardiovascular progenitors for severe ischemic left ventricular dysfunction. *J Am Coll Cardiol* 2018;71:429–38.
- [34] Dvir T, Kedem A, Ruvinov E, Levy O, Freeman I, Landa N *et al.* Prevascularization of cardiac patch on the omentum improves its therapeutic outcome. *Proc Natl Acad Sci USA* 2009;106:14990–5.
- [35] Tao H, Chen X, Wei A, Song X, Wang W, Liang L *et al.* Comparison of teratoma formation between embryonic stem cells and parthenogenetic embryonic stem cells by molecular imaging. *Stem Cells Int* 2018;2018: 1–9.
- [36] Campbell M, Chabria M, Figtree GA, Polonchuk L, Gentile C. Stem cell-derived cardiac spheroids as 3D *in vitro* models of the human heart microenvironment. In: Turksen K (ed). *Stem Cell Niche*. Methods in Molecular Biology. Totowa, NJ, USA: Humana Press, 2018, 51–9.
- [37] Groll J, Boland T, Blunk T, Burdick JA, Cho DW, Dalton PD *et al.* Biofabrication: reappraising the definition of an evolving field. *Biofabrication* 2016;8:013001.
- [38] Reid JA, Mollica PA, Johnson GD, Ogle RC, Bruno RD, Sachs PC. Accessible bioprinting: adaptation of a low-cost 3D-printer for precise cell placement and stem cell differentiation. *Biofabrication* 2016;8:025017.
- [39] Mironov V, Visconti RP, Kasyanov V, Forgacs G, Drake CJ, Markwald RR. Organ printing: tissue spheroids as building blocks. *Biomaterials* 2009; 30:2164–74.
- [40] Huang NF, Serpooshan V, Morris VB, Sayed N, Pardon G, Abilez OJ *et al.* Big bottlenecks in cardiovascular tissue engineering. *Commun Biol* 2018; 1:199.
- [41] Kim TY, Kofron CM, King ME, Markes AR, Okundaye AO, Qu Z *et al.* Directed fusion of cardiac spheroids into larger heterocellular microtissues enables investigation of cardiac action potential propagation via cardiac fibroblasts. *PLoS One* 2018;13:e0196714.
- [42] Polonchuk L, Chabria M, Badi L, Hoflack JC, Figtree G, Davies MJ *et al.* Cardiac spheroids as promising *in vitro* models to study the human heart microenvironment. *Sci Rep* 2017;7:7005.
- [43] Fleming PA, Argraves WS, Gentile C, Neagu A, Forgacs G, Drake CJ. Fusion of uniluminal vascular spheroids: a model for assembly of blood vessels. *Dev Dyn* 2010;239:398–406.
- [44] Shevach M, Zax R, Abrahamov A, Fleischer S, Shapira A, Dvir T. Omentum ECM-based hydrogel as a platform for cardiac cell delivery. *Biomed Mater* 2015;10:034106.
- [45] Mawad D, Artzy-Schnirman A, Tonkin J, Ramos J, Inal S, Mahat MM *et al.* Electroconductive hydrogel based on functional poly(ethylene-dioxy thiophene). *Chem Mater* 2016;28:6080–8.
- [46] Sawkins MJ, Saldin LT, Badyalak SF, White LJ. ECM hydrogels for regenerative medicine. In: Berardi AC (ed). *Extracellular Matrix for Tissue Engineering and Biomaterials*. Stem Cell Biology and Regenerative Medicine. New York, NY, USA: Springer International Publishing, 2018, 27–58.
- [47] Wang X, Ao Q, Tian X, Fan J, Tong H, Hou W *et al.* Gelatin-based hydrogels for organ 3D bioprinting. *Polymers* 2017;9:401.
- [48] Waters R, Alam P, Pacelli S, Chakravarti AR, Ahmed RPH, Paul A. Stem cell-inspired secretome-rich injectable hydrogel to repair injured cardiac tissue. *Acta Biomater* 2018;69:95–106.

- [49] Zhang YS, Pi Q, van Genderen AM. Microfluidic bioprinting for engineering vascularized tissues and organoids. *J Vis Exp* 2017;126:e55957.
- [50] Fujimoto KL, Tobita K, Merryman WD, Guan J, Momoi N, Stolz DB *et al.* An elastic, biodegradable cardiac patch induces contractile smooth muscle and improves cardiac remodeling and function in subacute myocardial infarction. *J Am Coll Cardiol* 2007;49:2292–300.
- [51] Mawad D, Mansfield C, Lauto A, Perbellini F, Nelson GW, Tonkin J *et al.* A conducting polymer with enhanced electronic stability applied in cardiac models. *Sci Adv* 2016;2:e1601007.
- [52] Noguchi R, Nakayama K, Itoh M, Kamohara K, Furukawa K, Oyama J *et al.* Development of a three-dimensional pre-vascularized scaffold-free contractile cardiac patch for treating heart disease. *J Heart Lung Transplant* 2016;35:137–45.
- [53] Zhang B, Montgomery M, Chamberlain MD, Ogawa S, Korolj A, Pahnke A *et al.* Biodegradable scaffold with built-in vasculature for organ-on-a-chip engineering and direct surgical anastomosis. *Nat Mater* 2016;15:669–78.
- [54] Cheng G, Liao S, Kit Wong H, Lacorre DA, di Tomaso E, Au P *et al.* Engineered blood vessel networks connect to host vasculature via wrapping-and-tapping anastomosis. *Blood* 2011;118:4740–9.
- [55] Domenech M, Polo-Corrales L, Ramirez-Vick JE, Freytes DO. Tissue engineering strategies for myocardial regeneration: acellular versus cellular scaffolds? *Tissue Eng Part B Rev* 2016;22:438–58.
- [56] Tu C, Chao B, Wu J. Strategies for improving the maturity of human induced pluripotent stem cell-derived cardiomyocytes. *Circ Res* 2018;123:512–14.
- [57] Jiang L, Gentile C, Lauto A, Cui C, Song Y, Romeo T *et al.* Versatile fabrication approach of conductive hydrogels via copolymerization with vinyl monomers. *ACS Appl Mater Interfaces* 2017;9:44124–33.
- [58] MacQueen LA, Sheehy SP, Chantre CO, Zimmerman JF, Pasqualini FS, Liu X *et al.* A tissue-engineered scale model of the heart ventricle. *Nat Biomed Eng* 2018;2:930–41.
- [59] Dor V, Saab M, Coste P, Kornaszewska M, Montiglio F. Left ventricular aneurysm: a new surgical approach. *Thorac Cardiovasc Surg* 1989;37:11–19.
- [60] Batista RJ. Reduction ventriculoplasty. *Z Kardiol* 2001;90(Suppl 1):35–7.
- [61] Menasché P, Vanneau V, Hagege A, Bel A, Cholley B, Cacciapuoti I *et al.* Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report. *Eur Heart J* 2015;36:2011–17.
- [62] Singelyn JM, Sundaramurthy P, Johnson TD, Schup-Magoffin PJ, Hu DP, Faulk DM *et al.* Catheter-deliverable hydrogel derived from decellularized ventricular extracellular matrix increases endogenous cardiomyocytes and preserves cardiac function post-myocardial infarction. *J Am Coll Cardiol* 2012;59:751–63.
- [63] Cossu G, Birchall M, Brown T, De Coppi P, Culme-Seymour E, Gibbon S *et al.* Lancet Commission: stem cells and regenerative medicine. *Lancet* 2018;391:883–910.
- [64] Chimenti I, Gaetani R, Forte E, Angelini F, De Falco E, Zoccai GB *et al.* Serum and supplement optimization for EU GMP-compliance in cardiomyocytes cell culture. *J Cell Mol Med* 2014;18:624–34.
- [65] Zhou G, Jiang H, Yin Z, Liu Y, Zhang Q, Zhang C *et al.* *in vitro* regeneration of patient-specific ear-shaped cartilage and its first clinical application for auricular reconstruction. *EBioMedicine* 2018;28:287–302.
- [66] Vagnozzi RJ, Maillet M, Sargent MA, Khalil H, Johansen AK, Schwaneckamp JA *et al.* An acute immune response underlies the benefit of cardiac stem-cell therapy. *Nature* 2020;577:405–9.
- [67] Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK *et al.* Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol* 2007;25:1015–24.
- [68] Romagnuolo R, Masoudpour H, Porta-Sanchez A, Qiang B, Barry J, Laskary A *et al.* Human embryonic stem cell-derived cardiomyocytes regenerate the infarcted pig heart but induce ventricular tachyarrhythmias. *Stem Cell Reports* 2019;12:967–81.
- [69] Hourd P, Medcalf N, Segal J, Williams DJ. A 3D bioprinting exemplar of the consequences of the regulatory requirements on customized processes. *Regen Med* 2015;10:863–83.