BJP British Journal of Pharmacology

DOI:10.1111/bph.12128
www.brjpharmacol.org

Themed Section:
Regenerative Medicine and Pharmacology: A Look to the Future

EDITORIAL

The birth of 'regenerative pharmacology': a clinical perspective

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This work forms part of the research themes contributing to the translational research portfolio of Barts and the London Cardiovascular Biomedical Research Unit, which is supported and funded by the National Institute of Health Research.

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Since the seminal paper by Orlic and colleagues in 2001, which demonstrated that local injection of bone marrow-derived stem cells leads to an improvement in cardiac function in a mouse model of myocardial infarction (MI; Orlic *et al.*, 2001), there has been considerable debate with regard to the mechanism by which cardiac repair is achieved. The results in the original paper suggested that the bone marrow-derived allogeneic cells underwent transdifferentiation into cardiomyocytes, thereby repairing the infarcted myocardium. Since then, other groups have shown that bone marrow-derived cells can affect cardiac repair, but no formation of new cardiomyocytes has been observed (Murry *et al.*, 2004; Nygren *et al.*, 2004; Balsam and Robbins, 2005). In order to reconcile these observations, several hypotheses have been proposed to explain cardiac repair in the absence of regeneration and are summarized in Figure 1 (Lovell and Mathur, 2010). Although all of the proposed mechanisms are based on associated evidence, the 'paracrine effect' appears to have the most data supporting it, irrespective of the cell type used (Kinnaird *et al.*, 2004; Gnecchi *et al.*, 2005; Xu *et al.*, 2007; Korf-Klingebiel *et al.*, 2008; Ratajczak *et al.*, 2012). The paracrine effect proposes that cells delivered to the site of organ injury (in this case MI) secrete a factor or a combination of factors that have a beneficial effect on cardiac function that is achieved either by enhancing surviving myocyte function, by preventing cell loss as a result of the ischaemic insult (e.g. activation of survival pathways) or stimulating 'resident' stem cell niches. The potential identification of individual factors as part of this hypothesis immediately raises the possibility of understanding the ligand–receptor interaction and hence the development of new pharmacological targets in the field of regenerative medicine. The development of an off-the-shelf drug that is capable of myocardial repair and can easily be applied to 'all comers' has clinical appeal and would revolutionize the application of regenerative medicine. Although considerable obstacles need to be overcome, more evidence is accumulating to bring the field closer to understanding whether the mechanisms of cell-derived therapy can be dissected down to the 'basic pharmacology' that controls them. The role that pharmacology plays as an adjunct to current approaches to cell therapy should also not be overlooked and demonstrates a significant partnership between the two fields. Below, this partnership will be explored using the clinical trials that have targeted cardiovascular disease as an example. Factors that have been identified as part of the paracrine hypothesis will be discussed and the potential for the development of a purely pharmacological approach considered.

Current clinical application

The application of cell therapy to treat cardiovascular disease provides one of the best examples of the challenges that investigators have needed to overcome as the results of basic research have been translated into human subjects. However, this translation into clinical trials has been criticized, due to the speed at which it has occurred and the fact that there is no agreed understanding of the mechanisms involved. This approach is nonetheless consistent with other advances in the treatment of cardiac disorders and is controlled by tight ethical and regulatory scrutiny. The recent development of

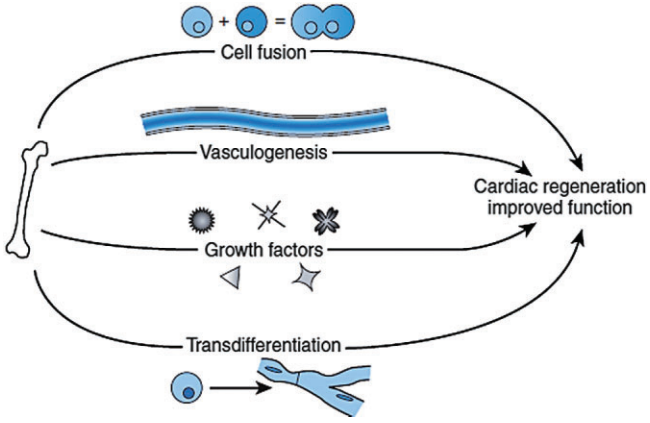

Figure 1

Potential mechanisms of stem cell therapy. Although initial research was focused on whether stem cells transdifferentiate into functioning cardiomyocytes, subsequent research has supported the existence of other mechanisms including the paracrine hypothesis.

industry-lead devices such as resynchronization therapy has shown mixed results with difficulties in identifying the target patient group, which further highlights the limited understanding we have about this mechanism. The implantation procedure itself is associated with a relatively high complication rate and surrogate end-points have only demonstrated modest, if any, benefit in phase II trials. Nonetheless, clinical outcome studies looking at survival and morbidity have demonstrated benefit to patients (Holzmeister and Leclercq, 2011) and, despite the costs involved, this therapy is now recommended by the National Institute of Clinical Effectiveness. The clinical trials in which cell therapy is used to treat myocardial dysfunction if anything compare more favourably with the results seen in the initial trials of resynchronization therapy, and, ultimately, the issues over safety are dealt with by the governance associated with performing clinical trials. In fact, the regulatory requirements have meant that to date few clinical trials of cell therapy have been initiated in the UK. In addition, the difficulties in securing funding for investigator lead trials in this area, with an average cost of approximately £8000 per patient recruited, have set a high bar that few have been able to reach. The development of a pharmacologically based regenerative strategy will probably increase the number of trials in this area while utilizing the established industry-derived infrastructure for drug discovery.

Beyond the regulatory and financial hurdles of initiating trials of cell-based regenerative therapies, the scientific approach has yet to identify the optimal cell type and delivery method (for more details, see the accompanying review by Jadczyk *et al.*, 2012). With respect to cell type (Table 1), clinical investigators have followed a pragmatic approach that started with the use of autologous bone marrow-derived cells, chosen due to the accessibility of the cell type and the regulatory requirements accompanying their use. This approach has been lead by academics that have faced the funding issues described above due to limited intellectual property associated with this technique and limited commercial support. The success of this approach was summarized in

Table 1

Stem/progenitor cell types used in clinical trials of cardiac repair

Allogeneic	Autologous (adult stem cells)
Embryonic stem cells	Induced pluripotent stem cells
Fetal cardiomyocytes	Adipose-derived stem cells
Human umbilical cord derived cells	Resident cardiac stem cells
	Epicardium-derived stem cells
	Skeletal myoblasts
	Bone marrow derived:
	Endothelial progenitor cells (EPC)
	Mononuclear/CD34+ fraction
	Mesenchymal stem cells

a recent meta-analyses that demonstrated 'activity' of cell-based approaches to cardiac repair in phase I/II clinical trials, thus heralding the next step – the design and initiation of phase III studies (Martin-Rendon *et al.*, 2008; Clifford *et al.*, 2012a,b). The initial results obtained with autologous bone marrow-derived cells have demonstrated the safety and feasibility of delivering cell therapy to the heart and have paved the way for the development of the next generation of cells – the so-called engineered cells, which have been developed to enhance the characteristics necessary for cell therapy. The main focus of producing these cells is to promote controlled differentiation into cardiomyocytes, although cells have also been created with the potential to enhance survival pathways via a paracrine mechanism (PI3K/Akt pathway, etc.) (Gnecchi *et al.*, 2005; 2006). The range of cells that are being 'engineered' thus covers the spectrum of mechanistic explanations from the paracrine effect (mesenchymal Akt expression) through transdifferentiation (induced pluripotent stem cell, a pluripotent cell artificially derived from a non-pluripotent cell) (Takahashi and Yamanaka, 2006; Mauritz *et al.*, 2008; Zhang *et al.*, 2009) and ending up with differentiation using cells that have a cardiomyocyte-like phenotype from the outset (e.g. cardiosphere-spherical clusters of cultured endogenous cardiac stem cells) (Smith *et al.*, 2007; Lee *et al.*, 2011; Makkar *et al.*, 2012).

An understanding of the pharmacological manipulation of these different cell types is crucial to the successful development of a new therapeutic strategy. As an example, the accompanying review by Atkinson *et al.* (2012) describes the important role of a pharmacological-based approach in controlling the complex transcription pathways involved in the differentiation of embryonic stem cells. However, the role of 'adjunctive' pharmacology in supporting the clinical applications of cell therapy has perhaps been underestimated. The interventional procedures carried out in cardiology to deliver cell therapy are performed on the background of existing protocols and the use of adjunctive pharmacological interventions that have been developed over the years to improve procedural success such as anti-platelet agents and anticoagulants. The introduction of cell therapy into this environment has occurred with little consideration of potential interactions between the cells and the drugs used to treat cardiac

Table 2

Possible explanations for the failure to translate the magnitude of myocardial recovery demonstrated in pre-clinical experiments into clinical trials in man

Not enough cells injected in man compared to animal models
Wrong cell type used – differences between stem cell characteristics in animals compared to man
Wrong timing relative to infarct age – remodelling in animal models has a different time course compared to man
Wrong delivery method – unable to achieve high enough concentration of cells at target
Animal models not representative – unlikely to reproduce results in man
Patients are old and diseased – cells reflect donor phenotype with little regenerative potential

disease. The animal experiments that were used to investigate the effects of cell therapy on cardiac function did not extensively examine these interactions. The translation of this work into human subjects has not been accompanied by the same improvements in cardiac function and this discrepancy has attracted several explanations (Table 2; Lovell and Mathur, 2010). Recent work has shed some light on how these interactions could be important and more far-reaching than originally suspected. In an attempt to explain why some clinical trials of cell therapy have been more successful in repairing the heart than others, investigators looked at the interaction between the migratory capacity of bone marrow-derived cells and heparin (routinely used in interventional cardiology and the preparation of cells). They demonstrated that the presence of clinically relevant concentrations of heparin in experiments testing the migratory capacity of progenitor cells leads to an inhibition of the response to chemoattractants. The mechanism for this effect was through heparin-mediated inhibition of stromal cell-derived factor-1 (SDF-1)/chemokine receptor type 4 signalling that is necessary for cell migration. This inhibition was not seen when an alternative anticoagulant – the thrombin inhibitor bivalirudin – was used. Given that the homing and engraftment of cells delivered as part of a therapeutic strategy is the key to a successful outcome in cardiac repair, this finding has important implications for the choice of anticoagulant when designing future clinical trials (Seeger *et al.*, 2012).

Other potentially negative interactions between cardiovascular drugs and cell therapy are not just confined to i.v. therapy used in interventional procedures but include oral medications that are widely used in primary and secondary prevention. For example, a 1–10 mmol·L^{-1} dose of aspirin has been shown to inhibit proliferation of mesenchymal stem cells (MSCs) in rats through a mechanism of action thought to involve inhibition of PGE_2 formation and subsequent down-regulation of the Wnt/β-catenin signalling pathway (Wang *et al.*, 2006). Treatment of MSCs with PGE_2 increases cell proliferation and enhances the activation of the Wnt/β-catenin pathway (Kleiveland *et al.*, 2008). Similarly, human embryonic stem cells are dependent on prostanoid synthesis, which is needed for differentiation and proliferation pathways. Treatment with a COX-2 inhibitor has been shown to substantially decrease PGE_2 production in the embryonic stem cell, potentially disrupting sensitive signalling pathways (Chillar *et al.*, 2011). Given the widespread use of aspirin in the treatment of cardiac disease and the important role of prostanoid synthesis in stem cell biology, an understanding of how this interaction could inhibit cardiac regeneration in the clinical setting is important, particularly if the clinical trials do not demonstrate the magnitude of effect seen in the animal models.

On a positive note, several drugs that are used in the treatment of cardiovascular disease exhibit beneficial effects on stem cells and may therefore enhance cardiovascular repair.

Several studies have shown statin therapy to have a beneficial effect on stem and progenitor cell survival and proliferation through multiple mechanisms (Assmus *et al.*, 2003, Spyridopoulos *et al.*, 2004, Yang *et al.*, 2009 and reviewed by Xu *et al.*, 2012). Two main mechanisms of action have been demonstrated – inhibition of cellular senescence and activation of the PI3K/Akt cellular survival pathway. Assmus *et al.* (2003) showed that the effect of statins on inhibiting cellular senescence was dose-dependent, with maximum inhibition of senescence occurring with 0.1 μmol·L^{-1} atorvastatin. Furthermore, Spyridopoulos *et al.* (2004)) showed that atorvastatin, mevastatin and simvastatin, all reduce loss of a protective telomere capping protein TRF2 (telomere repeat binding factor-2) and prevent functional impairment in the endothelial progenitor cell (EPC). They also showed that all three statins induced a threefold increase in TRF2 in cultured human EPCs. The effect was dose-dependent and maximal effects were obtained at 0.1 μmol·L^{-1} (atorvastatin), 1.0 μmol·L^{-1} (mevastatin) and ≥0.5 μmol·L^{-1} (simvastatin).

With respect to activation of survival pathways, both lovastatin and atorvastatin have been shown to be protective against hypoxia-induced MSC apoptosis in animal models (Xu *et al.*, 2008; Dong *et al.*, 2011). Lovastatin acts via the PI3K/Akt pathway, while atorvastatin exerts its protective effect via the AMPK (AMP activate kinase)/eNOS pathway.

Statins have also been shown to have beneficial effects on stem cell mobilization in clinical trials, which may confer a therapeutic advantage. High-dose atorvastatin (80 mg) post-primary or rescue percutaneous coronary intervention for ST-elevation myocardial infarction was associated with a significant and sustained rise in the number of EPCs at 4 months follow-up compared to that seen after a 'standard' dose of atorvastatin (20 mg) (Leone *et al.*, 2008).

Finally, statin therapy has been shown to increase stem cell survival and engraftment in a rat model of acute MI via activation of the JAK/STAT (signal transducer and activator of transcription 3) pathway (Xu *et al.*, 2011). MSCs transplanted into rats after ischaemic insult showed increased survival and engraftment in animals that had received rosuvastatin (8 mg·kg^{-1} the day before the MI and then 4 mg·kg^{-1}·day^{-1} for the next 5 weeks).

Another class of drugs used in the treatment of cardiovascular disease that interacts with stem cells are the angiotensin receptor blockers which have been shown to increase progenitor cell proliferation and homing to the heart in animal models (Bahlmann *et al.*, 2005; Ludwig *et al.*, 2012). Diabetic patients treated with 40 mg olmesartan and 300 mg irbe-

sartan showed a significant increase in circulating endothelial progenitor cell numbers. As with statins, the therapeutic benefit of this increase in circulating cells is speculative and the exact mechanism of their release is unknown.

Interestingly, although the majority of interactions between cardiovascular pharmacology and stem cells are generally class effects, there are exceptions. β-Blockers as a whole have been shown to have a beneficial interaction with stem cells in a regenerative setting. The β-blocker nebivolol has been shown to enhance EPC mobilization post-MI via an eNOS-dependent pathway in a mouse model. Carvedilol has been shown to improve MSC survival in an animal model of acute MI (carvidelol 5 $mg \cdot kg^{-1}$), but this was thought to be due to its antioxidant properties rather than its ability to block β-adrenoceptors. Also in tissue culture conditions carvidelol 2.5 μM was shown to reduce oxidative stress via inhibition of caspase-3 (a protein involved in cellular apoptosis). In animal models, this has lead to an improvement in ventricular function and reduced cardiac fibrosis, that is, positive remodelling (Hassan *et al.*, 2012). Interestingly, no such benefits were observed with atenolol, suggesting that the protective effects of carvidelol were a result of its antioxidant properties either alone or in combination with β blockade (Sorrentino *et al.*, 2011).

These examples of interactions between cardiovascular drugs and stem cells in the context of cardiac regeneration are by no means exhaustive but clearly demonstrate the need for thorough testing of all drugs that are used with cell-based therapies. As most cardiac patients will be taking several of the drugs mentioned earlier, it is important to understand whether the combination of these agents will have an overall positive or negative effect on the regenerative capacity of cell therapies. This would lead to a more personalized approach for each patient in order to optimize the outcome. These drug–stem cell interactions may in part explain why the results of cell therapy seen in pre-clinical experiments are lost when translated into humans.

As well as choice of cell for optimal cardiac repair, the optimal method of delivery of these cells still needs to be determined. Ultimately, it may well be that the best delivery method depends on the choice of cell used for therapy and patient characteristics. Although several mechanical delivery methods (Figure 2) have been tested in human subjects with varying degrees of success, pharmacological intervention could be used to improve the efficiency of homing and engraftment of these cells to the target sites. The interaction of the SDF-1 cytokine receptor, CXCR4 (Alexander *et al.*, 2011), with heparin has already been discussed as an example of how non-cell-based interventions can play a crucial role in determining the outcome of cell-based therapy. Several investigators have shown that an increase in CXCR4 expression at the target site leads to improved homing and engraftment of progenitor cells (Askari *et al.*, 2003; Yu *et al.*, 2010; Huang *et al.*, 2011; Dong *et al.*, 2012). The challenge, however, lies in developing a therapeutic approach in human subjects that could utilize this discovery to improve the homing and engraftment of injected cells. This would further extend the potential for adjunctive pharmacology in the development of successful cell therapy strategies.

So far, the interaction between clinical pharmacology and cell therapy has been discussed with respect to direct effects

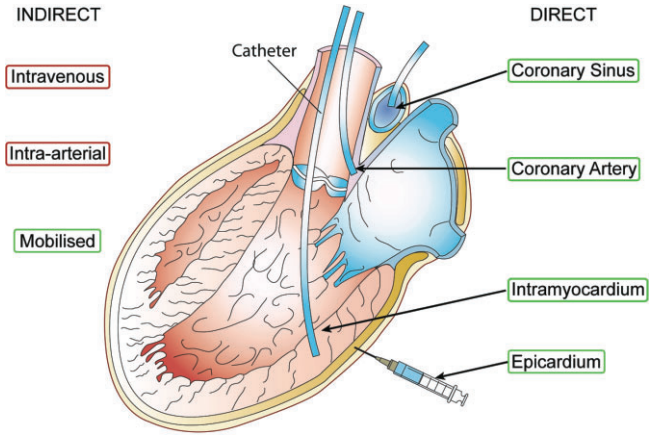

Figure 2

Delivery methods for cell therapy to the heart. Both direct and indirect methods of cell delivery have been tested in clinical studies. The most commonly used routes are highlighted in green boxes and compared to methods that have little use in man but have been tried in animals (red boxes).

on stem cell pathways. Some of the mechanisms that have been discussed are not only beneficial to stem cell therapy, for example, activation of survival pathways, remodelling, etc., but also have direct effects on the heart to promote myocardial repair. Therefore, a direct pharmacological approach in which these pathways are targeted to promote myocyte survival and enhance cardiac repair would bypass the need for the complexities of cell therapy. It should be remembered that the therapeutic objective is to improve current outcomes and thus any novel direct strategy either needs to further enhance molecular pathways that are already activated or find new targets. Several different agents have been found to activate the survival pathways during myocardial ischaemia and improve myocardial recovery. The PI3K/Akt and the ERK 1/2 anti-apoptotic pathways are two pro-survival pathways collectively termed the reperfusion injury salvage kinase pathway (Hausenloy and Yellon, 2004). Activation of these pathways inhibits pro-apoptotic proteins. The PI3K subunit γ (PI3K-γ) is also involved as a signalling intermediate in integrin-mediated homing of progenitor cells. The homing of progenitor cells to the site of ischaemia is an essential requirement for the induction of postischaemic neovascularization (Chavakis *et al.*, 2008; Madeddu *et al.*, 2008).

Several studies using different stem cell types have demonstrated activation of the PI3K/Akt pathway via paracrine effectors (Lovell *et al.*, 2010, Rosenberg *et al.*, 2012; Boomsma and Geenen, 2012). The latter includes VEGF, insulin-like growth factor-1, transforming growth factor (TGF) and chemokine ligand-12. The ERK 1/2 pathway has also been shown to have a role in myocardial regeneration (Hu *et al.*, 2007). However, it is likely that more diverse signalling pathways and paracrine effectors exist (Boomsma and Geenen, 2012), and understanding the precise biological actions of these effectors and pathways could help to identify target molecules to activate these pro-survival pathways and hence limit the extent of myocardial damage.

The paracrine hypothesis relating to the mechanism of cell therapy has identified several factors that have therapeutic potential for myocardial repair (see Table 3) (Kinnaird *et al.*, 2004; Xu *et al.*, 2007; Burdon *et al.*, 2011; Ratajczak *et al.*, 2012). The production of what is a predominately cytokine-based catalogue of reparative factors has been demonstrated from several different cell types, suggesting that progenitor/stem cells possess the same basic mechanism that can lead to cardiac repair. Hence, it is possible that the conversion of this cytokine-based catalogue into a pharmacological therapy could, in due course, make cell therapy redundant. However, several key elements to enable this are missing. The release of cytokines from cells is a controlled process that involves feedback mechanisms between target receptors and effector cells (Torella *et al.*, 2007). Understanding this precise relationship and how to control it without the 'black box' of the cell will be vital to ensure that the right balance of receptor–ligand interaction occurs at the required time. To date, no single factor has been identified that alone achieves the amount of cardiac repair in the same way as cell-derived therapies. Thus, developing a pharmacological approach that is derived from the paracrine hypothesis will be possible only once an understanding of, and solution to, mimicking the dynamic processes of the cell is achieved.

Finally, the 'holy grail' of regenerative medicine is to grow new functional cells to replace those that have been lost to injury. While the embryonic stem cell remains the most obvious candidate to produce the billions of cells needed for significant myocardial repair, considerable ethical and practical considerations still need to be overcome. Advances in the field of research associated with regenerative medicine have resulted in the identification of therapeutic regenerative potential in existing cell types (see accompanying review by Lee and Yoon, 2012). This could provide new pharmaco-

Table 3

Paracrine factors/cytokines secreted by stem/progenitor cells and implicated in myocardial repair

Paracrine factor	Mechanism
SDF-1 (stromal cell-derived factor-1) (Askari *et al.*, 2003)	Anti-apoptotic, cell migration, pro-angiogenic
VEGF (Kamihata *et al.*, 2001; Kinnaird *et al.*, 2004)	Pro-angiogenic, anti-apoptotic, cell proliferation and migration, contractility
bFGF (basic fibroblast growth factor) (Kamihata *et al.*, 2001, Kinnaird *et al.*, 2004)	Cell survival, pro-angiogenic, cell proliferation, contractility
IGF-1 (insulin-like growth factor) (Xu *et al.*, 2007)	Cell survival, pro-angiogenic, cell proliferation and migration
MCP-1 (monocyte chemoattractant protein) (Boomsma and Geenen, 2012)	Cell migration, pro-angiogenic, differentiation, survival
HGF (hepatocyte growth factor) (Kitta *et al.*, 2003)	Pro-angiogenic, cell survival, remodelling, differentiation
IL-1β (Kobayashi *et al.*, 2000)	Pro-angiogenic
IL-6 (Pricola *et al.*, 2009)	Cell proliferation,
IL-10 (Chen *et al.*, 2010)	Remodelling, cell survival
TGF-β (transforming growth factor-β) (Doyle *et al.*, 2008)	Cell hypertrophy, proliferation
TNF-α (Corallini *et al.*, 2010)	Pro-angiogenic, migration
MIP-1α (macrophage inflammatory protein-1α) (Boomsma and Geenen, 2012)	Cell migration
TIMP 1 and 2 (tissue inhibitor of metalloproteinase) (Singla and McDonald, 2007; Glass and Singla, 2012)	Cell migration, remodelling
SFRP-2 (secreted frizzled-related protein) (Alfaro *et al.*, 2008)	Cell survival
Thymosin β4 (Hinkel *et al.*, 2008)	Cell survival, remodelling
GCP-2 (granulocyte chemotactic protein) (Kim *et al.*, 2012)	Cell survival, pro-angiogenic, cell migration and proliferation
Angiopoietin-1/2 (Kamihata *et al.*, 2001)	Pro-angiogenic

logical targets once the relevant molecular pathways are identified. The induced pluripotent stem cell (iPS cell) has been identified from research into regenerative medicine (Takahashi and Yamanaka, 2006), and this cell may one day have the potential to fulfil the role of the embryonic stem cells but without the same ethical issues. However, for the time being technical issues prevent the use of these cells in human beings and the main application for the iPS cell at present is for drug discovery or testing *ex vivo* (see accompanying review by Khan *et al*., 2012). New methods are being developed to help identify the molecular pathways that are a key to developing new regenerative strategies (see accompanying review by Vieira and Riley 2012) and the potential for pharmacology to produce drugs that can directly stimulate resident stem cells to promote cardiac repair remains an enticing target. Single-agent strategies are already being tested (e.g. the peptide thymosin β4) and if successful will provide a much simplified approach to realizing the potential of regenerative medicine in human subjects. For now, the relationship between conventional pharmacology and cell-based regenerative medicine is crucial and further work is needed to understand these interactions to optimize cell-based therapies. Ultimately, the development of 'regenerative pharmacology' has the potential to revolutionize the field of regenerative medicine and overshadow current cell-based therapies. Until this new branch of pharmacology has matured, the cell-based approach continues to offer the best prospect for organ repair.

Acknowledgements

The clinical trials of cell therapy lead by Professor Mathur are supported by generous grants from the Heart Cells Foundation, the UK Stem Cell Foundation, Chugai Pharmaceutical, and Barts and the London Charity.

Conflict of interest

No conflict of interest.

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