Biomaterial-Enhanced Cell and Drug Delivery: Lessons Learned in the Cardiac Field and Future Perspectives.

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Question	**Response**
Please submit a plain text version of your cover letter here. **If you are submitting a revision of your manuscript, please do not overwrite your original cover letter. There is an opportunity for you to provide your responses to the reviewers later; please do not add them here**.	Dear Dr Dimitrios Zeugolis and Prof Abhay Pandit, On behalf of my co-authors, I am submitting the enclosed original article for consideration for publication in Advanced Materials. It has not been submitted for publication elsewhere nor has it been published in whole or in part elsewhere. All the authors were fully involved in the study and preparation of the manuscript and agree on its submission to Advanced Materials. None of the authors have any conflicts of interest. The above paper reviews the progresses made in cell and drug delivery for cardiac regeneration. Here, we review cardiac regenerative strategies with a particular focus on advanced delivery concepts as a potential means to enhance treatment efficacy and tolerability and ultimately, clinical translation. We specifically look at (i) trials and advancements in the biomaterial field, with an emphasis on acellular and cellular injectable hydrogels, (ii) localised, minimally invasive delivery options and advancements in the field and (iii) multimodal therapeutic strategies. We also look at the future perspectives of these advanced delivery strategies and their applications in chronic disease treatment. Specifically we look at advanced delivery strategies for the treatment of diabetes and chronic obstructive pulmonary disease. We believe this review article warrants consideration for publication in Advanced Materials. I look forward to your response.
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Abstract:	Heart failure is a significant clinical issue. It results in enormous healthcare costs worldwide and results in a significant source of morbidity and mortality. Cardiac regenerative therapy has progressed considerably from clinical and preclinical studies delivering simple suspensions of cells, biomolecules and small molecule drugs to more advanced delivery methods utilising biomaterial scaffolds as depots for delivery to the damaged and ischemic myocardium. Here, we review regenerative strategies for cardiac tissue engineering with a focus on advanced delivery strategies and the use of multi-modal therapeutic strategies. In addition, we look at these advanced delivery concepts and their future perspectives in chronic disease, specifically in diabetes and chronic obstructive pulmonary disease.

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Abstract

Heart failure is a significant clinical issue. It is the cause of enormous healthcare costs worldwide and results in a significant source of morbidity and mortality. Cardiac regenerative therapy has progressed considerably from clinical and preclinical studies delivering simple suspensions of cells, macromolecule and small molecules to more advanced delivery methods utilizing biomaterial scaffolds as depots for localized targeted delivery to the damaged and ischemic myocardium. Here, we review regenerative strategies for cardiac tissue engineering with a focus on advanced delivery strategies and the use of multi-modal therapeutic strategies.

Introduction

1.1 Overview of Ischemic Heart Disease

Cardiovascular disease (CVD) is one of the leading causes of morbidity and mortality in the world. According to data published by the World Health Organization (WHO) in 2014, both ischemic heart disease (IHD) and stroke are responsible for an estimated 14 million deaths annually. [1, 2] In 2010, 7million deaths were caused by IHD alone showing a significant 35% increase since 1990. [3] Both acute myocardial infarction (MI) and IHD may cause progressive myocardial remodelling and left ventricular dysfunction leading to the development of heart failure (HF) in a significant number of patients. [4] Over the past number of decades coronary artery disease has become the major cause of HF. [5, 6] A recently published study looked at the aetiology of HF among hospitalised patients across 319 hospitals in the United States (US) between 2005 and 2013. It was revealed that 59.2% of the patients had ischemic cardiomyopathy which was deemed to be the most prevalent aetiology of HF. [7] The estimated total cost of heart failure in the US was $39.2 billion in 2010, representing 1-2% of all health care expenditures. [8] Although the prevention of heart disease through the management and control of risk factors should always be a priority, the treatment of heart disease and its consequences will always be a significant aspect of medical care. In response to the increasing global burden of CVD, there is a critical need to develop novel therapeutic strategies which are capable of effectively preserving myocardial integrity and enhancing cardiac function in order to improve patient outcomes.

1.2 Ischemic heart disease leading to heart failure and need for ventricular stabilisation

Patients with a history of MI leading to left ventricular remodelling and reduced ejection fraction (EF) fall under the category, early stage of heart failure (Figure 1). [9, 10] The therapeutic goal in such patients is to prevent further cardiac remodelling and progression to later stages of heart failure. Selected patients may have to undergo revascularisation either by

coronary artery bypass grafting (CABG) or percutaneous intervention (PCI). [10] Additional remodelling of the left ventricle results in progression of heart failure.

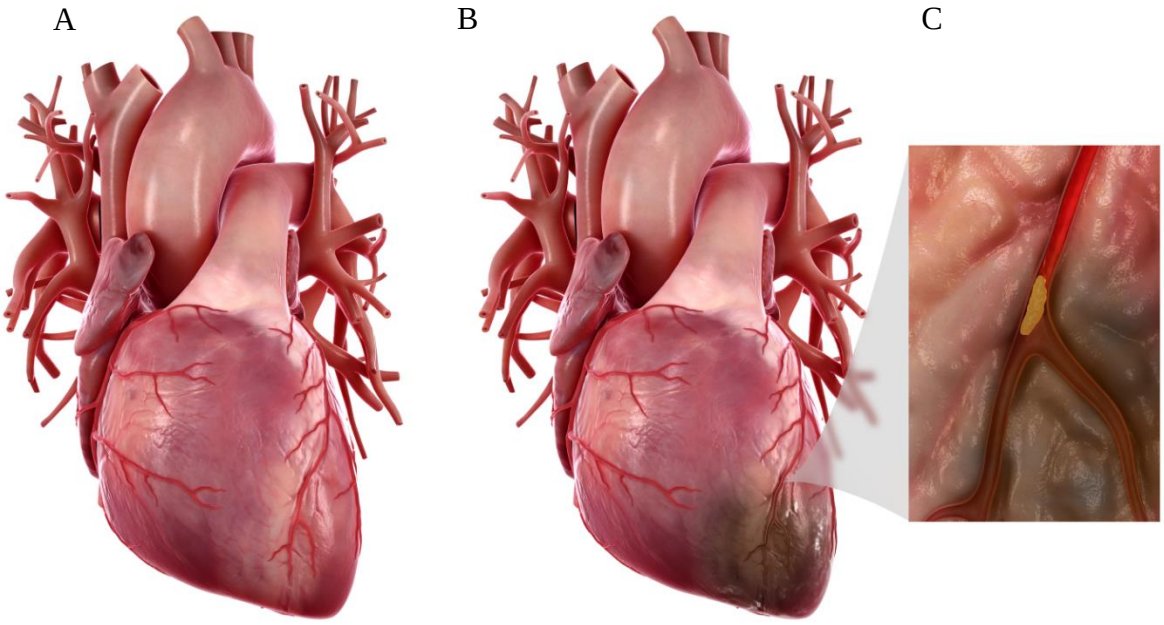

Figure 1 Comparison of heart tissue before (A) and after (B) an MI. Plaque accumulation in the coronary arteries can lead to a blockage (C). This blockage can prevent blood flow to the heart and tissue damage can be incurred due to the ischemic event. If the damage is significant, it can lead to a weakened heart muscle.

Ammar *et al.* conducted a study among residents over 45 years of age in Minnesota, US; investigating the rates of various stages of heart failure and reported a prevalence of 34% for early stage heart failure and 12% for overt heart failure. [11] Patients with overt heart failure may require ventricular stabilisation due to pauses in the cardiac cyce that are associated with the onset of pause-dependent ventricular tachyarrhythmias. Ventricular stabilisation can be achieved by mechanical means, such as cardiac resynchronisation therapy (CRT) devices or implantable cardioverter defibrillator (ICD). Refractory heart failure patients require heart transplant. [10]

Current treatment modalities slow down the progression of heart failure but do not reverse the process. [4, 12] Over the years, regenerative therapy for ischaemic cardiomyopathy is an extremely active area of research, a variety of potential treatment strategies have emerged

over recent decades which include stem cell delivery to the infarcted heart. [12] A recently published meta-analysis of adult bone marrow cell (BMC) therapy for ischemic heart disease looked at 48 randomised controlled trials (RCT's) enrolling 2602 patients. [4] Different imaging modalities including echocardiography, magnetic resonance imaging (MRI), left ventriculography and single-photon emission computed tomography (SPECT) were employed. This meta-analysis showed an improvement in left ventricular ejection fraction (LVEF), left ventricular end systolic volume (LVESV) and infarct size reduction. It also showed the persistence of these effects beyond 12 months. The greatest improvement was seen when BMC's were injected on 3-10 days post MI. [4] All of these strategies are directed towards ventricular stabilisation in order to improve cardiac function and halt the progression of heart failure and holds promise for not only decreasing the morbidity and mortality from CVD but will also improve the health related quality of life of patients following a heart attack. In addition to positive results observed with cellular therapy alone, drug delivery and biomaterial strategies have also been explored and recent advances in each area are discussed in detail in this progress report. The coupling of cells with biomaterial carriers and small molecules as a multimodal drug delivery system holds promise for not only decreasing the morbidity and mortality associated with CVDs but will also improve the health related quality of life of patients following a myocardial infarction.

2. Biomaterials Based Delivery to the Heart

2.1 Acellular material based scaffolds

As heart failure advances, the left ventricle (LV) undergoes progressive negative remodelling. This chronic pathology consists of LV dilation and wall thinning leading to increased wall stresses. Increased stress leads to local stress-induced apoptosis, which then propagates through a positive feedback loop of further dilation and wall thinning. [13, 14]

Injection of acellular hydrogels is an increasingly promising clinically transferable treatment for breaking this cascade (Figure 2). [15] Many materials have been investigated for use

including alginate, chitosan, fibrin, hyaluronic acid, collagen, Matrigel (BD Biosciences, San Jose, CA), keratin, calcium hydroxyapatite, decellularized extracellular matrix (ECM), and synthetic peptide or polyethylene glycol-based systems. Some systems have demonstrated preservation or increases of cardiac output or fractional shortening (FS) (Alginate, Fibrin, Collagen, ECM), [16-21] and fewer have progressed to phase I and II clinical trials, Algysil (LoneStar Heart, Inc.) (NCT00557531), IK-5001 (Bellerophon BCM LLC) (NCT00847964), and VentriGel (Ventrix, Inc.) (NCT02305602).

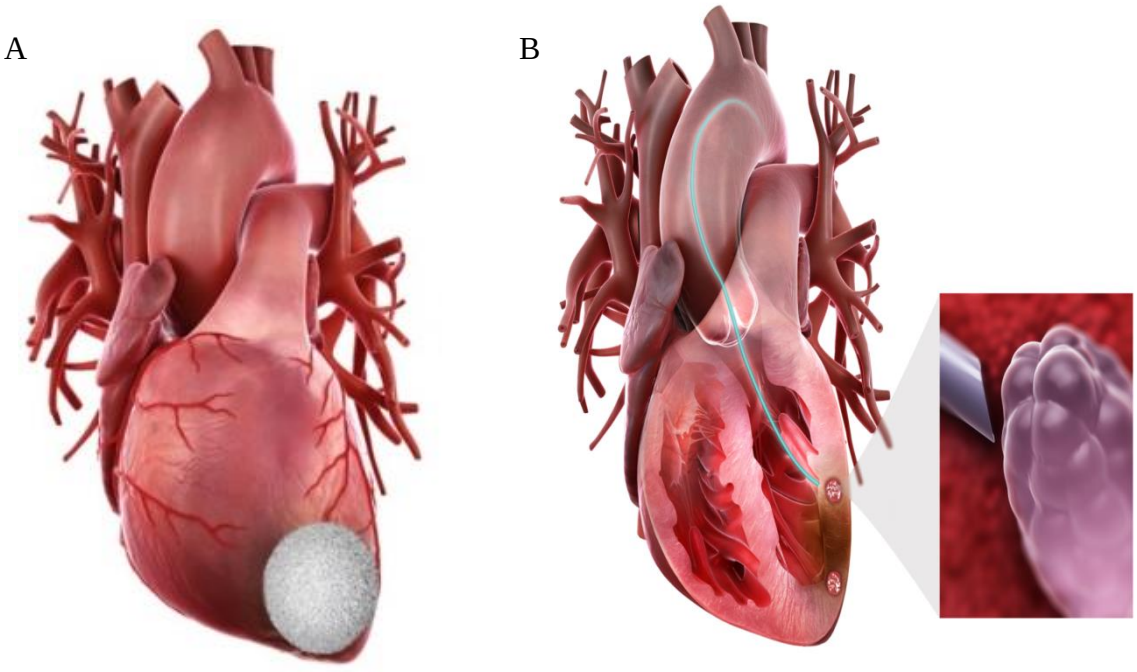

Figure 2 Biomaterial strategies for the treatment of myocardial infarction; cardiac patches and injectable biomaterials. Cardiac patches (A) and injectable materials (B) can be used as acellular scaffold to provide support to the dilated ventricular wall.

2.1.1.1 Progression of acellular biomaterials to clinical trials and their potential future directions

To date, two acellular biomaterials, alginate and ECM, have progressed to clinical trials (NCT00557531, NCT00847964 and NCT02305602). Alginate can be delivered by transcoronary infusion or intramyocardial injection, both approaches are currently in phase I or II clinical trials. During transcoronary infusion, a low viscosity calcium-alginate cross-

linked solution permeates through damaged infarct vasculature. It then crosslinks further, due to the high calcium content in the acute infarct environment, and becomes stiffer. The therapy, IK-5001 (BioLineRX, Jerusalem, Isreal), has been largely developed by Leor, Cohen and colleagues. After first demonstrating success in a rodent study [18] with intramyocardially delivered alginate, they then investigated transcoronary infusion of alginate in a porcine study. [19] In both studies, an increased number of myofibroblasts and connective tissue was observed in infarcts at follow up, (21 and 47 days post treatment respectively). In the acute infarct porcine study, reversal of LV enlargement, increased scar thickness and reduced hypertrophy was found, but no functional difference (fractional shortening was measured) was demonstrated when compared to the control. [19]

Following this study, an uncontrolled single arm phase I clinical trial (NCT00557531) of 27 acute MI patients who had also undergone successful revascularisation was initiated. [22] At six months post treatment, no adverse events related to the device, significant ventricular arrhythmias, or blood test abnormalities were found. Though efficacy data is limited due to the nature of the study, patients were shownto preserve their ventricular volumes and ejection fraction. A subsequent trial called PRESERVATION-1:IK-5001 (NCT01226563) has since been initiated. The phase of the trial has not been specified, and it also treats revascularised patients. The primary endpoint of this study is LV end diastolic volume index and it had an estimated primary endpoint of August 2015. If histology from the trial shows an increased presence of fibroblasts and connective tissue in infarcts, it will be the first time this has been reported after treatment with alginate. Furthermore, results will bring insight into whether stabilisation by minimally invasive intracoronary injection is sufficient for attenuation of heart failure in humans, or if intramyocardial injection in a set pattern, such as that with the Algysil treatment, is needed.

Subsequent to the alginate rodent work by Leor, Cohen and colleagues, [18] intramyocardial injection of alginate has largely been developed by Lee and colleagues. [15, 23-25] The groups

approach, Algysil (LoneStar Heart, Inc.), differs from others as it does not soley aim to stabilise the heart but also to restore the geometry of a healthy left ventricle, as outlined previously. [23] The efficacy of the circumferential mid-ventricular alginate injection pattern was investigated in two chronic infarction dog trials in 2009 and 2010. [24, 25] Both trials reported significantly reduced LV volumes and increased cardiac function (EF). In 2013, the results from the phase I/II clinical trial pilot study for the treatment were published (NCT00847964). [26] In the trial, three patients who were also undergoing revascularisation were treated. The trial was unblinded, non-randomised and had no control. Due to the small sample size it is difficultto draw many conclusions from the treatment, however it was noted that at six months, end diastolic volume (EDV) and end systolic volume (ESV) were both significantly reduced in two patients (the third did not complete the study), and that EF was significantly increased.

The trial found sufficient satisfactory tolerability and safety with the treatment to continue to a phase II multi-centre randomised study with a treatment (n=35) and control (n=38) group of advanced chronic heart failure patients respectively. [27] In the trial, treated patients did not have a significantly improved VO2 peak, from baseline at six months, (the primary endpoint of the trial) compared to control ($p = 0.014$). Despite this, an average increase of 10.2% over baseline was observed in treatment patients. In previous literature it has been reported that every 6% increase in peak VO2 resulted in an 8% reduction in cardiovascular mortality or HF hospitalization, and a 7% reduction in all-cause mortality. [28] Additionally, treated patients were categorised in the < 300m bracket in the 6 min walking test, this test is strongly prognostic of subsequent mortality and hospital admission in stable chronic HF [29, 30] and in patients with advanced HF. [31, 32] Improvement of >100m was demonstrated in treated patients, (improvement of >50 m has been reported as clinically very meaningful, [33] while decreases in walking distance were found in control patients. New York Heart Association (NYHA) index was shown to decrease from a mean of 2.9 in treated patients to 1.96 over six

months, while control patients were seen to decrease marginally from 2.8 to 2.7. It is notable that no statistical difference between treatment and control groups was found for the occurence of ventricular ectopy or ventricular arrhythmia. This helps to addresses some safety concerns for the treatment, that the injection of relatively large volumes of hydrogel into the left ventricle could be proarrhythmic. Furthermore, a reduction in rehospitalisation for major adverse cardiac events was seen for the treatment group.

Apart from the relative success of alginate ventricular injection seen in pre-clinical trials, the rapid movement of these therapies into the translational stage has been aided by the simplicity of the material used and the knock on effect that this has on time and cost associated with development and regulatory approval. Despite this, the therapies have very different approaches. The IK-5001 is minimally invasive as it avoids the need for surgical procedure and general anaesthetic. Contrarily, Algisyl is currently delivered by thoracotomy, reducing the ease of delivery. Interestingly, LoneStar Heart Inc. are currently developing a minimally invasive version of the treatment. A catheter based approach would certainly reduce invasiveness but would also bring bring technical challenges such as visualisation, injection accuracy and procedure length. Furthermore, if a new catheter design is being developed for this, it will add to the regulatory complexity of the treatment.

Intramyocardial delivery of decellularised myocardial matrix, developed by Christman *et al.*, has progressed through pre-clinical studies, [20, 21, 34] and is currently waiting to start phase I clinical trials (NCT02305602). Use of the native extracellular matrix is advantageous as it provides cells with the complex combination of proteins and polysaccharides seen *in vivo*. This complex combination not only guides cellular attachment but provides survival, migration, proliferation and differentiation cues [35-40] associated with significant changes in cell behaviour. [41, 42] The gel has been shown to retain its gylcosaminoglycan (GAG) content and to allow migration of fibroblasts *in vitro*. [21] Further studies have demonstrated the ability of the gel to stimulate and allow migration of endothelial and smooth muscle cells *in vitro* and

in vivo in rats. [21, 43] It also has a pore size sufficient to promote vasculature infiltration. Injection of the scaffold into healthy rats led to a significant increase in arteriole formation, this response was not observed following injection into infarcted rat and pig heart models. [43] Positive clinical outcomes of acellular strategies for cardiac repair highlight their inherent advantages in terms of simplicity as well as the lack of a required cell source. This simplicity may aid widespread clinical adoption in the future, particularly if the mode of action of these materials is not only structual but also pro-regenerative. A comparison to ongoing trials addressing the transplantation of stem cell populations for cardiac regeneration will be required to understand the optimal therapy to produce positive patient outcomes.

2.1.1.2 Current exploration of acellular hydrogel mechanisms of action

Despite the success of cardiac acellular biomaterial injection to date, the exact mechanisms that lead to functional improvements remain poorly understood. [44] Considerable work has been carried out to understand exactly how this beneficial effect is exerted. The benefit is hypothesised to be a function of mechanical properties [45-48] and injection pattern [48] of the therapy in question, or, less commonly, is attributed to the bioactive potential of the injected material. [49-52]

In vivo, acute infarct rodent studies by Dobner [53] and the Christman group have been completed. [49, 54] The studies investigated the effect of injecting a bio-inert non-degradable and degradable synthetic polymer polyethylene glycol (PEG) of low and high moduli (0.5+/-0.1 kPa and ≈ 10 kPa). They found treated groups had similar or worse progression of pathological remodelling and a decrease in cardiac function (ejection fraction was measured), to that of the control. This was despite the extra mechanical support provided by the significantly thicker heart walls seen in some treatment groups. [49, 54] Similar results were found in studies by the Burdick group aimed at investigating the relative influence of mechanical properties using degradable and non-degradable hyaluronic acid based gels of low and high storage moduli (approximately 8 kPa and 40 kPa respectively). [55, 56] In the acute

infarct ovine studies, significant decreases in ejection fraction (EF) from baseline was seen in all groups, including control. [55, 56] However, despite the decrease in EF, cardiac output (stroke volume) was only shown to be significantly lower in the control group, while increased infarct thickness and reduced infarct size was seen in both treatment groups.

Non-structural mechanisms of action of injected hydrogel remain largely unexplored and are broadly credited to the bioactive potential of gels. The hypothesis is largely based on the premises that many of the injected materials, such as fibrin, collagen, Matrigel, gelatin and decellularised tissue have extracellular matrix (ECM) components, and have been seen to degrade in 1-8 weeks *in vivo.* [57] These components contain multiple bioactive elements, such as proteins (e.g. peptide fragment hepIII of collagen IV [58]), glycosaminoglycan's (GAG's) that can bind and sequester growth factors [59] or contain fragments that influence cell recruitment (e.g. Fibrin fragment E is thought to contain angiogenic products). [57, 60] Furthermore, some materials, including non-ECM based materials, may promote protein adsorption leading to integrin binding and increased cellular cross-talk, which in turn may stimulate cell migration, matrix deposition and neovascularisation. [61] However, it has been demonstrated that enhanced integrin binding *in vitro* does not always lead to better *in vivo* therapeutic efficacy. [61, 62]

Given the data to date, it seems likely that both mechanical and bioactive properties of gels play potential roles in success with variability depending on such factors as gel type, timing of treatment and injection pattern. To expedite acellular hydrogel injection towards clinical adoption, further insight into the potential mechanisms of action of specific gels must be gained. This can be achieved with a more systematic approach focused on enhancing the understanding of the processes and parameters that lead to enhanced outcomes.

2.2 Cell Based Biomaterials

2.2.1 Cellular gels

The delivery of multipotent stem cells to the infarcted heart is an emerging strategy in the treatment of cardiovascular disease. This therapy aims to confer a cardioprotective effect through the release of paracrine signals to protect the myocardium in the case of acute myocardial infarction, or a cardiorestorative effect by stimulating tissue regeneration in patients with chronic ischaemic cardiomyopathy. [63] The efficacy and feasibility of cell therapy was initially comprised of lineage-unselected cell mixtures such as unfractionated bone-marrow-derived mononuclear stem cells BMMNCs. [64, 65] These cells have been predominantly used in clinical trials to date. [66] Recent studies have demonstrated a paradigm shift to purified cell populations, in order to eliminate non-regenerative cells and to accomplish a greater reparative potential. [67]

The injection of cardiac stem cells (CSCs), cardiopoetic mesenchymal stem cells and bone-marrow-derived mesenchymal stem cells (BMMSCs) have all shown promising results in terms of efficacy. [68, 69] Overall, a modest increase in cardiac function has been reported, which can be mainly attributed to paracrine signalling, as animal studies and clinical trials have unanimously shown poor retention and survival of implanted cells. [70] Poor cell survival is not surprising due to the fact that upon injection, cells are immediately faced with adverse conditions such as ischemia, inflammation and reactive oxygen species. [71] Stem cells are also subjected to the loss of cell-matrix interactions, which induces anoikis, a form of programmed cell death due to a lack of ECM support. [72]

Poor cell retention is likely to be a major factor underling the failure of cell-based therapies for MI to achieve consistent and substantial efficacy to date. [34, 73] Injected cells in a saline vehicle are lost extremely quickly with the majority lost within the first 24 hours. There has been significant variability in reported rates of retention in preclinical delivery, however, most studies confirm that the vast majority of cells are lost within the first few days post-

administration. The poor viability of a cell population at the diseased tissue might help to explain the unpredictable efficacy of treatments administered in clinical trials to date. One meta-analysis performed in 2012 of 33, randomised, controlled trials of 1765 patients who had received autologous stem or progenitor bone marrow-derived cell therapy to improve cardiac function after an acute myocardial infarction, revealed significant heterogeneity in results and reported no significant change in overall patient mortality or morbidity (as measured by hospital readmission, reinfarction or restenosis), although significant short-term improvement in left ventricular ejection fraction (LVEF) was reported, which persisted from 12-60 months. [74] Another meta-analysis published in 2015 analysed the safety and efficacy of intracoronary cell therapy after acute myocardial infarction (AMI), including individual patient data from 12 randomized trials (ASTAMI, Aalst, BOOST, BONAMI, CADUCEUS, FINCELL, REGENT, REPAIR-AMI, SCAMI, SWISS-AMI, TIME, LATE-TIME; n=1252) revealed that intracoronary cell therapy provided significant heterogeneity and no overall benefit, in terms of clinical events or changes in left ventricular function. [75]

The use of biomaterials as delivery vehicles aims to enhance stem cell therapy by increasing cell survival and retention, allowing the continuous release of paracrine signalling at the site of injury. [76] Injectable hydrogels can be used as a surrogate ECM for encapsulated cells, conferring several advantages over cells delivered in saline or media. Hydrogels can be optimised to provide excellent growing and attachment conditions, thereby helping to reduce cell death due to anoikis (Figure 3). The first study to demonstrate increased cellular retention and survival utilising an injectable hydrogel was performed in 2004. Christman *et al*. demonstrated that delivering skeletal myoblasts in an injectable fibrin hydrogel to infarcted rat hearts increased cell retention and survival with improved functional outcomes. [57] Since then a variety of materials and cell types have been utilised to improve cell retention over saline controls (table 1).

Table 1 Fold-increase in cell retention over intramyocardial saline delivery reported with various injectable hydrogels. Adapted from Hastings *et al.* [76]

Study	Hydrogel	Time(s) of analysis	Fold-increase in retention compared to saline control
[77]	Alginate Microspheres	24 hrs	1.3
[57]	Fibrin	5 weeks	~2
[78]	PEG based	4 weeks	2.5
[79]	Fibrin	90 min	1.77
[80]	Chitosan/β-GP	24 hrs	2
		4 weeks	~1.5
[81]	Chitosan/β-GP	24 hrs	~1.5
		1 week	~1.9
		2 weeks	~2

Naturally in the body, stem cells reside in a highly specialized, tissue specific, microenvironment known as a 'niche'. The stem cell niche is an important consideration for the encapsulation of cells within a surrogate 3D matrix as it functions to physically maintain cells and govern their fate. Within the niche, cells are exposed to biophysical properties and biochemical signalling which have major regulatory functions in the cells and in the tissue. The ideal hydrogel would not only improve cellular retention, but should also aim to provide cells with instructive cues to help control the fate of engrafted cells.

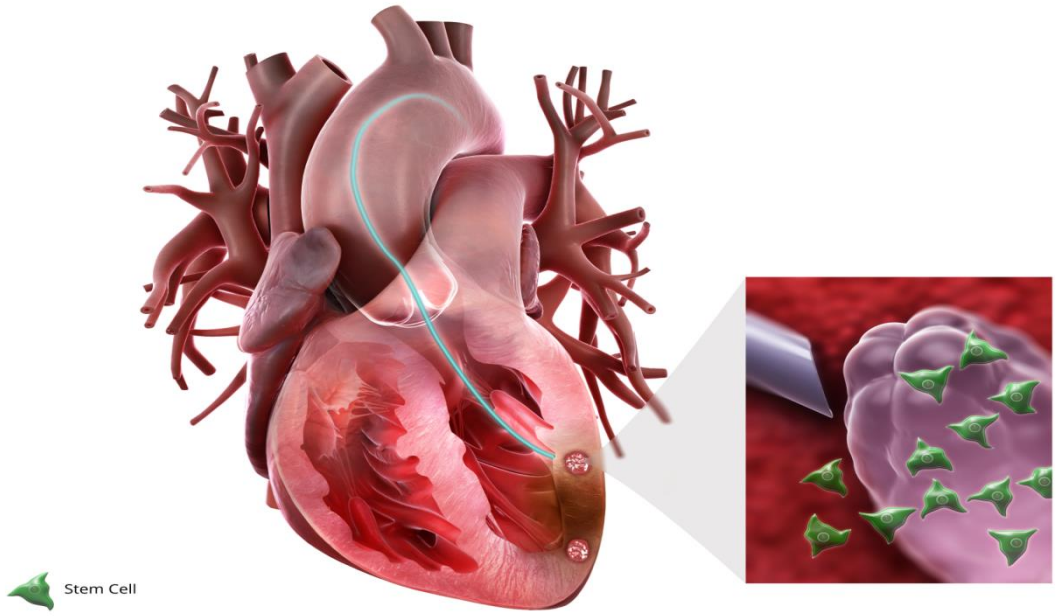

Figure 3 Injectable hydrogels can be utilised as cell delivery vehicles which can enhance cellular retention at the infarct site while proving a surrogate extracellular matrix which can be delivered in a minimally invasive manner.

2.2.1.1 Naturally derived hydrogels for cellular delivery

Hydrogels made from natural polymers are often employed as cell carriers for tissue regeneration as they closely resemble the natural ECM. Some examples commonly used of natural biomaterials are hyaluronic acid, fibrin, chitosan, alginate and agarose. [82] Naturally derived materials should ideally interact favourably within the body and have inherent bioactivity due to the presence of naturally occurring biological signals such as arginylglycylaspartic acid (RGD)-adhesion motifs. ECM-based materials have also emerged as a promising class of naturally derived biomaterial scaffolds. Given the tissue specificity of the ECM, these can closely mirror the target injection site once implanted. [20]

Encapsulation of human mesenchymal stem cells (hMSCs) in RGD-alginate microspheres has been shown to enhanced survival and retention in a rat model of myocardial infarction (MI). The microspheres were also found to provided structural support to the left ventricle and discourage negative remodelling post-MI. [77] Using a porcine model of healed MI, Panda *et al.* demonstrated the significantly enhanced engraftment and retention of MSCs when encapsulated in alginate. The presence of MSCs at the infarct site were also directly

associated with increased impulse conduction, while the presence of alginate causing no interference. [83] In attempting to design a substrate which can accurately mimic the native cardiac tissue, it is important to consider conductivity and electrical signalling. Spearman *et al.* have confirmed the biocompatibility of a conductive polypyrrole-polycaprolactone (PPy-PLC) film using HL-1 cells, a murine cardiomyocyte cell line. PPy-PLC was found to enhance cell attachment and support cell communication through the formation of connexin-43, a critical gap junction protein responsible for electrical coupling between cardiomyocytes. Cells cultured on conductive PPy-PLC films are subjected to enhanced substrate conductivity, creating a successful *in vitro* model to replicate the electric environment present in native cardiac tissue. [84]

Adipose-derived stem cells (ADSCs) possess similar properties to BMMSCs and are known to release the pro-angiogenic vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), an important signalling molecule involved in cell growth and migration. This cell type is of particular interest in terms of clinical translation, as adipose tissue contains a large quantity of stem cells, which can be extracted easily using a minimally invasive procedure. The encapsulation of ASDCs with alginate-poly-L-lysine-alginate (APA) microcapsules has demonstrated significant increases in cell retention in a rat model of MI. In a pig model of MI, porcine ADSCs were encapsulated in APA capsules labelled with Food and Drug Administration (FDA) approved SPIO nanoparticles in an attempt to track cell dispersal using magnetic resonance imaging (MRI). Interestingly, the APA capsules are so large in comparison to the surrounding blood vessels; they are unable to be forced into the blood stream. Although, this study confirmed enhanced retention using the magnetocapsules, there was no significant increase in heart function or infarct size when compared to non-encapsulated cells. [85]

Since the realisation that brown adipose tissue (BAT) persists after infancy, research into adipose tissue has grown rapidly. Brown adipose tissue, one of the two types fat in of adipose

tissue (the other being white), is activated to burn energy in non-shivering thermogenesis, a phenomenon observed in babies. Following the discovery of a CD29-positive population with the potential to differentiate into cardiomyocytes, this cell type has garnered interest in the field of cardiac regeneration. Chitosan has been confirmed as a suitable carrier for brown adipose-derived stem cells (BADSCs), demonstrating enhanced survival and differentiation of the cells following injection into infarcted rat hearts. These cells were also confirmed to have successfully engrafted into the host myocardium. BADSC delivery with chitosan also achieved enhanced angiogenesis and prevented the negative remodelling of the myocardium. [81] Interestingly in a separate study, a direct comparison of alginate and chitosan β-GP hydrogel using a rat model of MI was carried out, alginate was found to have a greater effect on the retention of hMSCs. [86]

The addition of simple peptides, such as the adhesion peptide RGD, [61, 87] may be used to further enhance efficacy. It is hypothesised that the addition would lead to increased cellular cross-talk, which in turn may stimulate cell migration, matrix deposition and neovascularisation. [61] Researchers from the Lee and Leor/Cohen research groups tested this in rodent models in 2009. [61, 62] In the study by Tsur-gang, addition of adhesion and non-specific peptides to alginate, led to an increase in *in vitro* cardio-fibroblast adhesion, but to a reduction in *in vivo* therapeutic effect (wall thickness, LV dilation and function). [62] Opposingly, a study by Yu *et al.* saw increased cell adhesion *in vitro* and also enhanced angiogenesis *in vivo.* [61] Both studies used unmodified alginate as the control. Discrepancies between results may be explained by differences in time of treatment and changes in stiffness with the modification of alginate. Enhancement in myocardial repair has also been observed with the binding of hepatocyte growth factor and insulin-like growth factor-1 to alginate. [88] These growth factors are known for their fibrosis inhibition, cytoprotection and induction of angiogenesis. Their addition in rodent studies was seen to improve tissue blood perfusion and induce mature blood vessel formation in a hind-limb ischemia model, and to also preserve

scar thickness, attenuate infarct expansion, reduce scar fibrosis, and increase angiogenesis in an acute MI model. In conclusion, preclinical evidence indicates that facilitation of increased cell retention translates to enhancements in efficacy. Naturally derived biomaterial delivery to the heart has demonstrated successfully that cell retention can be enhanced relative to simple systemic delivery as biomaterials can provide a protective and cohesive environment.

2.2.1.2 Synthetically derived hydrogels for cellular delivery

Disadvantages of the use of natural hydrogels include potential contamination, cross-species immunological issues, batch to batch variability and inherent difficulty in any possible modifications due to structural complexity. [89] In light of the variability of natural materials being used for therapeutic applications, semisynthetic and synthetic compounds are now being developed with a view to improving delivery of growth factors and cells to the ischaemic myocardium. Compared with natural polymers, synthetic polymers possess more reproducible physical and chemical properties. These hydrogels can be molecularly tailored with block structures, molecular weights, mechanical strength and biodegradability. [90, 91] Cai *et al.* developed a thermoresponsive hydrogel consisting of star shaped PEG complexed to poly(N-isopropylacrylamide) (PNIPAM) and a recombinant protein. At 37°C an increase in the gel strength occurred owing to the formation of a self-assembled secondary PNIPAM network. Further work showed that the properties of the gel could be easily modified by varying the concentration of the PNIPAM and thus the formation of the aforementioned secondary network. The gel was shown to undergo shear thinning followed by self-healing thus indicating that it can recover its original form following episodes of shear stress such as injection through a catheter system as would be required for cardiac applications. Subcutaneous injection of cells in the gel network showed cells were protected and remained viable *in vivo* up to seven days post injection. [92] Cardiac injection of this cell/gel mixture was not investigated as this was not the purpose of this study however the principles employed in the development of this gel may be applicable to the development of new polymer based

hydrogels for cardiac drug and cell delivery. Materials which respond to a physiological stimulus or stimuli may overcome some of the barriers currently encountered in drug and cell delivery. To date, thermoresponsive and pH responsive materials have been identified. These materials which should improve site specific deliveries of drugs and/or cells are relatively novel and thus much work will have to be done on determining their clinical usefulness as well as their safety.

2.2.2 Preformed scaffolds for cellular delivery

Generally, bioengineered cardiac tissue constructs can be divided into 2 categories: scaffold-based and scaffold-free. A number of three-dimensional (3D) scaffolds have been fabricated from natural biological materials, such as collagen, fibrin, and alginate, from naturally occurring Matrigel or decellularized heart matrix, and from synthetic polymers. They can be used for the delivery of a wide variety of therapeutics. The rational for the use of these patches as a myocardial cellular delivery vehicle is that cells will migrate from the patch into the myocardium over time, in response to infarct derived migratory stimuli, or in response to serum starvation as a result of patch avascularity. Alternatively, the patch can function as a sustained source of cell-derived paracrine signals or as a depot for the controlled release of implanted growth factors and other biotherapeutics. [72, 93]

Porous or fibrous preformed scaffolds are the most common way for creating 3D constructs for cell delivery (Figure 4). In many cases, cells are grown on these constructs pre-implantation and patches are surgically attached to the epicardial surface. Leor *et al*. used a 3D alginate scaffold to construct a bioengineered cardiac graft in a rat model of MI [94] and subsequently optimised it for cell seeding and distribution.

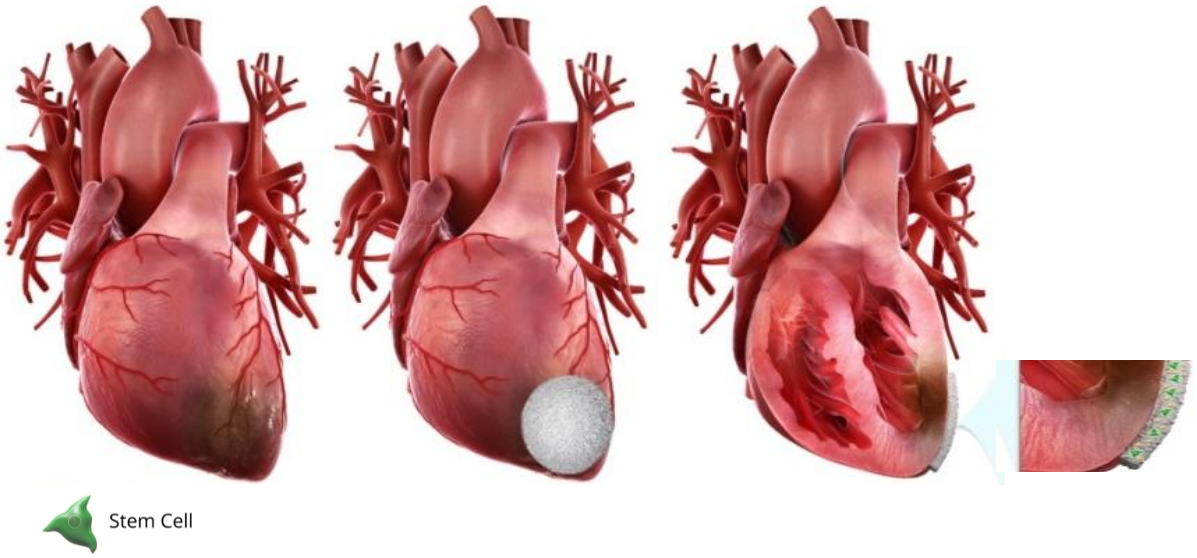

Figure 4 Stem cell delivery using epicardial patches are advantageous due to their defined pore size and architecture that can be tailor made depending on the shape and size of the infarct site.

Collagen type I is the most abundant protein in the human body as well as being a key element of the ECM where it offers strength to tissues such as blood vessels, tendon, cartilage and bone. [95, 96] Collagens primary structure results in helical self-assembly of tropocollagen subunits in order to create robust building blocks, which are further assembled into the microscale fibrils that are a fundamental component of the ECM of many tissues. In both native and engineered tissues, association of collagen with other proteins and ECM components is another important determinant of the structure and function of the matrix. These elements of collagen self-assembly and organization offer a rich set of variables that can be manipulated in order to create biomaterials with the desired architectures. [97]

Cell-free collagen patches were first conceived as replacments for lost tissue, where they were affixed to the epicardium to function as cardiac grafts. These epicardial patches were later developed as a dedicated cell delivery vehicle by affixing a collagen matrix containing MSCs to the epicardium of infarcted rat hearts. [98] The MAGNUM (Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium) trial investigated the delivery of MSCs via an epicardial collagen patch in patients presenting with left ventricular postischemic myocardial scars and indication for coronary artery bypass graft surgery. This collagen matrix

seeded with bone marrow cells demonstrated patient safety and improvement in several hemodynamic and functional parameters using this approach i.e. ejection fraction, left-ventricular end-diastolic volume and scar area thickness. [99] This trial compared isolated cardiomyoplasty with a combination of cardiomyoplasty and tissue engineering. The tissue engineering alternative was seen to offer better results in terms of functional recovery and ventricular remodelling.

Gelatin, derived from collagen, contains inherent peptide sequences that facilitate cell adhesion and enzymatic degradation. In addition, its low cost, lack of immunogenicity, and safety record in medicine as a blood volume expander makes gelatin an attractive implantable material. Modification of gelatin with pendant methacrylate groups (GELMA) allows cross-linked hydrogels to be formed using radical polymerization, these have been used extensively in cell culture and in tissue engineering studies. [100-103] This design confers an advantage over traditional preformed scaffolds, as the liquid precursor of hydrogels may result in leakage and pose difficulties in generating the desired implant geometry. GELMA is a preformed hydrogel scaffold with a defined geometry and microstructure that can be injected through the bore of a needle. These gels have 4 major criteria ideal for delivery through a port. The injectable scaffold can be compressible and flow under moderate pressure. The cryogel can maintain sufficient integrity and strength during injection for gel recovery. Once delivered, the cryogel can rapidly regain its original shape and size from the collapsed state. The scaffold can retain biomolecules/cells within itself during the injection process and allow their subsequent release *in vivo* in a controlled fashion. These characteristics make the material ideal for the implantation through a catheter that can combine the advanatages of minimally invasive delivery with the defined structure and architecture of a preformed scaffold, representing the next generation in cardiac scaffold design.

2.3 Combinational therapeutic approaches for cardiac regeneration

2.3.1 The case for combining mechanical and biological therapy

A recent study has shown that ventricular reloading can induce cardiomyocyte proliferation. It was hypothesized that an increase in mitochondrial content in response to mechanical load causes activation of DNA damage response (DDR) and permanent cell cycle arrest of cardiomyocytes. This impairs the ability of the heart to regenerate. The group showed that post-LVAD hearts (after "unloading" of the ventricle) showed a decrease in mitochondrial content and cardiomyocyte size compared with pre-LVAD hearts. [104] If this is the case, the administration of regenerative therapy while the heart is being unloaded should have a better chance of success compared to administration to a heart that is trying to compensate for a volume or pressure overload.

As such, there are numerous ongoing trials combining cell therapy with traditional mechanical assist devices. A multimodal combination of cells with mechanical assist devices (passive or active) represents a particularly attractive therapeutic strategy. This approach confers the potential for mechanical devices to act on co-delivered cells, as well as exert efficacy to the heart. Co-delivery in a biomaterial carrier can ensure that cells are kept in close proximity to the mechanical device for the duration of therapy to enhance synergistic interaction.

Ventricular restraint devices (VRD) represent a passive approach to mechanical assistance. Two of these devices have been commercially developed, a nitonol mesh designed by Paracor Medical (HeartNet) and a knitted polyester mesh (Corcap) developed by Acorn Cardiovascular. [105, 106] These devices wrap around the geometry of the heart and provide mechanical unloading; this helps prevent decrease in cardiac function post MI induced by change in spherical structure. In addition, the epicardial placement of the device facilitates targeted delivery of therapies to the heart.

In an interesting approach, Shafy *et al* showed that the combination of adipose-derived stem cells (injected into the infarct and seeded in a collagen matrix) with the polyester Corcap

VRD device resulted in significant improvements in ejection fraction, systolic and diastolic function in a sheep infarct model. [107] This semi-degradable ventricular bioprosthesis approach is an example of biomaterial-mediated cell therapy combined with a restraint device. In the past few years, the idea of combining active mechanical support and cellular therapy to work synergistically has emerged as a realistic alternative to heart transplantation. This is the subject of an abundance of research studies. [108, 109] The ASSURANCE Trial, which is examining the effect of Bone Marrow Derived Mononuclear Cells and ventricular assistance has the nearest estimated completion date, December 2016. [110] The MESAD trial plans to use autologous MSCS and mechanical assistance with the ultimate aim of weaning patients from the LVAD device. [111]

Both the MESAD and ASSURANCE trials use a needle and saline vehicle for cell delivery to the heart, which may not be suitable for such a fragile cargo. [112] Poor cell survival and retention involving traditional cell delivery methods necessitates multiple, invasive administrations of cells. A team based in the Wyss institute in Harvard is currently developing a refillable cell delivery device for the treatment of cardiac disease. [113] In this design a biomaterial based reservoir is connected to a subcutaneous port via a conduit, allowing multiple minimally invasive refills of cell therapy. This strategy addresses many limitations of current cell delivery strategies, poor cell survival and retention during delivery and the ability to survive and integrate in the harsh environment of the beating heart. Emerging hybrid mechanical assist devices that incorporate such a strategy may have a greater chance of successful clinical translation. Such a contemporary holistic hybrid approach for end-stage ischemic heart failure could address the issue of scarce donor hearts for transplantation and eventually lead to a recovery of native cardiac function.

2.3.2 The case for combining biological therapies

A number of advanced delivery strategies are being investigated for both growth factors and small molecule therapies. Dual drug delivery systems, which are capable of controlling the release behaviour of multiple drugs, are attractive for the combined administrations and optimization of a variety of therapeutic effects. Elucidating the powerful effects that drug combinations provide when applied synergistically, represents a powerful treatment platform. [114-117] Although the administration of single agents has been shown to support angiogenesis in animal models, there are still problems associated with vessel stability and maturity. [118, 119] The complex process of cell migration, differentiation and proliferation requires specific growth factors, which are both time-dependent and spatially distributed. For example, delivery of free loaded VEGF, involved in the initiation of angiogenesis, followed by the delivery of encapsulated PDGF-BB has been shown to stabilize newly formed blood vessels. [120-122] In a more recent study, the rapid release of VEGF and Ang-2, followed by the delayed release of PDGF and Ang-1 *in vivo* produced a dramatic increase in vessel formation and promotion of vessel maturation, demonstrating the importance of temporal control of release. [123]

With progress in time, homing of the stem cells in the infarct tissue becomes challenging due to homing signals like SDF-1 and VEGF which are poorly expressed in the acute stages. [124] To combat this, a semi-synthetic star Polyethyleneglycol-Heparin (starPEG-heparin) hydrogel has also been used for myocardial growth factor delivery. Customization of these hydrogels enable the support of various different tissue engineering schemes by loading heparin-binding growth factors that, for example, attract progenitor cells and stimulate angiogenesis. Stromal cell-derived factor 1α (SDF-1α) which plays a role in the recruitment of stem cells in ischaemic conditions is upregulated following myocardial infarction. However, endogenously produced levels are not high enough to prevent myocardial remodelling. Direct injection of SDF-1α resulted in rapid degradation and therefore is not useful. Baumann *et al.* incorporated

an SDF-1α variant into a starPEG-heparin hydrogel. The SDF-1α variant was bound to the heparin in the gel thus preventing both its premature release and its deactivation. As with the previously described sodium hyaluronate-HEMA hydrogel, an initial burst release of the SDF-1α was observed followed by prolonged release over a period of one week. [125] Early delivery of this SDF-1α post MI may result in the timely recruitment of endothelial progenitor cells which may reduce adverse cardiac remodelling.

Star shaped polymers such as that described above are increasingly being employed as drug or cell delivery vectors. Yan *et al.* reported on the synthesis of a hydrophilic star block copolymer which was composed of a branched polyethyleneimine core, an inner shell of poly(L-lysine) (PLL) and a poly(ethylene glycol) (PEG) outer shell. At physiological pH, the inner poly(L-lysine) shell is positively charged thus making it an ideal carrier for negatively charged proteins. In the procedure described, insulin was used as the protein of choice. It is negatively charged at a pH above 5.4 and thus was easily encapsulated by the positively charged PLL. A decrease in pH caused a change in charge of the insulin resulting in its sustained release. [126]

The synthesis of microgels responsive to both pH and temperature has also been described. Briefly, Poly (N-isopropylacrylamide-co-methacrylic acid) was synthesised by free radical cross-linking copolymerization of N-isopropylacrylamide (NIPAAm) and methacrylic acid (MA) with small amount of N,N‘-methylenebisacrylamide (MBAAm), as the cross-linker, and ammoinium persulfate (APS) and N,N,N‘,N‘-tetramethylethylenediamine (TEMED) were used as the redox initiator system, dried and crushed to form microgel particles. A number of drugs with different physicochemical characteristics were then loaded separately into the microgel particles. The results showed that the nature of the loaded drug determined the responsiveness of the microgel. pH and thermoresponsiveness occurred when a hydrophobic drug was encapsulated. This strategy requires further optimisation and characterisation but the

principle that the loaded molecule may affect the responsiveness of the microgel is one that should be borne in mind in the development of materials for cardiac regeneration. [127]

Cohen *et al*. synthesised a hydrogel designed to deliver a growth factor to the ischaemic myocardium. The gel consisted of sodium hyaluronate complexed with hydroxyethyl methacrylate (HEMA). Ammonium persulfate (APS) and N, N, N', N'-tetramethylethylenediamine (TEMED) were added as redox initiators to aid rapid formation of the hydrogel system on injection. The epidermal growth factor neuregulin which has been shown to promote cell-cycle re-entry of differentiated cardiomyocytes was incorporated into the resulting hydrogel. Analysis of the release of neuregulin in vitro showed that 60% was released in the first two days with the remainder released at a steady rate over the following twelve days. Degradation of the hydrogel occurred parallel to the release and was complete at day fourteen. *In vivo,* direct intramyocardial injection of the growth factor loaded gel resulted in significantly smaller left ventricular chamber area and greater left ventricular ejection fraction in mice that had received the treatment as opposed to those in the control groups. Post treatment peripheral blood levels of neuregulin were negligible which indicates that the gel was able to retain the growth factor in the myocardium. This is particularly important here where there are concerns over neuregulin's oncogenic potential. [128]

Bastings *et al*. synthesised a synthetic hydrogel consisting of an ureido-pyrimidinone (UPy) modified PEG hydrogel which consisted of UPy units coupled to PEG via alkyl-urea spacers. This hydrogel system was shown to be pH responsive. At a pH above 8.5 the hydrogel was fluid allowing it to be injected easily through a catheter system, the system then gelled on encountering the lower pH of the physiological environment. The authors proposed that this was due to the breaking of transient pH sensitive cross links between the fibres allowing a more rigid lateral conformation of fibres to form. The formed gel had a storage modulus of 24kPa which correlates with the mechanical stiffness of adult heart tissue. In vivo studies in a porcine model showed release of loaded growth factors following direct intramyocardial

injection. At four week follow up, treated animals showed areas of viable myocardium suggesting that more favourable myocardial remodelling may have occurred in these animals. [129] These Multimodal approaches show particular promise for myocardial regeneration.

2.4 Advanced delivery strategies

2.4.1 Catheter delivery requirements for injectable hydrogels

Current clinical trials ongoing in Europe demonstrate a preference for intramyocardial delivery of stem cells via the epicardial approach or transendocardially (Table 2). To add second-generation strategies with biomaterials carriers to this testing regime and clinical translation, it is important to understand the design requirements of catheters in relation to viscous hydrogels. The main objective of hydrogel delivery is to maximize the amount of active therapeutic delivered and retained at the target site, while minimizing the risk to the patient. Hydrogels can be injected intramyocardially, usually into the left ventricle, either using an epicardial or endocardial approach (Figure 5). Achieving a hydrogel that incorporates cells and is also compatible with catheter technology, for minimally invasive delivery and therefore shorter hospitalization time, is considered the ‘holy grail’ in the field. However, delivery of a gel through a minimally invasive approach presents a number of challenges for both material and catheter design.

Table 2 Phase II/III and III Clinical Trials of Cell-Based Therapy in Europe for Heart Disease using direct myocardial delivery. Adapted from Sanina & Hare, 2015. [130]

Trial ID; Trial Location; No. of Patients	Trial Name (Trial Stage)	Cardiac Indication	Delivery Method	Cell Type; Sponsor	Primary End Points
NCT01753440; Greece; 30 (estimated)	Allogeneic Stem Cells Implantation Combined With Coronary Bypass Grafting in Patients With Ischemic Cardiomyopathy (on- going)	Ischemic cardiomyopathy+CABG	Intramyocardial/ intraoperative	Allogeneic MSCs; AHEPA University Hospital	Change in LVEF by EchoCG and myocardial segmental perfusion
NCT00810238; Belgium, Serbia; 33 (final)	C-CURE (completed)	Chronic heart failure secondary to ischemic cardiomyopathy	Catheter-based transendocardial delivery	Autologous bone marrow–derived mesenchymal cardiopoietic cells; Celyad, Belgium	Change in LVEF
NCT01768702; Belgium, Hungary, Israel, Italy, Poland, Serbia, Spain, Sweden, Switzerland, United Kingdom; 240 (estimated)	CHART-1 (on-going, but no recruiting participants)	Chronic advanced ischemic heart failure	Catheter-based transendocardial delivery	Autologous bone marrow–derived mesenchymal cardiopoietic cells; Celyad, Belgium	MLHFQ 6-min walk test , LVESV (absolute change ≥4%) in LVEF

The material can be injected as a liquid and cross-linked once within the myocardial wall using various physical or chemical means including pH dependent, thermoresponsive, photo responsive or ionic cross-linking methods. Alternatively, the material can be injected in a partially cross-linked state, where cross-linking is completed *in situ*. Hydrogel formation parameters play a vital role in determining the behavior and properties of the material, both pre- and post-injection. Gelation time is an important factor to be considered when designing the hydrogel. The solution must gel rapidly at the site and remain structurally sound as to

avoid embolisation when the injection needle is removed. Any leakage of the cross-linked material into the circulatory system could cause a blockage and potentially lead to a stroke or heart attack for the patient. The mechanical properties of the gel when fully cross-linked should be suitable for supporting the ventricular wall. However, premature gelation of the material is not desired and will result in blocking the catheter tubing, rendering the therapeutic and catheter unusable. Pushability of the material through the catheter is another important feature that must be considered, and is directly related to the gelation time of the material. Partially cross-linked material may require high forces to be generated to push it through a length of catheter tubing. Mechanical/gearing systems can be installed in the catheter handle to reduce the amount of force applied by the user to inject the gel. Additionally, if cells are embedded within the gels, they may be protected from these harsh conditions by the gel as they move through the catheter tubing. There has been success in the catheter delivery of cells in a liquid carrier, typically saline, as the challenges discussed above are avoided. Several cell injection catheters are commercially available while clinical trials are ongoing. However, the main downfall of this approach is difficulties in retaining the cells and therapeutic at the target site. [131-133] Despite these challenges, there has been some progress in the catheter delivery of biomaterials alone whereby several pre-clinical studies have determined the feasibility using commercially available catheters. [34, 79, 134, 135] The challenge to date remains in combining these two approaches to develop a minimally invasive delivery strategy for advanced biomaterials, which incorporates cells.

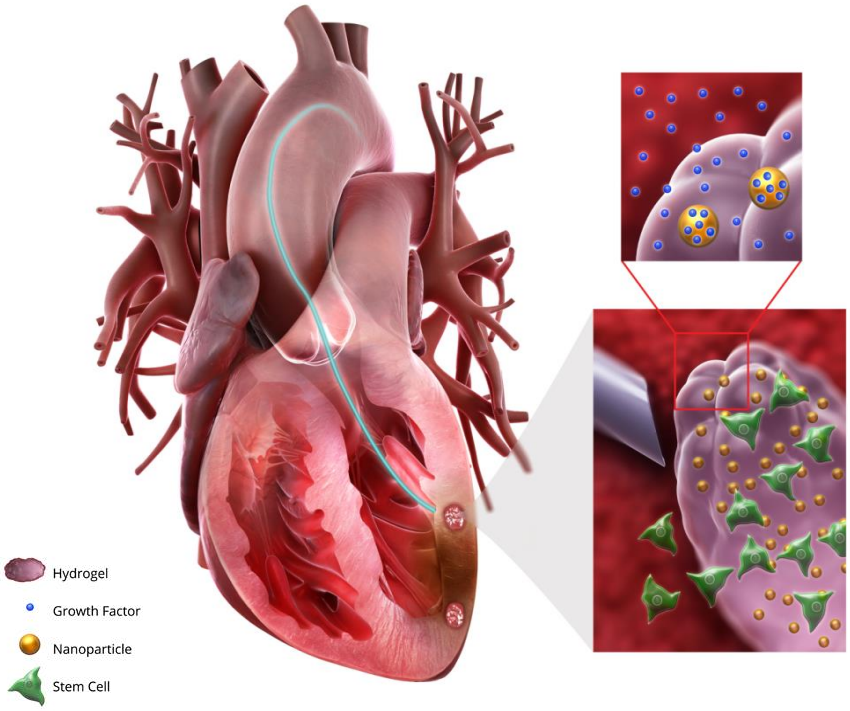

Figure 5 Minimally invasive hydrogel delivery can be accompolished by injecting into the myocardial wall from the endocardial side via transarterial catheter delivery.

2.4.2 Cardiac Patch delivery requirements

To address the issues of cell retention discussed above, tissue engineering research groups have been developing cardiac patches to be delivered to the epicardium via minimally invasive surgical procedures. Until now the preclinical trials have been performed using sternotomy which is an open chest procedure and is used as an approach for open heart procedures. [136, 137] It's complications may include sternal wound infection and dehiscence, dysrhythmias, haemorrhage, sternal instability and pseudoarthrosis, keloid and hypertrophic scars. Mechanical assist devices could be an option for delivering biomaterials. A few clinical trials have used the hybrid approach of ventricular unloading with cell delivery. S. Miyagawa *et al.* reported a case where combined autologous cellular cardiomyoplasty was carried out in a patient using skeletal myoblasts and bone marrow cells along with the implantation of with left ventricular assist system. Patient's cardiac performance was improved and reduction in fibrosis was observed. However, the delivery route was left

thoracotomy which involves a large incision and is not a minimally invasive technique. [138] Thoracotomy complications may include wound infection, haemorrhage, and lung injury.

Transapical delivery via mini thoracotomy could be a promising approach for delivering patches to the epicardial surface of the heart. [136] Mini thoracotomy involves a small incision and less muscle division than the classic one. Another potential minimally invasive approach could be video assisted thoracoscopy (VATS). VATS is currently used for various thoracic procedures and involves 2-3 ports. There may be some limitations to this technique including the issue of access to some areas of myocardium especially the posterior wall. It may be feasible to deliver the cells via epicardial injections through this route as Thompson *et al.* showed in their swine infarct heart model but delivering an epicardial patch could be challenging. [139]

Currently work is underway to develop epicardial patch delivery system compatible with minimally invasive surgical techniques. As part of Advanced Materials for Cardiac Regeneration (AMCARE) project, the consortium are working on deployment systems for epicardial patches. [140]

3 Development of advanced multimodal cardiac regeneration strategies

A multimodal combination of cells with an additional therapeutic agent represents a particularly attractive therapeutic strategy. This approach confers the potential for therapeutic agents to act on codelivered cells, as well as exert efficacy in target tissues. Cell therapy has progressed furthest as evidenced by the large number of clinical trials but is hampered by poor and unpredictable efficacy when implemented in large patient cohorts. A multimodal approach represents the next generation of treatment strategies, whereby the benefits of more than one therapeutic factor (e.g cells and growth factors) delivered in a biomaterial carrier are combined to maximise synergistic efficacy. Briefly, this approach amalgamates the effects of the therapeutic agents on both the delivered cells and the target tissue, together with the paracrine effects of the delivered cells, while maximising retention at the target site.

Multimodal approaches have been explored with promising results for the treatment of myocardial infarction using stem cells (neonatal rat cardiac cells, rat CPCs, human cardiosphere derived cells), growth factors (IGF-1, SDF-1, VEGF, bFGF) and biomaterials (alginate, peptide nanofibres, gelatin) in animal models. [141-143] Additionally, ALCADIA (AutoLogous human CArdiac-Derived stem cell to treat Ischemic cArdiomyopathy) a Phase I clinical trial demonstrated safety and feasibility of the approach. This hybrid biotherapy involves the delivery of biomaterials incorporating autologous cardiac stem cells with controlled bFGF delivery.

RECATABI (REgeneration of CArdiac Tissue Assisted by Bioactive Implants) a newly established European consortium which was recently created to develop cardiac bioengineering platforms which will combine elastomeric biomaterials (hydrogel-PuraMatrixTM) that improve the survival, delivery and proliferation of implanted cells. [144]

5 Future perspectives

Advanced delivery strategies for regenerative therpeutics represent particular promise for a variety of disease states. Inherently complex physiological and pathological processes in the heart require more sophisticated treatment strategies. Looking at the preclinical evidence, although single regenerative treatment strategies i.e. delivery of cells, growth factors, biomaterials, has demonstrated promise, the ideal therapeutic strategy will likely be a multimodel approach combined with advanced delivery procedures. Ideally the biomaterial should be implanted in as minimally invasive a manner as possible to increase patient comfort and reduce recovery time. The implanted material should ideally contain cells derived from the tissue source i.e cardiac derived cells or the use of progenitor cells in combination with biotherapeutics such as growth factors. The payload of growth factors should ideally be encapsulated in a particle delivery vehicle to control the rate of release and potentially enable a release profile that mimics release of endogenous reparative processes. This multimodal formulation should seek to mimic not only the biological cues but in addition the mechanical

cues in order to maximise efficacy. Finally, depending on the stage and severity of tissue damage, replenishments of therapy to the target site should be achieveable in as minimally invasive a manner as possible. If we, as a research field, can meet these prerequisites in ongoing research strategies, we can elicit an optimal efficacy response using biomaterial based approaches and positively impact patient outcomes in the future.

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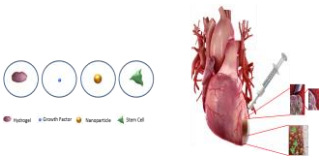
Graphical Abstract



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