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## CALL FOR PAPERS | *Cellular Plasticity in the Cardiovascular System*

### Cellular approaches to tissue repair in cardiovascular disease: the more we know, the more there is to learn

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THE RAPIDLY ADVANCING FIELD of cellular plasticity bears exciting implications for both basic understanding of cell biology and potentially important therapeutic approaches to a range of disease states that remain as areas of great patient need. Indeed, the use of stem cells, progenitor cells, or even mature cells for therapeutic intervention in cardiovascular disease holds great promise, as well as harboring significant controversy. This *Call for Papers* highlights the exciting advances that are being made in this broad area; and it illustrates the substantial breadth of cell types and approaches that have provided insights into the biology of cell-based repair processes and have promised implications for translation into the realm of patient therapy. The selections of the Call for Papers do indeed suggest several exciting potential therapeutic approaches, but they also help to underscore the many questions that remain to be answered about the underlying mechanisms that explain the beneficial effects that have been described after cell deliveries to vascular or cardiac targets. In this Prologue, a framework will first be provided by outlining key aspects currently under intense investigation in this area. The emphasis will be placed on some of the major questions that require consideration both with respect to the mechanism and future practical application. The manuscripts that comprise this Call will then be highlighted with regard to these issues.

#### *Therapy Recapitulates Physiology: A Working Hypothesis*

A foundational hypothesis that may be used to direct and evaluate cell therapy protocols is that the organism employs endogenous reparative processes, involving cells that have also been proposed or studied as potentially important in therapeutic approaches. It logically follows that most effective therapies will be modeled on the recapitulation of endogenous physiology and will assist in pathophysiological states by directing toward the normal condition. The study of endogenous cellular plasticity and repair mechanisms, which will presumably play a key role in the homeostatic response to repair or reverse damage, is thus very germane to advancing the field of cell therapies. Many lines of evidence now suggest that inadequate cell plasticity may impair the potential for cardiovascular tissue repair and regeneration in disease states, contributing to the development of atherosclerosis as well as cardiac disease and diseases of other organs critically dependent on blood flow. However, the precise limitations of the endogenous reparative process are not yet clear. It is therefore important to know

whether normal repair processes suffer deficits due to inadequate cell numbers or cell functions to consider thoughtfully whether more or indeed “better” cells must be provided.

Two fundamental potential activities of stem cells and progenitor cells, which are most relevant to cardiovascular disease, are, respectively, 1) the use of cell therapies to expand the coronary or peripheral vasculature (via vasculogenesis, angiogenesis, or arteriogenesis/collateral vessel formation; hereafter collectively referred to as angiogenesis) and 2) the improvement in myocardial function by formation/modification of injured myocardial tissue (myocardial regeneration).

Until recently, myocardial regeneration and angiogenesis have been treated as two distinct therapeutic goals. Recent studies, including the article below by Schuster et al. (Table 1, Article 6, Ref. 18a), however, suggest that they may be interrelated. For example, transdifferentiation may occur among relatively immature as well as mature cells (24); and endothelial cells can transdifferentiate into cardiomyocytes (6). Therefore, cell therapies directed to increases in the local numbers of vascular endothelial cells in the heart might result in enhanced formation of cardiomyocytes. Indeed, an interdependence between cell-induced myocardial regeneration and angiogenesis is suggested by the emerging concept that long-term engraftment and survival of delivered stem cells or progenitor cells will critically depend on establishing adequate blood flow through vascularizing the region surrounding the transplanted cells: a key point highlighted by the Schuster study.

#### *Major Issues to be Investigated in the Field of Cell Plasticity and Therapy*

Figure 1 provides a conceptual overview of key questions to be more completely answered regarding potential features of cell repair. The following features are some of the most prominent.

1) What are the major identities of the cells that undertake endogenous repair? In Fig. 1, several resident populations are depicted, but the actual number of relevant populations is unknown. Are these cells common among many tissues? Is there functional redundancy among multiple cell repair populations? Conversely, is an interactive synergy among multiple (>1) cell populations critical; i.e., must more than one population of repair cells be present and active to induce optimal tissue repair?

2) Where do the reparative cells grossly reside in the organism? Is this reservoir compartment the bone marrow or other extrahematopoietic tissue? Another possibility is that key

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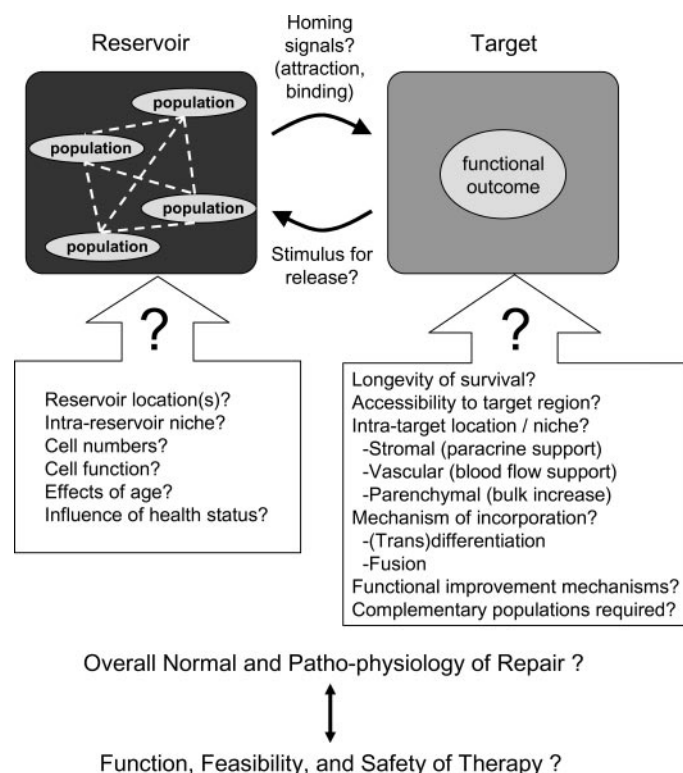


Fig. 1. Conceptual overview of key questions regarding the potential features of cell repair.

repair cells reside within the repair target tissue, as recent studies have suggested in the case of the heart (10, 15, 20). In cases such as the latter, will repair cells from other sources function as effectively (from a therapeutic perspective) in certain circumstances? What is the microscopic niche/location of these cells within their tissues? How do they gain access to their local niche in the target tissue?

3) With the onset of disease (or the presence of risk factors), to what extent do the relevant cells decrease in number? To what extent are they functionally impaired? Which of these issues is amenable to complementation by the input of additional cells? Can autologous cells actually complement the deficits, or must cells be provided from a nondiseased source (e.g., typically crossing immunologic barriers)? Alternatively, will the reparative function of autologous cells be enhanced by the incorporation of exogenous genetic material before their provision, so that the cells become gene vectors providing therapeutic levels of proteins to target tissues?

4) How do target sites stimulate the release of the cells from reservoir sites to make them potentially available for repair? Is this mechanism altered by disease status or the presence of risk factors?

5) What "recruits" or calls in the cells, once liberated, to home to target sites? What ligands function to bind them there?

6) For how long can/do the putative repair cells survive in the target tissue, once physiologically recruited? How long do they survive if placed artificially? Will redosing be necessary to achieve sustained benefit?

7) How do the cells function in the target tissue? Do they predominantly function to support the tissue by 1) providing

paracrine signals with informational or survival content [recruiter/manager (17, 18)/"software" (9) roles]; 2) enhancing the local vascular network (supplier role); or 3) adding to the parenchymal mass of the tissue ["building block"/"hardware" (9) role]?

8) If the cells incorporate into the parenchyma, do they do so by 1) a differentiation event from a relatively primitive precursor cell type; 2) a transdifferentiation event from another lineage; or 3) by fusion with the preexisting parenchymal cells?

9) How do the cells contribute to functional improvement in the target tissue (e.g., myocardial contractile or electrical function)? Which set of the above mechanisms plays a role in the functional behavior of these cells?

10) Is it in fact the case that the best approaches to therapy will employ elements that recapitulate normal physiological processes that are impaired due to disease?

11) How is delivery of putative repair cell populations best conducted to achieve maximal tissue investment with the cells in a fashion that contributed to functional improvement?

12) To what extent is it necessary to understand the answers to these questions before embarking on large clinical trials, when preclinical studies show intriguing evidence of functional improvements in blood flow or indexes of myocardial function?

#### *Can What You Don't Know Help You?*

The final point, the topic of *question 12*, is a key issue of debate. One distinct position holds that the positive preclinical data, coupled with the severity of disease found in cardiovascular patient populations that are not readily addressed by current therapy, justify the early clinical trials that have already begun. A clearly opposing viewpoint suggests that knowledge of the identity and mechanisms of action of endogenous repair cell populations must be much more fully developed before clinical trials are reasonable. However, a consideration of the history of the cell therapy, which is most firmly grounded in clinical practice, that of bone marrow transplantation, reveals that approaches to severe diseases have indeed met with significant successes even before the entire definition of hematopoietic stem cell identity. Accordingly, a moderate approach may be suggested, involving cautious attempts to pursue clinical trials directed to initially evaluate the safety of cell-based approaches, then to pursue efficacy after once establishing safe parameters of cell isolation and delivery.

Indeed, numerous clinical studies are presently underway with a variety of cell types or cell mixtures in patient populations that have experienced recent infarction or have manifestations of ischemic cardiomyopathy; despite the situation that the identity of the key therapeutic cell types and their mechanisms of function have yet to be fully characterized. A multitude of both controlled preclinical and largely uncontrolled clinical studies have suggested remarkable biological effects and potential improvements. Several of the manuscripts in this Call collection provide examples of impressive functional effects of cell delivery.

As this collection demonstrates, a very wide range of cell populations are being tested, raising the distinct question of why so many cell types appear to be able to confer benefit. It is conceivable that such observations have a basis in a set of

shared cellular mechanisms that exist more broadly than originally expected: the ability of many cell types to modulate their environment by the secretion of paracrine factors is one such mechanism. On the other hand, particular outcomes may require cellular properties, which are much less commonly held, such as the self-renewability and inducible differentiation, which are key hallmarks of stem or progenitor cells.

#### *Construction Managers or Building Blocks?*

Many recent studies indeed support the concept that cell-based repair mechanisms rely on the plasticity of their protein expression, leading them to conduct paracrine factor secretion facilitating angiogenesis or mediating cell survival. This has been described for a population of cells cultured from peripheral blood mononuclear cells, which have been variably termed circulating cultured angiogenic cells (CACs) [(17) and Call Ref. 3], culture-modified mononuclear cells (CMMCs) [(8) and Call Refs. 4 and 5], and endothelial progenitor cells (1, 3, 4, 7, 22, 23); for adipose stromal cells (18); and most recently for bone marrow-derived stromal cells (MSCs) (13). As alluded to above, this perspective in which such cells contribute their main effects by the synthesis of a complex mixture of bioactive molecules has led to their description as providing an instructive “manager” function (18) or “software” function (9).

Although several studies clearly support the ability of particular progenitor or cardiac-specified cells of embryonic origin to incorporate into adult myocardium (19) with resultant functional improvement after infarction (Table 1, *Article 7*, Ref. 9b), other studies that support an unexpected cardiomyocytic fate of bone marrow (16) have been recently challenged by findings that call into question the ability of such cells to transdifferentiate into cardiomyocytes. Some studies (2, 21) have suggested that significant mechanisms responsible for the appearance of cardiomyocyte fates involve cell fusion, particularly after marrow transplantation, although the functional outcomes of such fusion have not been documented in vivo, and the role of such fusion events in typical physiological repair of cardiovascular tissues is not yet known. More recent studies (5, 14) have not been able to confirm a cardiomyocytic fate of several marker-purified populations of hematopoietic-lineage cells after local intramyocardial injection. However, one of these studies did demonstrate a very low-frequency potential transdifferentiation event after bone marrow transplantation (14), highlighting the possibility that various methods of cell delivery to the putative niche for repair may lead to disparate biological outcomes.

In a parallel study directed toward understanding cell incorporation into nascent vasculature, it has been most recently suggested that bone marrow-derived cells do not incorporate into nascent neovasculature with high frequency in an ischemic hindlimb model (28) but rather accumulate around growing collateral arteries, expressing various angiogenic factors and chemokines.

It is important to recognize that these studies, which call into question particular mechanisms, such as transdifferentiation, do not imply the absence of potential for functional improvements in ischemic or other injury; they merely raise the awareness of the need for better assessment of mechanisms by which these improvements might be expected to occur.

#### *To Stem or Not to Stem?*

The redirection of the focus on cell repair processes from transdifferentiation and tissue incorporation toward paracrine effects naturally leads to a key question that also emerges from a consideration of the studies in the general literature as well as those within this Call. To succeed in a therapy, is it necessary to isolate cell populations with high-frequency stem or progenitor properties, or might less-specialized populations achieve important therapeutic goals by mechanisms including factor secretion and possibly enhancement of blood flow? Although the answer to this and many of the questions posed earlier have yet to emerge, the very possibility that more easily accessible, abundant populations may help in tissue repair is quite interesting from the perspective that ultimate clinical utilization is likely to depend not only on the functional effects and progressively defined mechanisms, but also critically on a reasonable modicum of practicality for widespread application of any therapy, which is put forward as a broadly useful approach.

#### *Implications of the Studies Featured Within This Call*

Table 1 lists the manuscripts of this Call by title and authors, and then provides key information about the manuscripts with respect to the cell source studied, the method of delivery where appropriate, the immune status of the cellular transplantations performed, the major end points of each study, and finally, the primary mechanisms by which the cells studied in each case mediated the observed end point effects. This table makes apparent the plurality of cell types, delivery approaches, and cell mechanisms suggested by these studies: these data are summarized at the bottom. It is shown that four studies studied cell populations isolated only by attachment or growth-selection phenotype, whereas four studies evaluated cells that were purified by their surface markers, or were relatively well-characterized discrete populations. Two studies evaluated systemic cell delivery, whereas the balance used some form of local delivery to place the cells where desired. Ultimately, six studies showed in vitro or in vivo incorporation of the studied cells into either vascular or cardiac structures, whereas five studies demonstrated an important role for paracrine stimulation by the cells. None of the studies directly evaluated for the possibility of cell fusion as a partial explanation for the findings of incorporation. Two studies explicitly modified cells by gene transfer, and one of these relied on cultured vascular smooth muscle cells, not even attempting to study cells with a stem/progenitor/precursor phenotype. The remainder of the studies studied various types of these latter cells, including umbilical cord-derived endothelial progenitors, outgrowth endothelial cells (OEC progenitors), which proliferate readily; CAC/CMMCs, which do not proliferate at all readily; granulocyte-colony stimulating factor (G-CSF) mobilized peripheral CD34+ cells; a murine embryonic stem cell line (CGR8); and finally bone MSC. Notwithstanding the range of cells studied among these 10 Call for Papers, each cell delivery protocol demonstrated convincingly important end points (Table 1, *Articles 1* and *3–9*, Refs. 8a, 8b, 9b, 9c, 13a, 15a, 18a).

The first six studies (Table 1, *Articles 1–6*, Refs. 8a, 8b, 9a, 9c, 18a, 24a) address various potential roles of progenitor cells in modulating vascular growth and function; whereas the last four studies (Table 1, *Articles 6–9*, Refs. 9b, 13a, 15a, 18a) all address myocardial function. The study by Schuster et al.

Table 1. Overview of articles presented

Article (Ref. No.)	Title	Authors	Cell Source/Purification	Delivery Method	Immune Status	End Points	Proposed Role of Cells
1 (24a)	Tissue-engineered microvessels on three-dimensional biodegradable scaffolds using human endothelial progenitor cells.	Wu, Aikawa-Rabkin, Gulserian, Perry, Masuda, Sutherland, Schoen, Mayer, Bischoff	Human smooth muscle cells and CD34+/CD133+ cells from Umbilical Cord	NA	NA	Structural assembly	Incorporation
2 (9a)	c-kit-Immunopositive vascular progenitor cells populate human coronary in-stent restenosis but not primary atherosclerotic lesions	Hibbert, Chen, O'Brien	NA	NA	NA	Local tissue and circulating cell pool numbers	NA
3 (8a)	Modulation of the vascular response to injury by autologous blood-derived outgrowth endothelial cells	Gulati, Jevremovic, Witt, Kleppe, Vile, Lerman, Simari	Rabbit outgrowth endothelial cells and culture-modified mononuclear cells	Local catheter-based application (20 mins)	Autologous	Cell incorporation, vasoreactivity, neointimal reduction	Incorporation into vessel lumen and paracrine stimulation
4 (9c)	Use of blood outgrowth endothelial cells as virus-producing vectors for gene delivery to tumors	Jevremovic, Gulati, Hennig, Diaz, Cole, Kleppe, Cosset, Simari, Vile	Human outgrowth endothelial cells and culture-modified mononuclear cells	Tail vein injection (systemic)	Athymic nude mice	Transfectability, homing, local vector dissemination	Paracrine stimulation via gene transfer
5 (8b)	Autologous vascular smooth muscle cell-based myocardial gene therapy to induce coronary collateral growth	Hattan, Warltier, Gu, Kolz, Chilian, Weihrauch	Canine jugular vein smooth muscle cells	Intracoronary	Autologous	Collateral flow, gene-product secretion	Paracrine stimulation via gene transfer
6 (18a)	Myocardial neovascularization by bone marrow angioblasts results in cardiomyocyte regeneration	Schuster, Kocher, Seki, Way, Xiang, Homma, Itescu	Human G-CSF mobilized, CD34+ enriched PBMC	Tail vein injection (systemic)	Athymic nude rats	Neovascularization, CMC proliferation, myocardial function	Incorporation (vascular), vascular nutritive function, and paracrine stimulation
7 (9b)	Stable benefit of embryonic stem cell therapy in myocardial infarction	Hodgson, Behfar, Zingman, Kane, Perez-Terzic, Alekseev, Puceat, Terzic	Murine embryonic stem cells (CGR8 line)	Direct intramyocardial injection	Immunocompetent rats	Cell incorporation, myocardial function, and depolarization	Incorporation into myocardium
8 (15a)	Bone marrow stromal cells improve cardiac performance in healed infarcted rat hearts	Olivares, Ribeiro, deCastro, Ribeiro, Matos, Goldenberg, Mill, Dohmann, de dos Santos	Rat adherent bone marrow stromal cells	Direct intramyocardial injection	Isogenic Wistar Rat donor/recipients	Cell incorporation, myocardial function, and depolarization	Incorporation into myocardium
9 (13a)	Autologous stem cell transplantation for myocardial repair	Liu, Hu, Wang, Gong, Mansoor, Hou, Zeng, Zhang, Jerosch-Herold, Guo, Bache, Zhang	Porcine adherent bone marrow stromal cells	Epicardial placement of pre-seeded fibrin matrix	Autologous	Neovascularization, cell incorporation, myocardial function, in vitro myocyte differentiation	Incorporation into neovasculature, vascular nutritive function, and paracrine stimulation

NA, not applicable.

(Table 1, *Article 6*, Ref. 18a) plays a bridging role in that it demonstrates that systemic delivery of mobilized mononuclear cells leads to cardiac effects presumably as a consequence of enhanced neovascularization.

The study of Wu et al. (Table 1, *Article 1*, Ref. 24a) is an *in vitro* exploration of a system that reveals the participation of an EPC population in vascular assembly in a manner dependent on smooth muscle, whereas the study of Hibbert et al. (Table 1, *Article 2*, Ref. 8a) describes clear distinctions between numbers of vessel-resident c-kit<sup>+</sup> cells and circulating CAC/CMC-type cells in patients with local atherosclerotic lesions, those with restenotic lesions, and nondiseased controls. These cell populations are clearly quite distinct, yet the observation that both exhibit significant alterations in these two states of vascular disease implies that these local macrovascular conditions are associated with systemically observable cellular perturbations. Whether the local vascular disease caused these quantitative alterations or occurred as a result of them cannot, however, be proven from this manuscript. The next study of Gulati et al. (Table 1, *Article 3*, Ref. 8a) evaluates rabbit EPCs of the OEC phenotype with regard to their ability to incorporate into a repairing vessel after injury. This study highlights the ability of EPCs to help repair macrovessels, which stands in contrast to the unknown, yet potentially adverse, effects of circulating smooth muscle progenitors as described.

The next two studies both describe aspects of using cells as gene carriers. Jevremovic et al. (Table 1, *Article 4*, Ref. 9c) directly compares the OEC endothelial progenitor and CMMC/CAC phenotype cells with regard to their transfectability and tumor homing. OECs were found to be more transfectable and to home more effectively into a tumor neovasculature, thus possibly pointing toward a tumor targeting strategy for the future. This manuscript also provides for a detailed comparison between the OEC and CMMC/CAC cell types; the use of the term "EPC" by different groups to describe both of these cell types reflects a lack of complete agreement on the defining characteristics of an EPC (e.g., primary reliance on surface marker, attachment, clonal proliferation, or other definitions). The study of Hattan et al. (Table 1, *Article 5*, Ref. 8b) is interesting in its identification of a robust enhancement of blood flow to canine myocardium in a model of intermittent ischemia, by the local instillation of autologous vascular smooth muscle cells, which were pretransfected with a plasmid expressing VEGF. This study has features of particular interest to clinical translation, including the relevance of the model to chronic angina, the easily conceived extension of the intracoronary instillation of such cells, and the choice of autologous cells as the carriers for the genes. It also establishes the potential utility of non-stemlike cells, which function primarily by factor secretion, as established by the lack of effect of nontransfected cell controls. Such an approach may also be of great interest with cells from other autologous sources not requiring expansion, such as adipose stromal cells (18).

The study of Schuster et al. (Table 1, *Article 6*, Ref. 18a) raises several interesting points: it employs systemic delivery of a CD34<sup>+</sup> subset of G-CSF mobilized cells 2 days after rat myocardial infarction, and suggested significant trafficking of such cells to the heart, with acceleration of neovascularization and associated improvement of cardiomyocyte survival as well as an increase of cardiomyocyte-marked cells showing bromodeoxyuridine incorporation and Ki67 stain by confocal

microscopy. It also showed functional and structural improvement at 15 wk post-MI. Although these remarkable findings will likely foster efforts using similar approaches, thoughtful caution will be present in light of the recent concerns raised with a protocol employing G-CSF mobilization (11).

Hodgson et al. (Table 1, *Article 7*, Ref. 9b) and Olivares et al. (*Article 8*, Ref. 15a) also employed rat myocardial models, which showed significant improvements in myocardial function, as well as evidence for incorporation of cells derived from the murine ES cell line and rat isogenic MSC cells, respectively. The models used were somewhat different, in that the Hodgson study tested cell delivery 8 wk after artery ligation and showed sustained functional improvement for 12 wk subsequent to delivery, whereas the Olivares study tested cell delivery 4 wk after ligation and evaluated functional outcomes after only 2 wk. The last study, that of Liu et al. (Table 1, *Article 9*, Ref. 13a), employed a novel tissue engineering approach reminiscent of the scaffolding described earlier (*Article 1*, Ref. 24), but tested the epicardial placement of incorporated autologous MSC in a porcine model of myocardial infarction with reperfusion. This stem cell patch approach evidenced both neovascularization and a correlated myocardial functional improvement along with the ingress of the MSCs into the myocardium, presumably reflecting a significant migratory ability of the MSCs in this milieu.

In conclusion, the use of local or systemic cellular approaches for the treatment of cardiovascular disease represents a very important therapeutic concept for further development. Much remains to be answered about the optimal cell types and methods, which will be useful and feasible to extend to widespread clinical practice. Indeed, mature differentiated cells have also been used for cell therapy and have at times shown therapeutic efficacy that is comparable (12, 25–27) to that found in studies using stem cells and progenitor cells. However, it does appear that cell-based therapies may have significant potential to address the substantial morbidities that still remain in patients with impaired cardiac function due to ischemia or other myocardial problems. As with other therapies, which require a mixture of biological and mechanical considerations, many details such as harvesting, selection, infusion, and potency will determine whether the transition can eventually be made from the promises represented in the Call for Papers to patients.

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