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ESC Working Group on Cellular Biology of the Heart: position paper for Cardiovascular Research: tissue engineering strategies combined with cell therapies for cardiac repair in ischaemic heart disease and heart failure

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Abstract

Morbidity and mortality from ischaemic heart disease (IHD) and heart failure (HF) remain significant in Europe and are increasing worldwide. Patients with IHD or HF might benefit from novel therapeutic strategies, such as cell-based therapies. We recently discussed the therapeutic potential of cell-based therapies and provided recommendations on how to improve the therapeutic translation of these novel strategies for effective cardiac regeneration and repair. Despite major advances in optimizing these strategies with respect to cell source and delivery method, the clinical outcome of cell-based therapy remains unsatisfactory. Major obstacles are the low engraftment and survival rate of transplanted cells in the harmful microenvironment of the host tissue, and the paucity or even lack of

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endogenous cells with repair capacity. Therefore, new ways of delivering cells and their derivatives are required in order to empower cell-based cardiac repair and regeneration in patients with IHD or HF. Strategies using tissue engineering (TE) combine cells with matrix materials to enhance cell retention or cell delivery in the transplanted area, and have recently received much attention for this purpose. Here, we summarize knowledge on novel approaches emerging from the TE scenario. In particular, we will discuss how combinations of cell/bio-materials (e.g. hydrogels, cell sheets, prefabricated matrices, microspheres, and injectable matrices) combinations might enhance cell retention or cell delivery in the transplantation areas, thereby increase the success rate of cell therapies for IHD and HF. We will not focus on the use of classical engineering approaches, employing fully synthetic materials, because of their unsatisfactory material properties which render them not clinically applicable. The overall aim of this Position Paper from the ESC Working Group Cellular Biology of the Heart is to provide recommendations on how to proceed in research with these novel TE strategies combined with cell-based therapies to boost cardiac repair in the clinical settings of IHD and HF.

Keywords

Cardiac tissue engineering • Ischaemic heart disease • Heart failure • Biomaterials • Cells

1. Tissue engineering and cell therapy: are they useful approaches?

Most complex organisms have lost the ability to fully regenerate the heart. However, stimuli to reactivate regenerative processes mammalian cells have been identified.¹ Despite this knowledge, we have learned over the years that reactivation of heart muscle regeneration is much more difficult than suggested by earlier studies.¹ Nature Biotechnology published an editorial in 2017, referring to 'A futile cycle in cell therapy'² because of the none-to-marginal benefits of cardiac cell therapy. The discredit on the entire research area has increased following the emergence of unreliable publications, especially in the infancy of the field, by unreliable unscrupulous scientists simply willing to ride a fashionable horse, recapitulating the story of gene therapy 20 years before. All that has prompted individual scientists declared 'the death of this research field'. Yet, it is not a novel observation that after an unreasonable hype and a phase of frustration that the true value of scientific discoveries unveil. And most importantly, in light of the huge clinical need it is in our view fully warranted to further scrutinize the possibility to regenerate the failing heart by remuscularization. Obvious roadblocks (e.g. poor cell retention and lack of proper integration) must be overcome to unfold the true potential of novel regenerative treatments. For this is important to ask: What have we learned so far and what needs to be achieved to obtain better result? In recent years, a consensus has been reached on several aspects that still require attention for the successful implementation of myocardial cell therapy.^{3,4} To this aim, different cell types endowed with various regenerative activities have been used, with various outcomes. Currently, we consider first-generation clinical cell therapy candidates, i.e. cell types that can be relatively easy prepared for clinical applications, but exhibit limited regenerative potential, including bone marrow-derived mononuclear cells or mesenchymal stromal cells (MSCs) with a focus on stimulation of endogenous regenerative responses.⁵ Second-generation clinical cell therapy candidates need more refined isolation and *ex vivo* amplification procedures, but have a higher regenerative potential, such as several cardiac derived progenitor cells in the form of cardiospheres and pluripotent stem cell-cardiac derivatives, including cardiac progenitor cells and cardiomyocytes and are considered more like an exogenous regenerative approach to replace lost myocardial cells. However, and irrespective of the cell source, a major problem for cell therapy is the low level of retention of infused

or injected cell products. Indeed, although encouraging results have been reported, most studies concur that only few of the transplanted cells survive in the hostile environment of the host tissue, such as that occurring after an infarction, and even fewer integrate and are retained in the host myocardium/myocardial scar. Transplanted cells quickly disappear from the injection site because they simply die in the disease struck and thus typically hostile environment or are 'washed out' into the circulation.⁶ The poor cell retention in the receiving tissue is primarily related to typically used delivery methods, such as intramyocardial (IM) injection, anterograde intracoronary perfusion, or retrograde delivery via the coronary venous (RV) delivery with short-term engraftment of approximately 10–15% can be detected, regardless of the dose of injected cells,⁷ long-term engraftment (>1 month) is reported to be less than 1%,⁶ questioning their direct contribution to myocardial remuscularization. Irrespective of the cell type, a significant fraction of cells (~35%) localizes to the lungs after IM delivery apparently due to clearance through venous myocardial drainage.⁸ MSCs applied attached to small gelatinous carriers resulted in reduced drainage from the myocardium compared with freely suspended MSC controls.⁸ Although such approaches are promising, initial high cell retentions may be lost when cells detach in time in the myocardium, subsequently causing a significant drop in cell numbers.⁹ More advanced tissue engineering (TE) approaches have led to long-term cell retentions of more than 80%, and therefore have gained much attention in recent years.¹⁰

The success of TE in the treatment of other medical conditions¹¹ should motivate the continuation of work in the cardiovascular field. In this position paper, we therefore discuss how new technologies, such as TE/biomaterials tools, can be used to promote the success rate of cell therapies for ischaemic heart disease (IHD) and heart failure (HF). In this context, some semantic considerations in terms of TE and regenerative medicine must be made to better understand how the two fields intersect and synergize each other.

TE aims at assembling functional constructs that restore, maintain, or improve damaged tissues or entire organs, through the combined use of scaffolds, cells, and biologically active molecules. Regenerative medicine includes TE, but, in addition, also includes research on self-healing—where the body uses endogenous mechanisms, sometimes with the help of foreign biological materials—to recreate cells and rebuild tissues and organs. TE emphasizes the starting materials and scaffolds used to create *de novo* tissue implants, while regenerative medicine encompasses the formation of new tissue induced by tissue-engineered materials. The Committee on the Biological and Biomedical Applications of Stem Cell

Research (<https://www.ncbi.nlm.nih.gov/books/NBK223688/>) stated that in the new era of TE combined with regenerative medicine, 'regenerative medicine seeks to understand how and why stem cells, whether derived from human embryos or adult tissues, are able to develop into specialized tissues, and seeks to find new ways of applying cells and their derivatives in order to empower cell based repair and regeneration that will restore lost function in damaged organs'. Therefore, for the purpose of this Position paper, we will discuss on TE as a strategy that can help the regenerative process initiated by the cellular component, providing a biomolecular and spatial environment conducive to cell survival, proliferation and vascularization, aimed at supporting cell and tissue growth. We will focus on how biomaterials, in combination with a cellular component (culture systems or cell products) can be used to increase the success rate of cell therapies for IHD and HF by inducing reparative processes.

2. Major properties and requirements of cell types

Cardiac cell-based TE aims to remuscularize post-infarct myocardial scars, provide paracrine support for activation of endogenous repair mechanisms, and add substitute tissue for failing hearts using a three-dimensional approach. Depending on the specific therapeutic goal, the cell requirements vary and different cell types are needed. If the primary objective is remuscularization, a large number of bona fide cardiomyocytes are required, which eventually couple with endogenous cardiomyocytes. The general feasibility of cardiomyocytes engraftment with electromechanical integration was demonstrated earlier for foetal¹² and pluripotent stem cell derived¹³ cardiomyocytes as well as engineered heart muscle (EHM)¹⁴ allografts, using either voltage indicator dyes and two-photon imaging or multi-array electrode recordings of the epicardial spread of electrical excitation. Murry *et al.*¹⁵ pioneered the use of genetically encoded calcium indicators to demonstrate electrical coupling of human ES-cell-derived cardiomyocyte grafts in injured guinea-pig and non-human primate.¹⁶ Whilst the original studies in only mildly injured non-human primate demonstrated ventricular arrhythmias in all investigated animals,¹⁶ recently published data suggest a lower risk of ventricular arrhythmias and ectopy rather than re-entry as the underlying mechanism.¹⁷ In case of activation of host-associated repair pathways via paracrine signalling, the flexibility of cell type is greater. TE is here mainly directed at optimizing the early cell retention, which allows the release of paracrine factors, a strategy which might later be replaced by the administration of their secretome only.¹⁸ Independent of the objective, the cells used for TE should be compliant with the 'Guideline on human cell-based medicinal products' from the European Medicines Agency, related to the identity of the cell population, potency, purity, viability, safety, efficacy, and suitability for intended use. For more details see ref.⁴

2.1 Cells and their requirements for TE-directed remuscularization

With the advent of human pluripotent stem cells (hPSC), including human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC), the availability of large-scale production in bioreactors and differentiation/purification protocols, sufficient quantities of essentially pure human cardiomyocytes can be produced according to GMP requirements and applied in the engineering of heart muscle.¹⁹

Human EHM rings and patches^{19–21} as well as human engineered heart tissue strips²² from human embryonic (hESC)- and hiPSC-derived cardiomyocytes could be implanted and used to partially repair large muscle defects in rat and guinea-pig hearts. These studies collectively demonstrated long-term cardiomyocyte retention (>200 days), maturation, graft vascularization, and a variable degree of proliferation; further electrical coupling to the host myocardium was observed in some, but not all investigated guinea-pigs,²² in line with earlier studies demonstrating the isolation of human engineered muscle xenograft grafts by scar formation in athymic rats.²³ Three-dimensional culture and maturation of hiPSC-cardiomyocytes can be stimulated in clinical scalable 3D cultures, displaying robust electromechanical coupling, consistent H-zones, I-bands, and evidence for T-tubules and M-bands, and there is a general agreement that engineered three-dimensional heart muscle cultures achieve a greater resemblance to bona fide human myocardium in comparison to standard 2D monolayer cultures.^{21,22,24–33} Several specific interventions have been identified to improve the maturation of hPSC-derived cardiomyocytes including (i) addition of thyroid hormone,³⁴ (ii) application of electrical stimulation,^{33,35,36} (iii) mechanical loading^{21,27,37–39}, or (iv) co-culture with cardiac-specific cell types including endothelial cells²² and cardiac fibroblasts^{21,31,40–42} or a mixture of fibroblast-like stromal cells.^{37,43} A good understanding of the optimal cellular make up as well as reliable quality markers for cardiomyocytes and non-myocyte maturation should be instrumental to further improve the sophisticated architecture and function engineered myocardium.

Current protocols mainly give rise to ventricular-like cardiomyocytes. Going forward, the field would benefit from the development of precise, directed standardized protocols for cardiomyocytes subtype specification and functional maturation. Accordingly, several steps have been taken, including the formation of atrial myocytes specification,⁴⁴ epicardial progenitor cells,^{45,46} and sinoatrial node cells.⁴⁷ Recently, protocols for the engineering of EHM with distinct atrial and ventricular properties have been reported.⁴⁸ Non-cardiomyocytes are included in most TE studies—either by their addition or by a contamination of the cardiomyocyte pool. Whether and in particular how they contribute to tissue assembly, maturation, and survival after implantation needs further mechanistic studies. It seems clear that endothelial cells can support angiogenesis⁴⁹ and fibroblasts contribute to extracellular matrix (ECM) homeostasis as well as its viscoelastic properties.²¹ In addition, there is certainly paracrine cross-talk which may be instrumental in the heart muscle tissue formation process.

Additional open questions include: Are there any circumstances in which non-cardiomyocytes can be damaging to graft or host? Is there an ideal mixture of cardiomyocytes with the multiple stroma cell species found in the healthy heart? Recently Gao *et al.*⁵⁰ reported the use of human induced PS derived cardiomyocytes (hiPSC), smooth muscle cells (hiPS-SMC), and endothelial cells (hiPS-EC) that were mixed into a fibrin scaffold for 7 days to create a cardiac muscle patch. The patch was used to cover an infarct area in a pig heart. Beneficial effects, likely mediated by paracrine cross-talk, were observed after 4 weeks.⁵⁰ It is clear that the proximity between capillary endothelial cells and cardiomyocytes in the healthy adult heart has a functional role beyond lining of the capillary,⁵¹ which includes pro-angiogenic paracrine signalling.⁵²

2.2 Cells and their requirements for TE-directed endogenous regeneration

Cells committed to a cardiac lineage such as right ventricle-derived cardiosphere-derived cells,⁵³ bone marrow-derived MSC engineered to

express cardiac transcription factors,⁵⁴ and ESC-derived cardiac progenitor cells⁵⁵ may be used in TE to stimulate endogenous regeneration by the release of paracrine factors. ESC/iPSC-derived cells carry the risk of teratoma formation due to contamination with residual pluripotent cells that have not responded to lineage-specific instructive cues, and therefore have retained their state of uncontrolled proliferative potential. To avoid this contamination, a selection step based on surface antigens is mandatory for obvious safety reasons.⁵⁶ From a translational point of view a more realistic approach is to control the differentiation and proliferation processes properly to exclude the presence of teratogenic cells. With regards to the prediction of the efficacy of cells to be used for TE, a cardiopoietic index was recently established.⁵⁷ This biomarker-based index relies on canonical cardiac transcription factors employing gene expression profiles as a means to assess the regenerative quotient of patient-derived cells.

2.3 Allogeneic vs. autologous cells

The use of allogeneic cells is preferential over autologous cells since they are not exposed to patient risk factors (i.e. age, diabetes mellitus, and smoking), known to impair the potential of autologous stem cells⁵⁸ and allow off-the-shelf use: streamlined logistics, consistency and immediate availability of the product and guaranteed dosage. However, allogeneic cells will always induce an immune response. Alloreactivity depends on foreign peptide presentation by major histocompatibility complex (MHC) on antigen presenting cells and detection by T cells⁵⁹ and can be modulated by T cell suppressors, including calcineurin inhibitors (cyclosporine and tacrolimus are commonly used) and corticosteroids. Immunosuppressive treatment is associated with a number of unwanted effects, including hypertension due to kidney damage, transaminitis due to liver damage, an increased risk for infections, and if used long term with malignancies). These are the same risks heart transplant patients are exposed to and have been proven to be manageable. An alternative strategy to avoid the need for long-term immunosuppression is modulation of the innate immune system. A very recent paper from Braza *et al.*⁶⁰ demonstrated the feasibility of promoting long-term organ transplant acceptance by inhibiting macrophage activation with nano therapeutics.

Another strategy to prevent allogeneic cell transplantation-related immune rejection is the use of MHC-matched donor cells or to engineer grafts with immune tolerant properties such as recently demonstrated by a functional HLA knock-out via beta-2-microglobulin (B2M) deletion with subsequent insertion of a B2M-HLA-E fusion to overcome T- and NK-cell mediated killing.⁶¹ Although similarly appealing as autologous cells from the immunology point of view, these cells will be more difficult to eradicate if unwanted side effects occur, because they will not be detected as foreign by the endogenous immune system. A potential strategy for eradication of immune tolerant cells is the use of suicide genes, as pioneered by Malcom Brenner and Helen Heslop for treatment of graft vs. host disease following adoptive cell therapy.⁶²

Systematic review and meta-analysis of cell therapy in large animal studies concluded effects of autologous and allogeneic cell therapy for IHD were similar, irrespective of immunosuppressive therapy⁶³ which can be taken as an additional indirect evidence for a similar cell loss with predominantly paracrine mechanisms of action in the reported studies.

In the context of TE, strict immunologic monitoring as well as for example monitoring circulating cell-free allograft DNA⁶⁴ will be helpful to if needed adapt immune suppression protocols for enhanced graft survival. It appears likely that a differentiated use and need for

immunosuppressive therapy and MHC matching will depend also on the cell type and TE approach used.

For a TE strategy directed at stimulating cardiac endogenous regeneration via paracrine effects, a short-term immunosuppressive treatment would be recommended with the premise that the transplanted cells would be likely short-lived and act via paracrine signalling.¹⁸ This short-term immunosuppressive regime is underscored by the finding that although allogeneic cells are expected to be eliminated more rapidly than autologous cells, their transient presence shortly after transplantation is sufficient to yield equivalent long-term benefits.⁶⁵ In the case of TE-directed remuscularization with iPS- or ESC-derived cardiomyocytes, durable engraftment of the cells/graft is required along with long-term state-of-the-art immunosuppressive therapies. MHC matching of iPS-derived cardiomyocytes has been shown to enhance cell engraftment in non-human primates.⁶⁶ Translated to patients, a large repository with a variety of MHC-homozygous stem cell lines, collected from donors or engineered, may solve this issue.⁶⁷

3. Methods for engineering myocardial tissue

As indicated, major hurdles to therapeutic applications have been observed, including low cellular survivals, and poor localization to the target area. To further facilitate integration and prolonged activities, several approaches are under development that each have various properties and advantages. These approaches include (i) seeding of cells on preformed scaffolds, (ii) self-assembly of cells in hydrogels, and (iii) cell sheet engineering (reviewed in ref.^{68,69}).

3.1 Hydrogels

Hydrogel-based injection and TE are promising techniques in which hydrophilic structures are used, made of either synthetic or natural polymers, which can assemble into a three-dimensional polymeric network.⁷⁰ Hydrogels have a high number of essential scaffold requirements, including the exchange of oxygen, nutrients and metabolites due to their porosity and the possibility of including growth factors and other molecules to mediate cross talk between cells.⁷¹ However, in general the hydrogel structures lack structural supportive characteristics and the correct stiffness of cardiac materials.⁷²

3.2 Engineered myocardial tissue

Engineered myocardial tissue can be used to aim at the restoration and improvement of cardiac function in terms of tissue regeneration, as indicated above, however, they can also be used to develop three-dimensional *in vitro* models able to mimic the native heart muscle. Construction of engineered heart tissue/muscle (EHT/M) was pioneered and continuously developed by the laboratories of Eschenhagen and Zimmermann^{21,73–76} and became widely used recently.^{49,77–79} To this end, a range of innovative three-dimensional culture *in vitro* systems for cardiac disease modelling has been developed. In both approaches overlapping characterizations are needed, including the presence of (i) native-like biochemical, electrophysiological, and mechanical cell-ECM and cell-cell interactions, (ii) dynamic *in vivo* like conditions such as fluid flow and shear stress, and (iii) correct cell characteristics and morphologies and structural micro-architectures.

3.3 Extracellular matrix

In addition to the cellular components of myocardial tissue, the ECM is a major player in normal cardiac functioning and homeostasis and cellular behaviour. Ideally, a hydrogel or engineered construct should perfectly mimic native cardiac ECM and provide a physiological micro-environment for cells. The cardiac ECM consists of a complex network of structural and non-structural (matricellular) proteins, of which three-dimensional hydrogel scaffolds have been generated from decellularized cardiac ECM⁸⁰ and used as injectable biomaterial for myocardial repair,⁸¹ currently under clinical investigation (NCT02305602).

In addition to the native cardiac ECM, naturally occurring ECM proteins are suitable materials to be used as a hydrogel-based scaffold in cardiac TE due to their bio-mimicking and bioactive properties.⁸² Some of the frequently used hydrogels from natural sources include collagen,²¹ fibrin,³⁵ gelatin,⁸³ hyaluronic acid,⁸⁴ and alginate.¹⁰ Matrigel was originally introduced as being supportive in the engineering of rat EHT⁷⁶ with also subsequent application in human TE, but will not be applicable in clinical applications.

While the primary advantage of these materials is to maintain transplanted cells alive in the myocardium and retain them *in situ* following transplantation, a potential shortcoming is the possibility that even small modifications of local mechanical compliance could activate fibrosis due to the emerging stiffness-sensitivity of myocardial resident stromal cells.⁸⁵ This problem may be overcome by finely controlling the visco-elastic properties of the materials employed to encapsulate cells, or by providing them with sufficiently controlled level of degradation to avoid changes in myocardium compliance. In this respect, employment of 'bio-ink' materials, such as gelatin methacryloyl (GelMA) hydrogels, whose stiffness may be easily adjusted by, e.g. pre-injection photo-polymerization⁸⁶ offer a new opportunity for advanced myocardial TE applications.

3.4 Cell sheets

Alternatively, non-cardiomyocytes can be employed for endogenous ECM production, resulting in a replacement of exogenous scaffold material.⁸⁷ Similarly, in cell sheet engineering⁸⁸ it is well established that cell-secreted ECM plays a key role in sheet assembly and epicardial connection after implantation.⁸⁹ Cell sheet engineering makes use of temperature-responsive polymer surfaces to enable the controlled release of cell monolayers; free-floating sheet of cohesive cells that can, for example, be placed onto the epicardium with or without stitches.⁸⁹ Using this approach 3–4 monolayers can be fused without a palpable core necrosis. The cell sheet approach can be applied to all cell types, which are capable of forming a biomechanically interconnected monolayer, such as cardiomyocytes for contractile support and non-myocytes for the delivery of secreted factors.^{90–92} This technology has been recently tested clinically for the delivery of skeletal myoblasts to the failing human heart⁹³ and is presently further exploited for the delivery of allogeneic iPSC-derived cardiomyocytes.⁹⁴ Issues with this approach are the frailty of these sheets which may cause their folding or tearing during manipulations and the limited number of sheets which can be stacked on each other without cell death.⁹⁵

3.5 Biofabrication

The major concern with classical TE, which entails the seeding of pre-fabricated scaffolds with cells, is that only inhomogeneous cell densities can be achieved, because of the cells propensity to remain at the scaffold surface, and thus only weakly contracting cardiac tissues can be

fabricated. This caveat may not apply to the delivery of non-cardiomyocytes with a primary mode of action related to their paracrine activity. In hydrogel approaches, cell content is typically more homogeneous, and anisotropic growth is typically guided by mechanical stimuli. A better understanding of how to achieve dense anisotropic muscle structures with capillarization would be important for the development of the next generation of tissue-engineered products. Accordingly, tremendous effort is invested into improved biofabrication technologies combining additive manufacturing techniques with cell printing to create hierarchical tissue-like structures; commonly known as three-dimensional printing.^{96,97} In principle, biofabrication allows the production of cardiac tissues layer by layer utilizing multiple print heads and inks (e.g. shear thinning gels) containing distinct cell-types. In this way, various parameters can be controlled such as correct cellular composition, the positioning of various cell-types and materials, vascularization, and the incorporation of bioactive substances.⁹⁸ Currently, the field focusses on improving the hardware, to provide more additional gels for printing, and to include materials that can change their shape after printing upon defined stimuli (4D biofabrication) to generate for example vascular-like networks.^{99,100} Although promising and potentially very versatile in its applicability, it remains a key challenge to printing tissues at meaningful dimensions for heart repair applications.

4. How biomaterials and myocardial engineering can influence cell therapy in the repair and regeneration of the heart

Two substantially different strategies can be endeavoured in myocardial TE: (i) cell-based methods in which differentiated myocardium tissue patches, or progenitor cells 'niches', can be implanted directly in areas lacking contractile tissue; or (ii) *in situ* strategies, in which composite biomaterials (cells + ECM) may be designed to support the regeneration capacity of endogenous myocardial cells.

4.1 Cell-based methods

For clinical translation, the first discussed strategy requires not only fine-tuning of materials/cells combinations to recapitulate the physiology of the normal tissue environment, but also an enhancement of the propensity for electromechanical and vascular coupling with the recipient myocardial tissue. The latter aspect is crucial to promote stable patch engraftment and coordinated action potential propagation from pre-existing myocardium (reviewed in ref.⁸⁰). A second problem inherent to the generation of myocardial patches concerns the status of cell maturity inside the engineered grafts. Commonly used stem or progenitor cell derivatives have a variable level of proliferation and phenotypic immaturity, which results in the formation of rather immature muscle *in vitro*. Maturation occurs after implantation *in vivo*, rendering the cells indistinguishable from native myocardium in an allograft setting.¹³ Whether similar factors would drive human cardiomyocyte maturation in the diseased heart remains an open question. Data on advanced maturation after implantation even in a xenograft setting suggest that similar maturation capacities exist in human PSC-derived cardiomyocytes.^{19,69} The underlying process of *in vivo* maturation are however not well understood and further optimization of engineering paradigms may benefit from further mechanistic insight. A possible implementation of engineering systems to overcome these

shortcomings may consist in using biomaterial fabrication criteria supporting coordinated multi-layered vascular and myocardial cell growth,¹⁰⁰ systems favouring electro-mechanical coupling of cells in the patch^{101,102} and, finally, materials with defined biophysical characteristics (e.g. stiffness) promoting maturation of myocyte action potential propagation.¹⁰³

An emerging area of interest in myocardial engineering is the modelling of the so-called cardiac niches, which can be defined as individual 'functional units' that include spatially prearranged biochemical/biophysical information. These functional units can instruct the coordinated differentiation of stem/progenitor cells into terminally differentiated cardiomyocytes, while maintaining stable amounts of self-renewing cells in its core.¹⁰⁴ By exploiting the natural stem/progenitor cardiogenic differentiation process, this strategy may lead to a new 'organoid'-based approach to cardiac (re)generation.¹⁰⁵ In case standardization and scalability will be demonstrated, this method could be employed to produce large amounts of allogenic or even personalized tissue 'building blocks', that could be transplanted into the recipient myocardium using appropriate delivery systems. *Trans*-endocardial injection guided by real-time electrophysiology myocardial mapping (e.g. NOGA⁴) is an example of delivery systems, with the caveat that endocardial deliveries of a bulk material need a careful safety assessment because of the potential of systemic embolization of the material end-products. An alternative without this limitation is the percutaneous delivery of tissue-engineered products with a shape-memory onto the epicardium.¹⁰⁶

4.2 *In situ* strategies

A systematic investigation of the conditions promoting stem/progenitor cells cardiogenic differentiation could either lead to improved existing regenerative approaches by 'combining' materials/cells, or even cell-free *in situ* strategies. A first possibility is offered by embedding therapeutic cells into unstructured biomaterials (e.g. hydrogels) and combining them with survival/cardiogenic factors which may preserve their vitality, proliferation and/or enhance differentiation capacity in the potentially hostile environment.¹⁰⁷ In a second formulation, the injection of 'smart' materials that instruct *in situ* cellular specifications has been demonstrated, at least at a proof of concept level.¹⁰⁸ In this formulation, the therapeutic approach could consist of hydrogels containing instructing signals for cardiac cell reprogramming (e.g. slowly releasing miRNAs¹⁰⁹), or stem cell-derived secretome survival factors.¹¹⁰ In the case where the combination of materials/bioactive factors includes formulations with stiffness characteristics consistent with myocardium elasticity,¹¹¹ materials making them electrically conductive^{102,112} or topologically arranged substrates,¹¹³ there will be valuable options to operate cell-free cardiac regeneration that could lead to the delivery of clinically effective therapies. In fact, these applications would not only boost the inefficient myocardium regeneration process,¹¹⁴ but would also permit a coordinated and spatially-organized regeneration of myocardial contractile tissue in areas where cardiomyocyte death and myofibroblast-dependent fibrosis typically limit the replacement with new contractile cells. In this regard, the inclusion of materials (e.g. silk-derived proteins¹¹⁵) or drugs (e.g. anti-inflammatory molecules¹¹⁶) capable of suppressing pathways mediating the early inflammatory response leading to myofibroblast activation in response to myocardium damage, or of stimulating cardiomyocyte cell-cycle re-entry in the myocardium,^{116,117} could be effective enhancement strategies limiting the extent of post-ischaemic damage and, at the same time, promoting cardiac regeneration.

4.3 How to prevent inflammatory/immune reactions in the host environment

One of the most important challenges in the field of cardiac TE is to avoid adverse foreign-body host immune response to implanted scaffolds. Novel biocompatible, immunomodulatory biomaterials can reduce the foreign-body response and improve engraftment.¹¹⁸ Furthermore, because inflammation is a critical component influencing cardiac regeneration,^{118,119} immunomodulation by smart biomaterials is a potential strategy to overcome this major challenge in cardiovascular regenerative medicine. The modification of the physicochemical properties of biomaterials can modify the host inflammatory response, which can improve biomaterial integration and their interaction with immune cells including the reparative cells such as macrophages and MSC, as well as the controlled delivery of anti-inflammatory small molecules and cytokines.¹¹⁸

5. Cell-free (secretome) approaches

5.1 Acellular biomaterials

These cell-free materials include the use of material itself for directing cardiac responses or as delivery vehicles for biologics. The material is typically natural (e.g.: alginate, collagen, hyaluronic acid, chitosan, decellularized ECM), or less commonly, a synthetic polymer (reviewed in ref.120). The proposed mechanisms of benefit include reduction of wall stress by mechanical support, and modulation of host cellular responses including inflammation, neovascularization, fibroblast activity, stem cell recruitment, and protection against cell death. The potential advantages of this approach are that it is cost-effective, localized (vs. systemic), and avoids the need to consider cell survival in the injected biomaterial. The method of delivery is an important factor, with injectable methods that have the advantage of a minimally invasive delivery route, though the need for injection confers several design limitations on the gels.¹²⁰ To date, results of the few clinical trials of acellular biomaterials have been mixed.^{121–123} Another promising approach is the injection of VentiGelTM (Ventrix), an injectable hydrogel derived from porcine myocardial ECM, which showed benefits in pigs⁸¹ and is currently in a Phase I clinical trial in patients with HF post-myocardial infarction (MI) (NCT02305602).

Biomaterials can be used to deliver biologics such as growth or survival factors. This places further limitations on the biomaterial, necessitating carefully controlled rates of degradation and release. In a successful example, an epicardial collagen patch was used to deliver recombinant follistatin-like 1 (Fstl1), stimulating cardiomyocyte proliferation, and improving cardiac function and survival in mouse and swine models of myocardial infarction.¹²⁴ To improve the localization and retention in the heart once the factors are released, the biomaterial can be modified to bind the factors. This approach has seen some success in small and large animals,¹²⁰ but clinical translation may be limited by the expense of synthesizing and incorporating peptide growth factors.

5.2 Exosomes and microvesicles with biomaterials

The secretome of stem cells contains a rich cocktail of growth factors and other pro-regenerative molecules including extracellular vesicles (EVs) such as exosomes and microvesicles.¹²⁵ In fact, the secretome may mediate much of the benefit in cardiac function that has been observed after the injection of stem cells into the heart, via mechanisms that include paracrine stimulation of pro-angiogenesis pathways, alterations of

macrophage phenotype and anti-fibrotic effects.^{3,126} In a recent head-to-head comparison, EVs from human cardiovascular progenitors outperformed the cells themselves at improving cardiac function in mice with HF post-MI.¹²⁷ Although some long-lasting benefits have been observed after a single injection of EVs, it may be presumed that controlled release of the secretome from an injected biomaterial would allow for maximum and/or prolonged benefit.¹²⁸ Although there are few studies using this approach to date, its feasibility has been demonstrated by the use of a hydrogel based on polyethylene glycol (PEG), end-modified with ureidopyrimidinone moieties to deliver growth factors in the soluble phase with no exosome delivery to pig myocardium.^{129,130} A gelatin and laponite nanocomposite hydrogel has been used to deliver the complete secretome of human adipose-derived stem cells (hASC) to the perinfarct myocardium of rats, and was found to reduce relative infarct size after 21 days recovery, and significantly improve EF.¹¹⁰ Finally, in a small feasibility study in patients, hESC-derived cardiovascular progenitors were encapsulated in a fibrin patch which was then sewn to the epicardial surface of the hearts of six patients with HF. Although this was a safety study, an improvement in cardiac regional systolic contractile function was seen, which was most likely mediated via secreted paracrine factors.⁵⁵

6. Modes of cell applications and retention with biomaterial carriers

6.1 *In vitro* and *in vivo* engineering and the three routes of cell delivery

The ideal strategy to deliver cells in biomaterial carriers depends on multiple factors including features of the diseased heart (type of cardiomyopathy, location of scarring), the clinical setting [e.g. whether or not it is combined with coronary artery bypass grafting (CABG)], biomaterial characteristics, and to a lesser extent cell characteristics. The actual TE process can take place *in vitro* where the cells are seeded or grown into a pre-formed structure, or *in vivo* after injection of cells in conjunction with a biomaterial. The stimulation of endogenous repair by transplanted cells, or the direct reprogramming of other cell types into cardiomyocytes, is sometimes also referred to as *in vivo* TE.

IM injection can be performed via the endocardial or epicardial route. The epicardial route is the only one that allows direct visualization, and therefore, usually used in preclinical (small animal) research or in combination with open chest surgery, most often CABG for ischaemic cardiomyopathy. The endocardial application, via a percutaneous approach, is less invasive and allows for precisely targeted therapy if a special electro-mechanical mapping system (e.g. NOGA) is used, although these mappings have their limitations in their anatomical visualizations. The transvenous approach represents a combination of the two techniques, as cells are injected through a coronary vein puncture¹³¹ under IntraVascular UltraSound guidance. IM injection thus has the advantage of delivery to a specific location but the risk of rapid wash-out of cells via venous drainage is high⁸ and should be overcome with suitable biomaterials.

The epicardial application can also be in the form of an engineered tissue patch that is sutured on top of the heart. This ensures an excellent cell retention mechanically, but electromechanical integration via this route may be more challenging and the technique currently depends on open chest surgery. Future technical developments should allow for less invasive epicardial (and potentially endocardial) application, parallel with

developments in minimal invasive valve surgery. Intracoronary infusion is minimally invasive and easy to perform with standard catheterization lab equipment, but caution should be taken not to induce embolization, especially when thicker biomaterials are used. In addition, the coronary arteries may be inaccessible due to the nature of the disease. The interstitial retrograde coronary venous infusion may then serve as an alternative. It is performed by placing a balloon-catheter in the coronary sinus or one of the coronary veins and occluding the distal side temporarily to allow the cells to disseminate into the heart.¹³² In each case, the cells need to be able to migrate across the microvascular endothelium to reach their planned site of action. Finally, because repeated dosing may be required to achieve a maximal therapeutic benefit,¹³³ less invasive approaches likely need to be considered and in this setting it might be worth further investigating the intravenous route.

6.2 Injectable hydrogels

Non-gelated, switchable hydrogels can be administered using a syringe and incorporated into any catheter-based application. Cells, and additives such as growth factors, are suspended in or co-injected with the liquid material provided it features visco-elastic properties allowing it to take a liquid form as the shear stress increases. The solution should be designed to polymerize/crosslink quickly after arrival *in vivo* in the heart, usually due to pH or temperature change, and remain stable afterwards to prevent embolization. Degradation should occur without toxic byproducts.¹³¹ This has been reported to enhance cell retention significantly and possibly improve differentiation and functional effects in pre-clinical models.¹⁰⁷

6.3 Porous scaffolds

Therapeutic cells can be grown into a three-dimensional construct on a porous or fibrous scaffold, which usually needs additional treatment to maximize cell attachment.⁶⁹ In cell sheets, usually cardiomyocytes or progenitor cells are combined with support cells which enhance tissue organization and robustness.^{134–136} Until now, patches have been placed on the epicardial side of the myocardium via open chest surgery but the development of less invasive techniques may change this requirement in the future.

6.4 Microcapsules

Sized in the order of hundreds of microns or less, microcapsules consist of cells encapsulated with nanoporous materials.¹³⁷ The capsules protect the cells from recognition by the immune system and may enhance the long-term paracrine action of the transplanted cells. Although intracoronary infusion of encapsulated glucagon-like peptide-1-eluting mesenchymal stem cells preserved left ventricular function in a porcine model of acute MI, the suggestion of coronary occlusion halted these preclinical studies. For cardiac applications, biodegradable hydrogels such as gelatin seem most appropriate.⁹ So far, application has been via IM injection due to the size of the cell-laden capsules and the necessity to pass the endothelial barrier in intravascular routes. On top of microcapsules or carriers, also (mixed) aggregates¹³⁸ of iPSC-cardiomyocytes in capsules is a potential route to improve cellular retention and thereby increase myocardial function.¹³⁹

7. Towards clinical applications

The clinical use of tissue-engineered constructs in myocardial regeneration is still at an early phase. Although the regulatory framework needed

to achieve authorization of a tissue engineering product is similar to that of cell-based therapy, the combination of cells and materials in an advanced therapy medicinal products will require more stringent quality criteria, and therefore, more advanced preclinical testing before it can be approved. This includes a careful assessment of potential benefits and obvious risks in a well-defined patient population and ultimately also economic considerations.^{136,140}

7.1 Factors influencing results/limitations

In fact, although effective carrier materials and/or engineering approaches may enhance cell or tissue retention, the problems with sources of autologous cell and survival in the host tissue remains problematic, similarly to any cell-based therapies. The quality and number of cells may diminish in patients who are older or have comorbidities (together with specific medications for the comorbidity) or genetic defects. Moreover, cell/tissue survival is also effected by the host tissue environment with comorbidities, aging, gender, etc. (for review, see ref.³). Unfortunately, little is known about the effect of major comorbidities and risk factors on quality of cell source and cell survival in the host tissue.^{141,142}

Other limitations are the logistics and high costs of such advanced therapy medicinal products as well as the failure of many biomaterials to meet translationally relevant requirements. The first in man phase one trials using products of TE will require dedicated GMP-production and clinical infrastructure for the implantation procedure and follow-up. GMP-production according to the relevant regulatory demands would for preclinical trials ideally be at one site to ensure quality and comparability of a TEP. This will be particularly important in case of multicentre studies. This requires qualified protocols and carriers for TEP delivery to the point-of-care. In this context, recent data on transatlantic shipping of EHM without an apparent loss in potency and quality is encouraging.¹⁹ Early clinical trials would also in light of the high costs associated with the set-up of GMP-production clearly be facilitated by single production units. If later phase clinical testing and market authorization is considered based on compelling clinical data, production logistics will have to be reconsidered. A general concern is related to the high cost of advanced therapy medicinal products in general, leading to their withdrawal from the market despite approval by the European Medical Agency (EMA) and no safety or efficacy concerns.¹⁴³ Thus, setting up cost effective production processes will be at least as important as compelling data from clinical trials.

7.2 Potential risks: arrhythmias, tumour development, rejection, calcification

Since myocardial tissue is subject to the coordinated propagation of electromechanical stimuli, the development of arrhythmias is a key risk for all cell therapeutics and especially so for conductive myocyte-containing therapeutics. This caveat has been clearly recognized as clinically relevant in the first clinical trials on skeletal muscle cells for heart remuscularization.¹⁴⁴ A number of important preclinical large animal studies have further underscored this risk in case of the application of cardiomyocytes.^{16,145,146} Conversely, a recent study on fibrin-patch mediated delivery of ESC-derived cardiac progenitors did not provide evidence for arrhythmia,¹⁸ which is in line with the primarily secretome associated mechanism of action but also suggests that avoidance of IM punctures may mitigate the risk of uneven integration of the grafted cells and the attendant predisposition of inhomogeneous coupling at the graft-host interface to trigger arrhythmias.

Potential risks that could constitute a serious obstacle to the clinical translation of tissue-engineered myocardium could arise from uncontrolled proliferation and unwanted differentiation of cells in the constructs that may lead to, e.g., tumour formation in case of iPSC/ESC,^{147,148} or to calcification of the host myocardium by MSC.¹⁴⁸ These issues remain essentially the same as those of classical cell-based cardiomyoplasty approaches, with the caveat that an increased efficiency of cell survival and engraftment, resulting from embedding cells into hydrogels or structured tissue patches, could enhance the risks of side effects. Another relevant issue that may need to be solved is the problem of immune-rejection against cells/materials combinations. In this regard, the materials causing no inflammatory or foreign body reactions would be ideal (reviewed in ref.¹⁴⁹). This class of materials includes, (i) naturally derived polymers with an innate anti-inflammatory activity (e.g. silk), (ii) ECM matrix components deriving from decellularization or lyophilization procedures, or finally, (iii) materials with controlled release of anti-inflammatory/immune-suppressive molecules. A final strategy, which can be used for cardiac TE with allogenic cells could derive from the exploitation of cell combinations that maximize the suppression of immune responses *in vivo*.¹⁵⁰

7.3 Safety, regulatory and ethical frameworks related to the use of tissue engineering products in myocardial repair

According to the EMA classification (EC No. 1394/2007), tissue-engineered constructs and combinations of cells/biomaterials fall in the definition of advanced therapy medicinal product. A conceptually similar definition (Human Cells, Tissues, or Cellular and Tissue-Based Products HCT/Ps) is adopted by the Food and Drug Administration in United States.¹⁵¹ In either cases, the adoption of stringent quality criteria complying with the Good Manufacturing Practice (GMP) production under a manufacturing authorization by the local competent authority is the basis to ensure standardization, safety, traceability, and potency of the final product. Recognizing the diverse nature of advanced therapy medicinal products, including the tissue-engineered products (TEP), EU guidelines (e.g. Guidelines on Safety and Efficacy Follow-up: Risk Management of Advanced Therapy Medicinal Products—EMEA/149995/2008) provide guidance as to how to (i) ensure quality of the production process, (ii) evaluate potential risks, and (iii) demonstrate potency and efficacy of the final product with *in vitro/in vivo* tests. Issues that are particularly recognized include: (i) 'transmission of infectious agents to the patient and to close contacts', (ii) 'graft dysfunction and/or rejection', (iii) 'induction of autoimmunity or immunogenic reactions', (iv) 'induction of malignancies', and (v) 'impossibility of discontinuing or removal of the product'. In case of cell/materials combinations, the same guideline additionally recommends specific testing of biodegradation and mechanical factors, thus asking for an evaluation of long-term patient/graft interactions possibly affecting the graft performance at long term. These latter recommendations may be particularly important for setting myocardial therapy using, for example, cellularized patches. These patches should, alternatively, be produced with fully bio-absorbable materials, able to release therapeutic cells without eliciting inflammatory responses secondary to material degradation, or be designed to resist to biodegradation, thereby prompting a full functional integration in the host's myocardium. Finally, each TEP has to be evaluated by the responsible regulatory authority to specify the required manufacturing and preclinical strategy before embarking on first-in-patient trial.

At the time of the preparation of our current paper, the available EMA 'Scientific Recommendations' lists (www.ema.europa.eu/ema/in)

Table 1 Ongoing human clinical trials for cardiac tissue engineering

Clinical trial name	Material/cells/tissue	Trial identifier www.clinicaltrials.gov	Phase (enrolled patients)— (publication)
Epicardial infarct repair using CorMatrix [®] -ECM: clinical feasibility study (EIR)	CorMatrix-extracellular matrix	NCT02887768	Phase 1 ¹¹⁹
Transplantation of human embryonic stem cell-derived progenitors in severe heart failure (ESCORT)	Human ESC-derived progenitors embedded into a fibrin patch	NCT02057900	Phase 1 ^{57,116}
A study of VentriGel in post-MI patients	VentriGel extracellular matrix hydrogel	NCT02305602	Phase 1 (recruiting)
Myocardial assistance by grafting a new bioartificial upgraded myocardium (MAGNUM trial)	Collagen matrix seeded with bone marrow cells	NCT01429415	Completed ¹⁴⁰

dex.jsp?curl=pages/regulation/general/general_content_000301.jsp&mid=WC0b01ac05800862c0) the following advanced therapy medicinal products for myocardial regeneration, such as e.g. autologous/allogenic hematopoietic stem cells (CD34⁺/CD133⁺), adult bone marrow/adipose tissue-, or placental (Wharton Jelly)-derived mesenchymal stem cells, none of which is based on material/cells combinations.

7.4 Ongoing clinical trials

We are aware of six clinical trials (according to www.clinicaltrials.gov), which have been organized up to date with a TE approach in myocardial repair. In Table 1, we report the five clinical trials according to www.clinicaltrials.gov. In an actively recruiting trial, a CorMatrixTM ECM sheet is applied on the epicardium of patients undergoing bypass implantation after an acute ischaemic event. This study was corroborated by a preclinical investigation performed in pigs supporting the cardioprotective and *pro*-vasculogenic effect of the matrix. In another recently completed trial, a combination of allogeneic embryonic stem cell-derived cardiac progenitor cells and fibrin was employed to create an engineered tissue patch that was sutured to the necrotic portion of the ventricle cover by a pericardial flap. Results of this study in six patients were recently published,¹⁸ and showed a promising outcome as to safety (the primary endpoint of the trial) and hints supporting the concept of functional recovery of the heart. In a different approach, therapeutic material (a myocardium specific ECM matrix) is delivered inside the myocardium using a minimally invasive procedure. In this case, the choice of the regions of the myocardium to be treated is guided by the mapping of myocardial viability, available by electric voltage mapping (e.g. NOGA system). The clinical trial approved in Japan and previously mentioned (see Section 4.3) is registered in the Japanese clinical trial registry. It will for the first time test human iPSC-derived cardiomyocytes implanted epicardially as cell sheets in patients with HF. As such it is a follow-up study to the completed skeletal myoblast cell sheet trial by the same group of investigators.⁹³ The propensity for arrhythmia induction and tumour formation as well as immune responses to the allograft will have to be monitored carefully. Following the fast track developmental strategy of the Japanese government, the study is planned to be performed in three patients under a conditional approval of the cardiomyocyte cell sheet for application in HF repair.⁹⁴

8. Recommendations

- The application of non-myocytes appears to be safe in patients with IHD and HF, but so far largely ineffective. Low cell retention at the site of injury and false expectations as to the outcome of the mostly

small clinical trials, which were naturally designed to test for safety and feasibility, but often misinterpreted as efficacy trials, contributed to the perception of futile outcome. These underpowered trials were successful in all cases, but not designed with sufficient power to test efficacy. Sufficiently long retention of the biologically active constituent, i.e. either cardiomyocytes or cells with paracrine activity, must be ensured to render tissue-engineered products effective in the remuscularization/regeneration of the failing heart;

- TE strategies should be further explored and optimized as they offer means to significantly enhance cell retention at the site of injury.
- Advanced preclinical development including manufacturing of tissue-engineered products should be in close interaction with the relevant regulatory authorities to be in-line with regulatory demands. In addition to an agreement on the GMP-manufacturing process this also includes a detailed discussion of typically rodent and large animal experiments.
- Further refinement of TE strategies, including the testing of three-dimensional printing, are attractive to optimize the biological activity of tissue-engineered products, i.e. either contractile performance or secretome activity or both, to continuously develop the next generation tissue-engineered products as well as strategies for their optimal administration.

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References

1. Iismaa SE, Kaidonis X, Nicks AM, Bogush N, Kikuchi K, Naqvi N, Harvey RP, Husain A, Graham RM. Comparative regenerative mechanisms across different mammalian tissues. *NPJ Regen Med* 2018;**3**:6.
2. A futile cycle in cell therapy. *Nat Biotechnol* 2017;**35**:291.
3. Madonna R, Van Laake LW, Davidson SM, Engel FB, Hausenloy DJ, Lecour S, Leor J, Perrino C, Schulz R, Ytrehus K, Landmesser U, Mummery CL, Janssens S, Willerson J, Eschenhagen T, Ferdinandy P, Sluijter JPG. Position paper of the European Society of Cardiology Working Group Cellular Biology of the Heart: cell-based therapies for myocardial repair and regeneration in ischemic heart disease and heart failure. *Eur Heart J* 2016;**37**:1789–1798.
4. Fernández-Avilés F, Sanz-Ruiz R, Climent AM, Badimon L, Bolli R, Charron D, Fuster V, Janssens S, Kastrup J, Kim HS, Lüscher TF, Martin JF, Menasché P, Simari RD, Stone GW, Terzic A, Willerson JT, Wu JC; TACTICS (Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes) Writing Group; Authors/Task Force Members. Chairpersons; Basic Research Subcommittee; Translational Research Subcommittee; Challenges of Cardiovascular Regenerative Medicine Subcommittee; Tissue Engineering Subcommittee; Delivery, Navigation, Tracking and Assessment Subcommittee; Clinical Trials Subcommittee; Regulatory and funding strategies subcommittee; Delivery, Navigation, Tracking and Assessment Subcommittee. Global position paper on cardiovascular regenerative medicine. *Eur Heart J* 2017;**38**:2532–2546.
5. Eschenhagen T, Bolli R, Braun T, Field LJ, Fleischmann BK, Frisén J, Giacca M, Hare JM, Houser S, Lee RT, Marbán E, Martin JF, Molkentin JD, Murry CE, Riley PR, Ruiz-Lozano P, Sadek HA, Sussman MA, Hill JA. Cardiomyocyte regeneration: a consensus statement. *Circulation* 2017;**136**:680–686.
6. Nguyen P, Neofytou E, Rhee J, Wu J. Potential strategies to address the major clinical barriers facing stem cell regenerative therapy for cardiovascular disease: a review. *JAMA Cardiol* 2016;**1**:953–962.
7. Hou D, Youssef EA-S, Brinton TJ, Zhang P, Rogers P, Price ET, Yeung AC, Johnstone BH, Yock PG, March LJ. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. *Circulation* 2005;**112**:I150–I156.
8. van den Akker F, Feyen DAM, van den Hoogen P, van Laake LW, van Eeuwijk ECM, Hoefer I, Pasterkamp G, Chamuleau SAJ, Grundeman PF, Doevendans PA, Sluijter JPG. Intramyocardial stem cell injection: go(ne) with the flow. *Eur Heart J* 2017;**38**:184–186.
9. Feyen DAM, Gaetani R, Deddens J, van Keulen D, van Opbergen C, Poldervaart M, Alblas J, Chamuleau S, van Laake LW, Doevendans PA, Sluijter JPG. Gelatin microspheres as vehicle for cardiac progenitor cells delivery to the myocardium. *Adv Health Mater* 2016;**5**:1071–1079.
10. Gaetani R, Feyen DAM, Verhage V, Slaats R, Messina E, Christman KL, Giacomello A, Doevendans PAFM, Sluijter JPG. Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction. *Biomaterials* 2015;**61**:339–348.
11. Pintus E, Baldassarri M, Perazzo L, Natali S, Ghinelli D, Buda R. Stem cells in osteochondral tissue engineering. *Adv Exp Med Biol* 2018;**1058**:359–372.
12. Rubart M, Pasumarthi K, Nakajima H, Soonpaa M, Nakajima H, Field L. Physiological coupling of donor and host cardiomyocytes after cellular transplantation. *Circ Res* 2003;**92**:1217–1224.
13. Didié M, Christalla P, Rubart M, Muppala V, Döker S, Unsöld B, El-Armouche A, Rau T, Eschenhagen T, Schwoerer AP, Ehmke H, Schumacher U, Fuchs S, Lange C, Becker A, Tao W, Scherschel JA, Soonpaa MH, Yang T, Lin Q, Zenke M, Han D-W, Schöler HR, Rudolph C, Steinemann D, Schlegelberger B, Kattman S, Witty A, Keller G, Field LJ, Zimmermann W-H. Parthenogenetic stem cells for tissue-engineered heart repair. *J Clin Invest* 2013;**123**:1285–1298.
14. Zimmermann W-H, Melnychenko I, Wasmeier G, Didié M, Naito H, Nixdorff U, Hess A, Budinsky L, Brune K, Michaelis B, Dhein S, Schwoerer A, Ehmke H, Eschenhagen T. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. *Nat Med* 2006;**12**:452–458.
15. Shiba Y, Fernandes S, Zhu W-Z, Filice D, Muskheili V, Kim J, Palpant NJ, Gantz J, Moyes KW, Reinecke H, Van Biber B, Dardas T, Mignone JL, Izawa A, Hanna R, Viswanathan M, Gold JD, Kotlikoff MI, Sarvazyan N, Kay MW, Murry CE, Laflamme MA. Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. *Nature* 2012;**489**:322–325.
16. Chong JH, Yang X, Don CW, Minami E, Liu Y-W, Weyers JJ, Mahoney WM, Van Biber B, Cook SM, Palpant NJ, Gantz JA, Fugate JA, Muskheili V, Gough GM, Vogel KW, Astley CA, Hotchkiss CE, Baldessari A, Pabon L, Reinecke H, Gill EA, Nelson V, Kiem H-P, Laflamme MA, Murry CE. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature* 2014;**510**:273–277.
17. Liu B, Shi R, Li X, Liu Y, Feng X, Chen X, Fan X, Zhang Y, Zhang W, Tang J, Zhou X, Li N, Lu X, Xu Z. Downregulation of L-type voltage-gated Ca²⁺ and voltage-gated K⁺, and large-conductance Ca²⁺-activated K⁺ channels in vascular myocytes from salt-loading offspring rats exposed to prenatal hypoxia. *J Am Heart Assoc* 2018;**7**:pii: e008148.
18. Menasché P, Vanneau V, Hagege A, Bel A, Chollet B, Parouchev A, Cacciapuoti I, Al-Daccak R, Benhamouda N, Blons H, Agbulut O, Tosca L, Trouvin J-H, Fabreguettes J-R, Bellamy V, Charron D, Tartour E, Tachdjian G, Desnos M, Larghero J. Transplantation of human embryonic stem cell-derived cardiovascular progenitors for severe ischemic left ventricular dysfunction. *J Am Coll Cardiol* 2018;**71**:429–438.
19. Riegler J, Tiburcy M, Ebert A, Tzatzalos E, Raaz U, Abilez OJ, Shen Q, Kooreman NG, Neofytou E, Chen VC, Wang M, Meyer T, Tsao PS, Connolly AJ, Couture LA, Gold JD, Zimmermann WH, Wu JC. Human engineered heart muscles engraft and survive long term in a rodent myocardial infarction model. *Circ Res* 2015;**117**:720–730.
20. Qin X, Riegler J, Tiburcy M, Zhao X, Chour T, Ndoe B, Nguyen M, Adams J, Ameen M, Denney TS Jr, Yang PC, Nguyen P, Zimmermann WH, Wu JC. Magnetic resonance imaging of cardiac strain pattern following transplantation of human tissue engineered heart muscles. *Circ Cardiovasc Imaging* 2016;**9**:pii:e004731.
21. Tiburcy M, Hudson JE, Balfanz P, Schlick S, Meyer T, Chang Liao M-L, Levent E, Raad F, Zeidler S, Wingender E, Riegler J, Wang M, Gold JD, Kehat I, Wettwer E, Ravens U, Dierickx P, van Laake LW, Goumans MJ, Khadjeh S, Toischer K, Hasenfuss G, Couture LA, Unger A, Linke WA, Araki T, Neel B, Keller G, Gepstein L, Wu JC, Zimmermann W-H. Defined engineered human myocardium with advanced maturation for applications in heart failure modeling and repair. *Circulation* 2017;**135**:1832–1847.
22. Weinberger F, Breckwoldt K, Pecha S, Kelly A, Geertz B, Starbatty J, Yorgan T, Cheng K-H, Lessmann K, Stolen T, Scherrer-Crosbie M, Smith G, Reichenspurner H, Hansen A, Eschenhagen T. Cardiac repair in guinea pigs with human engineered heart tissue from induced pluripotent stem cells. *Sci Transl Med* 2016;**8**:363ra148.
23. Gerbin KA, Yang X, Murry CE, Coulombe KL. Enhanced electrical integration of engineered human myocardium via intramyocardial versus epicardial delivery in infarcted rat hearts. *PLoS One* 2015;**10**:e0131446.
24. Shadrin IY, Allen BW, Qian Y, Jackman CP, Carlson AL, Juhas ME, Bursac N. Cardiopatch platform enables maturation and scale-up of human pluripotent stem cell-derived engineered heart tissues. *Nat Commun* 2017;**8**:1825.
25. Ulmer BM, Stoehr A, Schulze ML, Patel S, Gucek M, Mannhardt I, Funcke S, Murphy E, Eschenhagen T, Hansen A. Contractile work contributes to maturation of energy metabolism in hiPSC-derived cardiomyocytes. *Stem Cell Reports* 2018;**10**:834–847.
26. Abilez OJ, Tzatzalos E, Yang H, Zhao M-T, Jung G, Zöllner AM, Tiburcy M, Riegler J, Matsa E, Shukla P, Zhuge Y, Chour T, Chen VC, Burrige PW, Karakikes I, Kuhl E, Bernstein D, Couture LA, Gold JD, Zimmermann WH, Wu JC. Passive stretch induces structural and functional maturation of engineered heart muscle as predicted by computational modeling. *Stem Cells* 2018;**36**:265–277.
27. Ruan J-L, Tulloch NL, Razumova MV, Saiget M, Muskheili V, Pabon L, Reinecke H, Regnier M, Murry CE. Mechanical stress conditioning and electrical stimulation promote contractility and force maturation of induced pluripotent stem cell-derived human cardiac tissue. *Circulation* 2016;**134**:1557–1567.
28. Kana K, Song H, Laschinger C, Zandstra PW, Radisic M. PI3K phosphorylation is linked to improved electrical excitability in an *in vitro* engineered heart tissue disease model system. *Tissue Eng Part A* 2015;**21**:2379–2389.
29. Godier-Furnémont AF, Tiburcy M, Wagner E, Dewenter M, Lämmle S, El-Armouche A, Lehnart SE, Vunjak-Novakovic G, Zimmermann WH. Physiologic force-frequency response in engineered heart muscle by electromechanical stimulation. *Biomaterials* 2015;**60**:82–91.
30. Maidhof R, Tandon N, Lee EJ, Luo J, Duan Y, Yeager K, Konofagou E, Vunjak-Novakovic G. Biomimetic perfusion and electrical stimulation applied in concert improved the assembly of engineered cardiac tissue. *J Tissue Eng Regen Med* 2012;**6**:e12–e23.
31. 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**:5813–5820.
32. Yang X, Pabon L, Murry CE. Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. *Circ Res* 2014;**114**:511–523.

33. Ronaldson-Bouchard K, Ma SP, Yeager K, Chen T, Song LJ, Sirabella D, Morikawa K, Teles D, Yazawa M, Vunjak-Novakovic G. Advanced maturation of human cardiac tissue grown from pluripotent stem cells. *Nature* 2018;**556**:239–243.
34. Yang X, Rodriguez M, Pabon L, Fischer KA, Reinecke H, Regnier M, Sniadecki NJ, Ruohola-Baker H, Murry CE. Tri-iodo-L-thyronine promotes the maturation of human cardiomyocytes-derived from induced pluripotent stem cells. *J Mol Cell Cardiol* 2014;**72**:296–304.
35. Hirt MN, Boeddinghaus J, Mitchell A, Schaaf S, Börnchen C, Müller C, Schulz H, Hubner N, Stenzig J, Stoehr A, Neuber C, Eder A, Luther PK, Hansen A, Eschenhagen T. Functional improvement and maturation of rat and human engineered heart tissue by chronic electrical stimulation. *J Mol Cell Cardiol* 2014;**74**:151–161.
36. Nunes SS, Miklas JW, Liu J, Aschar-Sobbi R, Xiao Y, Zhang B, Jiang J, Massé S, Gagliardi M, Hsieh A, Thavandiran N, Laflamme MA, Nanthakumar K, Gross GJ, Backx PH, Keller G, Radisic M. Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. *Nat Methods* 2013;**10**:781–787.
37. Soong P, Tiburcy M, Zimmermann W. Cardiac differentiation of human embryonic stem cells and their assembly into engineered heart muscle. *Curr Protoc Cell Biol* 2012; Chapter 23:Unit23.8. doi: 10.1002/0471143030.cb2308s55.
38. Liaw NY, Zimmermann WH. Mechanical stimulation in the engineering of heart muscle. *Adv Drug Deliv Rev* 2016;**96**:156–160.
39. Schaaf S, Shibamiya A, Mewe M, Eder A, Stöhr A, Hirt MN, Rau T, Zimmermann W-H, Conradi L, Eschenhagen T, Hansen A. Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology. *PLoS One* 2011;**6**:e26397.
40. Kensah G, Roa Lara A, Dahlmann J, Zweigerdt R, Schwanke K, Hegermann J, Skvorc D, Gawol A, Azizian A, Wagner S, Maier LS, Krause A, Dräger G, Ochs M, Haverich A, Gruh I, Martin U. Murine and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue *in vitro*. *Eur Heart J* 2013;**34**:1134–1146.
41. Pedrotty DM, Klinger RY, Kirkton RD, Bursac N. Cardiac fibroblast paracrine factors alter impulse conduction and ion channel expression of neonatal rat cardiomyocytes. *Cardiovasc Res* 2009;**83**:688–697.
42. Naito H, Melnychenko I, Didié M, Schneiderbanger K, Schubert P, Rosenkranz S, Eschenhagen T, Zimmermann WH. Optimizing engineered heart tissue for therapeutic applications as surrogate heart muscle. *Circulation* 2006;**114**:172–178.
43. Detert S, Stamm C, Beez C, Diedrichs F, Ringe J, Van Linthout S, Seifert M, Tschöpe C, Sittler M, Haag M. The atrial appendage as a suitable source to generate cardiac-derived adherent proliferating cells for regenerative cell-based therapies. *J Tissue Eng Regen Med* 2018;**12**:e1404–e1417.
44. Devalia HD, Schwach V, Ford JW, Milnes JT, El-Haou S, Jackson C, Gkatzis K, Elliott DA, Chvu de Sousa Lopes SM, Mummery CL, Verkerk AO, Passier R. Atrial-like cardiomyocytes from human pluripotent stem cells are a robust preclinical model for assessing atrial-selective pharmacology. *EMBO Mol Med* 2015;**7**:394–410.
45. Witty AD, Mihic A, Tam RY, Fisher SA, Mikryukov A, Shoichet MS, Li R-K, Kattman SJ, Keller G. Generation of the epicardial lineage from human pluripotent stem cells. *Nat Biotechnol* 2014;**32**:1026–1035.
46. Guadix JA, Orlova VV, Giacomelli E, Bellin M, Ribeiro MC, Mummery CL, Pérez-Pomares JM, Passier R. Human pluripotent stem cell differentiation into functional epicardial progenitor cells. *Stem Cell Reports* 2017;**9**:1754–1764.
47. Protze SI, Liu J, Nussinovich U, Ohana L, Backx PH, Gepstein L, Keller GM. Sinoatrial node cardiomyocytes derived from human pluripotent cells function as a biological pacemaker. *Nat Biotechnol* 2016;**35**:56–68.
48. Cyganek L, Tiburcy M, Sekeres K, Gerstenberg K, Bohnenberger H, Lenz C, Henze S, Stauske M, Salinas G, Zimmermann WH, Hasenfuss G, Guan K. Deep phenotyping of human induced pluripotent stem cell-derived atrial and ventricular cardiomyocytes. *JCI Insight* 2018;**3**:pii:99941.
49. Tulloch NL, Muskheili V, Razumova MV, Korte FS, Regnier M, Hauch KD, Pabon L, Reinecke H, Murry CE. Growth of engineered human myocardium with mechanical loading and vascular coculture. *Circ Res* 2011;**109**:47–59.
50. Gao L, Gregorich ZR, Zhu W, Mattapally S, Oduk Y, Lou X, Kannappan R, Borovjagin AV, Walcott GP, Pollard AE, Fast VG, Hu X, Lloyd SG, Ge Y, Zhang J. Large cardiac muscle patches engineered from human induced-pluripotent stem cell-derived cardiac cells improve recovery from myocardial infarction in swine. *Circulation* 2018;**137**:1712–1730.
51. Segers VFM, Brutsaert DL, De Keulenaer GW. Cardiac remodeling: endothelial cells have more to say than just no. *Front Physiol* 2018;**9**:382.
52. Li Y, Asfour H, Bursac N. Age-dependent functional crosstalk between cardiac fibroblasts and cardiomyocytes in a 3D engineered cardiac tissue. *Acta Biomater* 2017;**55**:120–130.
53. Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LEJ, Berman D, Czer LSC, Marbán L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marbán E. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. *Lancet* 2012;**379**:895–904.
54. Bartunek J, Behfar A, Dolatabadi D, Vanderheyden M, Ostojic M, Denis J, El Nakadi B, Banovic M, Beleslin B, Vrolix M, Legrand V, Vrints C, Vanoverschelde JL, Crespo-Diaz R, Homsy C, Tendera M, Waldman S, Wijns W, Terzic A. Cardiopoietic stem cell therapy in heart failure: the C-CURE (Cardiopoietic stem Cell therapy in heart failure) multicenter randomized trial with lineage-specified biologics. *J Am Coll Cardiol* 2013;**61**:2329–2338.
55. Menasche P. Cell therapy trials for heart regeneration—lessons learned and future directions. *Nat Rev Cardiol* 2018;**15**:659–671.
56. Hentze H, Graichen R, Colman A. Cell therapy and the safety of embryonic stem cell-derived grafts. *Trends Biotechnol* 2007;**25**:24–32.
57. Crespo-Diaz R, Yamada S, Bartunek J, Perez-Terzic C, de Waele P, Mauën S, Terzic A, Behfar A. Cardiopoietic index predicts heart repair fitness of patient-derived stem cells. *Biomarkers Med* 2015;**9**:639–649.
58. Dimmeler S, Leri A. Aging and disease as modifiers of efficacy of cell therapy. *Circ Res* 2008;**102**:1319–1330.
59. Felix NJ, Allen PM. Specificity of T-cell alloreactivity. *Nat Rev Immunol* 2007;**7**:942–953.
60. Braza MS, van Leent MMT, Lameijer M, Sanchez-Gaytan BL, Arts RJW, Pérez-Medina C, Conde P, Garcia MR, Gonzalez-Perez M, Brahmachary M, Fay F, Kluza E, Kossatz S, Dress RJ, Salem F, Rialdi A, Reiner T, Boros P, Strijkers GJ, Calcagno CC, Ginhoux F, Marazzi I, Lutgens E, Nicolaes GAF, Weber C, Swirski FK, Nahrendorf M, Fisher EA, Duivenvoorden R, Fayud ZA, Netea MG, Mulder WJM, Ochando J. Inhibiting inflammation with myeloid cell-specific nanobiologics promotes organ transplant acceptance. *Immunity* 2018;**49**:819–828.e6.
61. Gornalusse GG, Hirata RK, Funk SE, Riobobos L, Lopes VS, Manske G, Prunkard D, Colunga AG, Hanafi L-A, Clegg DO, Turtle C, Russell DW. HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. *Nat Biotechnol* 2017;**35**:765–772.
62. Di Stasi A, Tey S-K, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, Straathof K, Liu E, Duret AG, Grilley B, Liu H, Cruz CR, Savoldo B, Gee AP, Schindler J, Krance RA, Heslop HE, Spencer DM, Rooney CM, Brenner MK. Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med* 2011;**365**:1673–1683.
63. Jansen OF, Lorkeers SJ, Eding JEC, Vesterinen HM, van der Spoel TIG, Sena ES, Duckers HJ, Doevendans PA, Macleod MR, Chamuleau SAJ. Similar effect of autologous and allogeneic cell therapy for ischemic heart disease: systematic review and meta-analysis of large animal studies. *Circ Res* 2015;**116**:80–86.
64. Beck J, Oellerich M, Schulz U, Schauerer V, Reinhard L, Fuchs U, Knabbe C, Zittermann A, Olbricht C, Gummert JF, Shipkova M, Birschmann I, Wieland E, Schütz E. Donor-derived cell-free DNA is a novel universal biomarker for allograft rejection in solid organ transplantation. *Transplant Proc* 2015;**47**:2400–2403.
65. Malliaras K, Li T-S, Luthringer D, Terrovitis J, Cheng K, Chakravarty T, Galang G, Zhang Y, Schoenhoff F, Van Eyk J, Marbán L, Marbán E. Safety and efficacy of allogeneic cell therapy in infarcted rats transplanted with mismatched cardiosphere-derived cells. *Circulation* 2012;**125**:100–112.
66. Kawamura T, Miyagawa S, Fukushima S, Maeda A, Kashiwama N, Kawamura A, Miki K, Okita K, Yoshida Y, Shiina T, Ogasawara K, Miyagawa S, Toda K, Okuyama H, Sawa Y. Cardiomyocytes derived from MHC-homozygous induced pluripotent stem cells exhibit reduced allogeneic immunogenicity in MHC-matched non-human primates. *Stem Cell Reports* 2016;**6**:312–320.
67. Taylor CJ, Peacock S, Chaudhry AN, Bradley JA, Bolton EM. Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. *Cell Stem Cell* 2012;**11**:147–152.
68. Zimmermann WH. Remuscularizing failing hearts with tissue engineered myocardium. *Antioxid Redox Signal* 2009;**11**:2011–2023.
69. Weinberger F, Mannhardt I, Eschenhagen T. Engineering cardiac muscle tissue: a maturing field of research. *Circ Res* 2017;**120**:1487–1500.
70. Camici-Unal G, Cuttica D, Annabi N, Demarchi D, Khademhosseini A. Synthesis and characterization of hybrid hyaluronic acid-gelatin hydrogels. *Biomacromolecules* 2013;**14**:1085–1092.
71. Wang C, Varshney RR, Wang DA. Therapeutic cell delivery and fate control in hydrogels and hydrogel hybrids. *Adv Drug Deliv Rev* 2010;**62**:699–710.
72. El-Sherbiny IM, Yacoub MH. Hydrogel scaffolds for tissue engineering: progress and challenges. *Glob Cardiol Sci Pract* 2013;**2013**:316–342.
73. Eschenhagen T, Fink C, Remmers U, Scholz H, Wachtow J, Weil J, Zimmermann WH, Dohmen HH, Schäfer H, Bishopric N, Wakatsuki T, Elson EJ. Three-dimensional reconstitution of embryonic cardiomyocytes in a collagen matrix: a new heart muscle model system. *FASEB J* 1997;**11**:683–694.
74. Zimmermann W-H, Schneiderbanger K, Schubert P, Didié M, Münzel F, Heubach JF, Kostin S, Neuberger WL, Eschenhagen T. Tissue engineering of a differentiated cardiac muscle construct. *Circ Res* 2002;**90**:223–230.
75. Hansen A, Eder A, Bönstrup M, Flato M, Mewe M, Schaaf S, Aksehirliglob B, Schwoerer AP, Schwörer A, Uebeler J, Eschenhagen T. Development of a drug screening platform based on engineered heart tissue. *Circ Res* 2010;**107**:35–44.
76. Zimmermann WH, Fink C, Kralisch D, Remmers U, Weil J, Eschenhagen T. Three-dimensional engineered heart tissue from neonatal rat cardiac myocytes. *Biotechnol Bioeng* 2000;**68**:106–114.
77. Li RA, Keung W, Cashman TJ, Backeris PC, Johnson BV, Bardot ES, Wong AOT, Chan PKW, Chan CWY, Costa KD. Bioengineering an electro-mechanically functional miniature ventricular heart chamber from human pluripotent stem cells. *Biomaterials* 2018;**163**:116–127.
78. Leonard A, Bertero A, Powers JD, Beussman KM, Bhandari S, Regnier M, Murry CE, Sniadecki NJ. Afterload promotes maturation of human induced pluripotent stem cell derived cardiomyocytes in engineered heart tissues. *J Mol Cell Cardiol* 2018;**118**:147–158.

79. Sun J, Lu Y, Huang Y, Zhang L, Ma Y. Engineered heart tissue transplantation alters electrical-conduction function in rats with myocardial infarction. *Life Sci* 2014;**118**: 34–38.
80. Yanamandala M, Zhu W, Garry DJ, Kamp TJ, Hare JM, Jun H-W, Yoon Y-S, Bursac N, Prabhu SD, Dorn GW, Bolli R, Kitsis RN, Zhang J. Overcoming the roadblocks to cardiac cell therapy using tissue engineering. *J Am Coll Cardiol* 2017;**70**:766–775.
81. Seif-Naraghi SB, Singelyn JM, Salvatore MA, Osborn KG, Wang JJ, Sampat U, Kwan OL, Strachan GM, Wong J, Schup-Magoffin PJ, Braden RL, Bartels K, DeQuach JA, Preul M, Kinsey AM, DeMaria AN, Dib N, Christman KL. Safety and efficacy of an injectable extracellular matrix hydrogel for treating myocardial infarction. *Sci Transl Med* 2013;**5**:173ra25.
82. Dawson E, Mapili G, Erickson K, Taqvi S, Roy K. Biomaterials for stem cell differentiation. *Adv Drug Deliv Rev* 2008;**60**:215–228.
83. Nawroth JC, Scudder LL, Halvorson RT, Tresback J, Ferrier JP, Sheehy SP, Cho A, Kannan S, Sunyovszki I, Goss JA, Campbell PH, Parker KK. Automated fabrication of photopatterned gelatin hydrogels for organ-on-chips applications. *Biofabrication* 2018;**10**:025004.
84. Shin SR, Jung SM, Zalabany M, Kim K, Zorlutuna P, Kim S, B, Nikkhah M, Khabiry M, Azize M, Kong J, Wan K-T, Palacios T, Dokmeci MR, Bae H, Tang X(S), Khademhosseini A. Carbon-nanotube-embedded hydrogel sheets for engineering cardiac constructs and bioactuators. *ACS Nano* 2013;**7**:2369–2380.
85. Mosqueira D, Pagliari S, Uto K, Ebara M, Romanazzo S, Escobedo-Lucea C, Nakanishi J, Taniguchi A, Franzese O, Di Nardo P, Goumans MJ, Traversa E, Pinto-do-Ó P, Aoyagi T, Forte G. Hippo pathway effectors control cardiac progenitor cell fate by acting as dynamic sensors of substrate mechanics and nanostructure. *ACS Nano* 2014;**8**:2033–2047.
86. Yue K, Trujillo-de Santiago G, Alvarez MM, Tamayol A, Annabi N, Khademhosseini A. Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. *Biomaterials* 2015;**73**:254–271.
87. Tiburcy M, Didié M, Boy O, Christalla P, Döker S, Naito H, Karikkineth BC, El-Armouche A, Grimm M, Nose M, Eschenhagen T, Zieseniss A, Katschinski DM, Hamdani N, Linke WA, Yin X, Mayr M, Zimmermann W-H. Terminal differentiation, advanced organotypic maturation, and modeling of hypertrophic growth in engineered heart tissue. *Circ Res* 2011;**109**:1105–1114.
88. Shimizu T, Sekine H, Yang J, Isoi Y, Yamato M, Kikuchi A, Kobayashi E, Okano T. Polysurgery of cell sheet grafts overcomes diffusion limits to produce thick, vascularized myocardial tissues. *FASEB J* 2006;**20**:708–710.
89. Yang J, Yamato M, Nishida K, Ohki T, Kanzaki M, Sekine H, Shimizu T, Okano T. Cell delivery in regenerative medicine: the cell sheet engineering approach. *J Control Release* 2006;**116**:193–203.
90. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, Ishino K, Ishida H, Shimizu T, Kangawa K, Sano S, Okano T, Kitamura S, Mori H. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. *Nat Med* 2006;**12**:459–465.
91. Narita T, Shintani Y, Ikebe C, Kaneko M, Campbell NG, Coppen SR, Uppal R, Sawa Y, Yashiro K, Suzuki K. The use of scaffold-free cell sheet technique to refine mesenchymal stromal cell-based therapy for heart failure. *Mol Ther* 2013;**21**:860–867.
92. Masumoto H, Matsuo T, Yamamizu K, Uosaki H, Narazaki G, Katayama S, Marui A, Shimizu T, Ikeda T, Okano T, Sakata R, Yamashita JK. Pluripotent stem cell-engineered cell sheets reassembled with defined cardiovascular populations ameliorate reduction in infarct heart function through cardiomyocyte-mediated neovascularization. *Stem Cells* 2012;**30**:1196–1205.
93. Sawa Y, Yoshikawa Y, Toda K, Fukushima S, Yamazaki K, Ono M, Sakata Y, Hagiwara N, Kinugawa K, Miyagawa S. Safety and efficacy of autologous skeletal myoblast sheets (TCD-51073) for the treatment of severe chronic heart failure due to ischemic heart disease. *Circ J* 2015;**79**:991–999.
94. Cyranoski D. ‘Reprogrammed’ stem cells approved to mend human hearts for the first time. *Nature* 2018;**557**:619–620.
95. Shimizu T, Yamato M, Isoi Y, Akutsu T, Setomaru T, Abe K, Kikuchi A, Umezumi M, Okano T. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. *Circ Res* 2002;**90**:e40.
96. Groll J, Boland T, Blunk T, Burdick JA, Cho D-W, Dalton PD, Derby B, Forgacs G, Li Q, Mironov VA, Moroni L, Nakamura M, Shu W, Takeuchi S, Vozzi G, Woodfield TB, Xu T, Yoo JJ, Malda J. Biofabrication: reappraising the definition of an evolving field. *Biofabrication* 2016;**8**:013001.
97. Cui H, Miao S, Esworthy T, Zhou X, Lee SJ, Liu C, Yu ZX, Fisher JP, Mohiuddin M, Zhang LG. 3D bioprinting for cardiovascular regeneration and pharmacology. *Adv Drug Deliv Rev* 2018;**132**:252–269.
98. Bejleri D, Streeter BW, Nachlas ALY, Brown ME, Gaetani R, Christman KL, Davis ME. A bioprinted cardiac patch composed of cardiac-specific extracellular matrix and progenitor cells for heart repair. *Adv Healthc Mater* 2018;**7**:1800672.
99. Ionov L. 4D biofabrication: materials, methods, and applications. *Adv Healthc Mater* 2018;**7**:1800412.
100. Schaefer JA, Guzman PA, Riemenschneider SB, Kamp TJ, Tranquillo RT. A cardiac patch from aligned microvessel and cardiomyocyte patches. *J Tissue Eng Regen Med* 2018;**12**:546–556.
101. Jackman CP, Ganapathi AM, Asfour H, Qian Y, Allen BW, Li Y, Bursac N. Engineered cardiac tissue patch maintains structural and electrical properties after epicardial implantation. *Biomaterials* 2018;**159**:48–58.
102. Dvir T, Timko BP, Brigham MD, Naik SR, Karajani SS, Levy O, Jin H, Parker KK, Langer R, Kohane DS. Nanowired three-dimensional cardiac patches. *Nat Nanotechnol* 2011;**6**:720–725.
103. Boothe SD, Myers JD, Pok S, Sun J, Xi Y, Nieto RM, Cheng J, Jacot JG. The effect of substrate stiffness on cardiomyocyte action potentials. *Cell Biochem Biophys* 2016;**74**: 527–535.
104. Roeder I, Loeffler M, Glauche I, Other P. Towards a quantitative understanding of stem cell-niche interaction: experiments, models, and technologies. *Blood Cells Mol Dis* 2011;**46**:308–317.
105. Chimenti I, Massai D, Morbiducci U, Beltrami AP, Pesce M, Messina E. Stem cell spheroids and ex vivo niche modeling: rationalization and scaling-up. *J Cardiovasc Transl Res* 2017;**10**:150–166.
106. Montgomery M, Ahadian S, Davenport Huyer L, Lo Rito M, Civitarese RA, Vanderlaan RD, Wu J, Reis LA, Momen A, Akbari S, Pahnke A, Li R-K, Calderone CA, Radisic M. Flexible shape-memory scaffold for minimally invasive delivery of functional tissues. *Nat Mater* 2017;**16**:1038–1046.
107. Sepantafar M, Maheronnaghsh R, Mohammadi H, Rajabi-Zeleti S, Annabi N, Aghdami N, Baharvand H. Stem cells and injectable hydrogels: synergistic therapeutics in myocardial repair. *Biotechnol Adv* 2016;**34**:362–379.
108. Madonna R, Petrov L, Teberino MA, Manzoli L, Karam J-P, Renna FV, Ferdinandy P, Montero-Menei CN, Ylä-Herttuala S, De Caterina R. Transplantation of adipose tissue mesenchymal cells conjugated with VEGF-releasing microcarriers promotes repair in murine myocardial infarction. *Cardiovasc Res* 2015;**108**:39–49.
109. Wang LL, Liu Y, Chung JJ, Wang T, Gaffey AC, Lu M, Cavanaugh CA, Zhou S, Kanade R, Atluri P, Morrissy EE, Burdick JA. Local and sustained miRNA delivery from an injectable hydrogel promotes cardiomyocyte proliferation and functional regeneration after ischemic injury. *Nat Biomed Eng* 2017;**1**:983–992.
110. 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.
111. Liu H, Paul C, Xu M. Optimal environmental stiffness for stem cell mediated ischemic myocardium repair. *Methods Mol Biol* 2017;**1553**:293–304.
112. Bao R, Tan B, Liang S, Zhang N, Wang W, Liu W. A pi-pi conjugation-containing soft and conductive injectable polymer hydrogel highly efficiently rebuilds cardiac function after myocardial infarction. *Biomaterials* 2017;**122**:63–71.
113. Morez C, Nosedà M, Paiva MA, Belian E, Schneider MD, Stevens MM. Enhanced efficiency of genetic programming toward cardiomyocyte creation through topographical cues. *Biomaterials* 2015;**70**:94–104.
114. Ebrahimi B. In vivo reprogramming for heart regeneration: a glance at efficiency, environmental impacts, challenges and future directions. *J Mol Cell Cardiol* 2017;**108**:61–72.
115. Song Y, Zhang C, Zhang J, Sun N, Huang K, Li H, Wang Z, Huang K, Wang L. An injectable silk sericin hydrogel promotes cardiac functional recovery after ischemic myocardial infarction. *Acta Biomater* 2016;**41**:210–223.
116. Kim BH, Park M, Park HJ, Lee SH, Choi SY, Park CG, Han SM, Heo CY, Choy YB. Prolonged, acute suppression of cysteinyl leukotriene to reduce capsular contracture around silicone implants. *Acta Biomater* 2017;**51**:209–219.
117. Kimura W, Xiao F, Canseco DC, Muralidhar S, Thet S, Zhang HM, Abderrahman Y, Chen R, Garcia JA, Shelton JM, Richardson JA, Ashour AM, Asaithamby A, Liang H, Xing C, Lu Z, Zhang CC, Sadek HA. Hypoxia fate mapping identifies cycling cardiomyocytes in the adult heart. *Nature* 2015;**523**:226–230.
118. Vishwakarma A, Bhise NS, Evangelista MB, Rouwkema J, Dokmeci MR, Gaemmaghami AM, Vrana NE, Khademhosseini A. Engineering immunomodulatory biomaterials to tune the inflammatory response. *Trends Biotechnol* 2016;**34**:470–482.
119. Aurora AB, Porrello ER, Tan W, Mahmoud AI, Hill JA, Bassel-Duby R, Sadek HA, Olson EN. Macrophages are required for neonatal heart regeneration. *J Clin Invest* 2014;**124**:1382–1392.
120. Hernandez MJ, Christman KL. Designing acellular injectable biomaterial therapeutics for treating myocardial infarction and peripheral artery disease. *JACC Basic Transl Sci* 2017;**2**:212–226.
121. Mann DL, Lee RJ, Coats AJS, Neagoe G, Dragomir D, Pusineri E, Piredda M, Bettari L, Kirwan B-A, Dowling R, Volterrani M, Solomon SD, Sabbah HN, Hinson A, Anker SD. One-year follow-up results from AUGMENT-HF: a multicentre randomized controlled clinical trial of the efficacy of left ventricular augmentation with Algisyl in the treatment of heart failure. *Eur J Heart Fail* 2016;**18**:314–325.
122. Anker SD, Coats AJS, Cristian G, Dragomir D, Pusineri E, Piredda M, Bettari L, Dowling R, Volterrani M, Kirwan B-A, Filipatos G, Mas J-L, Danchin N, Solomon SD, Lee RJ, Ahmann F, Hinson A, Sabbah HN, Mann DL. A prospective comparison of alginate-hydrogel with standard medical therapy to determine impact on functional capacity and clinical outcomes in patients with advanced heart failure (AUGMENT-HF trial). *Eur Heart J* 2015;**36**:2297–2309.
123. Frey N, Linke A, Süsselbeck T, Müller-Ehmsen J, Vermeersch P, Schoors D, Rosenberg M, Bea F, Tuvia S, Leor J. Intracoronary delivery of injectable bioabsorbable scaffold (IK-5001) to treat left ventricular remodeling after ST-elevation myocardial infarction: a first-in-man study. *Circ Cardiovasc Interv* 2014;**7**:806–812.

124. Wei K, Serpooshan V, Hurtado C, Diez-Cuñado M, Zhao M, Maruyama S, Zhu W, Fajardo G, Nosedá M, Nakamura K, Tian X, Liu Q, Wang A, Matsuurá Y, Bushway P, Cai W, Savchenko A, Mahmoudi M, Schneider MD, van den Hoff MJB, Butte MJ, Yang PC, Walsh K, Zhou B, Bernstein D, Mercola M, Ruiz-Lozano P. Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. *Nature* 2015;**525**: 479–485.
125. Sluijter JPG, Davidson SM, Boulanger CM, Buzás EI, de Kleijn DPV, Engel FB, Giricz Z, Hausenloy DJ, Kishore R, Lecour S, Leor J, Madonna R, Perrino C, Prunier F, Sahoo S, Schiffelers RM, Schulz R, Van Laake LW, Ytrehus K, Ferdinandy P. Extracellular vesicles in diagnostics and therapy of the ischaemic heart: position Paper from the Working Group on Cellular Biology of the Heart of the European Society of Cardiology. *Cardiovasc Res* 2018;**114**:19–34.
126. Barile L, Cervio E, Lionetti V, Milano G, Ciullo A, Biemmi V, Bolis S, Altomare C, Matteucci M, Di Silvestre D, Brambilla F, Fertig TE, Torre T, Demertzis S, Mauri P, Moccetti T, Vassalli G. Cardioprotection by cardiac progenitor cell-secreted exosomes: role of pregnancy-associated plasma protein-A. *Cardiovasc Res* 2018;**114**: 992–1005.
127. El Harane N, Kervadec A, Bellamy V, Pidal L, Neametalla HJ, Perier MC, Lima Correa B, Thiébault L, Cagnard N, Duché A, Brunaud C, Lemitre M, Gauthier J, Bourdillon AT, Renault MP, Hovhannisyán Y, Paiva S, Colas AR, Agbulut O, Hagege A, Silvestre JS, Menasché P, Renault NKE. Acellular therapeutic approach for heart failure: in vitro production of extracellular vesicles from human cardiovascular progenitors. *Eur Heart J* 2018;**39**:1835–1847.
128. Chen CW, Wang LL, Zaman S, Gordon J, Arisi MF, Venkataraman CM, Chung JJ, Hung G, Gaffey AC, Spruce LA, Fazelinia H, Gorman RC, Seeholzer SH, Burdick JA, Atluri P. Sustained release of endothelial progenitor cell-derived extracellular vesicles from shear-thinning hydrogels improves angiogenesis and promotes function after myocardial infarction. *Cardiovasc Res* 2018;**114**:1029–1040.
129. Pape AC, Bakker MH, Tseng CC, Bastings MM, Koudstaal S, Agostoni P, Chamuleau SA, Dankers PY. An injectable and drug-loaded supramolecular hydrogel for local catheter injection into the pig heart. *J Vis Exp* 2015:e52450.
130. Koudstaal S, Bastings MMC, Feyen DAM, Waring CD, van Slochteren FJ, Dankers PYW, Torella D, Sluijter JPG, Nadal-Ginard B, Doevendans PA, Ellison GM, Chamuleau SAJ. Sustained delivery of insulin-like growth factor-1/hepatocyte growth factor stimulates endogenous cardiac repair in the chronic infarcted pig heart. *J Cardiovasc Transl Res* 2014;**7**:232–241.
131. Hastings CL, Roche ET, Ruiz-Hernandez E, Schenke-Layland K, Walsh CJ, Duffy GP. Drug and cell delivery for cardiac regeneration. *Adv Drug Deliv Rev* 2015;**84**: 85–106.
132. Gathier WA, van Ginkel DJ, van der Naald M, van Slochteren FJ, Doevendans PA, Chamuleau SAJ. Retrograde coronary venous infusion as a delivery strategy in regenerative cardiac therapy: an overview of preclinical and clinical data. *J Cardiovasc Transl Res* 2018;**11**:173–181.
133. Tang XL, Nakamura S, Li Q, Wyszczynski M, Gumpert AM, Wu WJ, Hunt G, Stowers H, Ou Q, Bolli R. Repeated administrations of cardiac progenitor cells are superior to a single administration of an equivalent cumulative dose. *J Am Heart Assoc* 2018;**7**:pii:e007400.
134. Chaudhuri R, Ramachandran M, Moharil P, Harumalani M, Jaiswal AK. Biomaterials and cells for cardiac tissue engineering: current choices. *Mater Sci Eng C Mater Biol Appl* 2017;**79**:950–957.
135. Bel A, Planat-Bernard V, Saito A, Bonnevie L, Bellamy V, Sabbah L, Bellabas L, Brinon B, Vanneaux V, Pradeau P, Peyrard S, Larghero J, Pouly J, Binder P, Garcia S, Shimizu T, Sawa Y, Okano T, Bruneval P, Desnos M, Hagege AA, Casteilla L, Pucéat M, Menasché P. Composite cell sheets: a further step toward safe and effective myocardial regeneration by cardiac progenitors derived from embryonic stem cells. *Circulation* 2010;**122**:S118–S123.
136. Kawatou M, Masumoto H, Fukushima H, Morinaga G, Sakata R, Ashihara T, Yamashita JK. Modelling Torsade de Pointes arrhythmias in vitro in 3D human iPSC cell-engineered heart tissue. *Nat Commun* 2017;**8**:1078.
137. Qi C, Yan X, Huang G, Melerzanov A, Du Y. Biomaterials as carrier, barrier and reactor for cell-based regenerative medicine. *Protein Cell* 2015;**6**:638–653.
138. Kempf H, Kropp C, Olmer R, Martin U, Zweigerdt R. Cardiac differentiation of human pluripotent stem cells in scalable suspension culture. *Nat Protoc* 2015;**10**:1345–1361.
139. Rojas SV, Kensah G, Rotaermeil A, Baraki H, Kutschka I, Zweigerdt R, Martin U, Haverich A, Gruh I, Martens A. Transplantation of purified iPSC-derived cardiomyocytes in myocardial infarction. *PLoS One* 2017;**12**:e0173222.
140. Chachques JC, Trainini JC, Lago N, Cortes-Morichetti M, Schussler O, Carpentier A. Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium (MAGNUM trial): clinical feasibility study. *Ann Thorac Surg* 2008;**85**:901–908.
141. Ferdinandy P, Hausenloy DJ, Heusch G, Baxter GF, Schulz R. Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning. *Pharmacol Rev* 2014;**66**:1142–1174.
142. Hausenloy DJ, Garcia-Dorado D, Botker HE, Davidson SM, Downey J, Engel FB, Jennings R, Lecour S, Leor J, Madonna R, Ovize M, Perrino C, Prunier F, Schulz R, Sluijter JPG, Van Laake LW, Vinten-Johansen J, Yellon DM, Ytrehus K, Heusch G, Ferdinandy P. Novel targets and future strategies for acute cardioprotection: position Paper of the European Society of Cardiology Working Group on Cellular Biology of the Heart. *Cardiovasc Res* 2017;**113**:564–585.
143. Corbett MS, Webster A, Hawkins R, Woolacott N. Innovative regenerative medicines in the EU: a better future in evidence? *BMC Med* 2017;**15**:49.
144. Menasché P, Hagege AA, Vilquin J-T, Desnos M, Abergel E, Pouzet B, Bel A, Sarateanu S, Scorsin M, Schwartz K, Bruneval P, Benbunan M, Marolleau J-P, Duboc D. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol* 2003;**41**:1078–1083.
145. Shiba Y, Gombuchi T, Seto T, Wada Y, Ichimura H, Tanaka Y, Ogasawara T, Okada K, Shiba N, Sakamoto K, Ido D, Shiina T, Ohkura M, Nakai J, Uno N, Kazuki Y, Oshimura M, Minami I, Ikeda U. Allogeneic transplantation of iPSC cell-derived cardiomyocytes regenerates primate hearts. *Nature* 2016;**538**:388–391.
146. Liu Y-W, Chen B, Yang X, Fugate JA, Kalucki FA, Futakuchi-Tsuchida A, Couture L, Vogel KW, Astley CA, Baldessari A, Ogle J, Don CW, Steinberg ZL, Seslar SP, Tuck SA, Tsuchida H, Naumova AV, Dupras SK, Lyu MS, Lee J, Hailey DW, Reinecke H, Pabon L, Fryer BH, MacLellan WR, Thies RS, Murry CE. Human embryonic stem cell-derived cardiomyocytes restore function in infarcted hearts of non-human primates. *Nat Biotechnol* 2018;**36**:597–605.
147. Madonna R. Human-induced pluripotent stem cells: in quest of clinical applications. *Mol Biotechnol* 2012;**52**:193–203.
148. Breitbach M, Bostani T, Roell W, Xia Y, Dewald O, Nygren JM, Fries JWU, Tiemann K, Bohlen H, Hescheler J, Welz A, Bloch W, Jacobsen SEW, Fleischmann BK. Potential risks of bone marrow cell transplantation into infarcted hearts. *Blood* 2007;**110**:1362–1369.
149. Chung L, Maestas DR Jr, Housseau F, Elisseeff JH. Key players in the immune response to biomaterial scaffolds for regenerative medicine. *Adv Drug Deliv Rev* 2017;**114**:184–192.
150. Natsumeda M, Florea V, Rieger AC, Tompkins BA, Banerjee MN, Golpanian S, Fritsch J, Landin AM, Kashikar ND, Karantalis V, Loescher VY, Hatzistergos KE, Bagno L, Sanina C, Mushtaq M, Rodriguez J, Rosado M, Wolf A, Collon K, Vincent L, Kanelidis AJ, Schulman IH, Mitrani R, Heldman AW, Balkan W, Hare JM. A combination of allogeneic stem cells promotes cardiac regeneration. *J Am Coll Cardiol* 2017;**70**:2504–2515.
151. Marks P, Gottlieb S. Balancing safety and innovation for cell-based regenerative medicine. *N Engl J Med* 2018;**378**:954–959.