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## Where have all the stem cells gone?

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myocardial repair; cell therapy; mesenchymal stem cell; microcirculation

Acute myocardial infarction is the most generally accepted cause of morbidity and mortality in developed countries. Despite rapid restoration of perfusion, postinfarction heart failure remains a major challenge. Considerable experimental and clinical evidence indicates the feasibility of therapeutic implantation of bone marrow-derived cells in reversing abnormal cardiac remodeling with the generation of cardiac myocytes as well as the stimulation of neovascularization within the infarcted area.<sup>1–3</sup> While therapeutic delivery of stem cells following myocardial infarction and heart failure is being explored in clinical trials (see [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) for the list of current trials), little is yet known about what determines the fate of stem cells and how they move into and within tissues to provide a regenerative effect. The acute fate of these cells in the microcirculation seems of particular interest, since the small blood vessels are the site of adhesion and migration of circulating cells, including systemically or locally administered stem cells, into the tissues. One would expect that an intimate interaction of stem cells with elements of the microcirculation plays a crucial role in integration and survival of cells allowing for potential tissue repair.

Among the various cell types investigated, bone marrow-derived mesenchymal stem cells (MSCs) are self-renewing clonal precursors of nonhematopoietic stromal tissues.<sup>4, 5</sup> MSCs represent a type of adult stem cell that can easily be isolated from various tissues and expanded *in vitro*.<sup>4</sup> Evidence indicates that MSCs give rise to osteoblasts and chondroblasts; and that under appropriate conditions they can also express phenotypic characteristics of endothelial, neural and smooth muscle cells as well as skeletal myoblasts and cardiac myocytes.<sup>4</sup> The MSCs, with their ease of isolation, high expansion potential, genetic stability and potential to promote tissue repair appear to be an appealing source for stem cell therapy, but the lack of common criteria of MSC identity and characteristics as well as the lack of universal standards for preparation of MSCs seem to hamper further progress in this field of investigation.<sup>6</sup> The low survival rate of MSCs after administration into an ischemic tissue further limits their therapeutic efficacy.<sup>7, 8</sup> Perhaps, due to the fact that current imaging techniques used to track implanted stem cells do not allow for direct visualization of individual cells, knowledge of the exact mechanisms responsible for the limited survival of MSCs is still missing.

Ideal imaging technology used for stem cell tracking would have single-cell sensitivity and would permit quantification of cell numbers at any anatomic location. The current imaging techniques, such as X-ray, ultrasound, single-photon emission CT, positron emission tomography, magnetic resonance and optical imaging, to track stem cells *in vivo*, are far from

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### Disclosures

None.

ideal.<sup>9, 10</sup> Thus the pattern of migration of stem cells even after local administration remains essentially unknown. It would be important to determine the acute fate of stem cells following intravascular administration to be able better to predict their survival in order to ensure incorporation into the tissue for regeneration. Although studies have measured MSC survival and apoptosis following injection,<sup>11, 12</sup> no studies have examined the time course of cell infiltration through the vascular wall in relationship to cell survival. Also, there is no direct assessment of the ability of MSCs to pass through microvascular networks.

Previous studies aimed at following MSC distribution upon systemic delivery have shown that most of the cells became entrapped in the lung.<sup>8, 13</sup> Only a direct injection of MSCs into the ischemic myocardium enhanced migration and colonization of the implanted cells.<sup>8</sup> Interestingly, a study by Vulliet et al. showed that intracoronary injection of bone marrow-derived MSCs causes myocardial infarction in a dog model, as indicated by ECG changes, increased troponin I levels and postmortem histological data.<sup>14</sup> Although clinical studies have shown that intracoronary infusion of MSCs at the time of or after myocardial infarction is safe and could be of benefit to patients,<sup>3</sup> the aforementioned study raised the possibility that MSCs are easily entrapped in the microcirculation, a phenomenon, the importance of which is unknown.

In the present issue of *Circulation Research* Toma et al.<sup>15</sup> describe an innovative approach that aims to assess the acute fate of intra-arterially injected MSCs in the rat cremaster muscle microcirculation. The authors used intravital microscopy to observe cellular migration in this skeletal muscle microcirculation under conditions that preserve the local microvessel architecture. The results show that intra-arterial injection, most of the *in vitro* expanded MSCs, whose average diameter was 23  $\mu\text{m}$ , became entrapped in precapillary vessels, resulting in cessation of blood flow in the feeding artery. The majority of the entrapped cells became non-functional and exhibited cytoplasmic fragmentation and nuclear condensation. In spite of the substantial cell loss, 14 % of the surviving cells became integrated into the microvascular wall or were seen at perivascular locations at a precapillary level within the 72-hour period of observation, indicating that integration of MSCs occurred at the point of initial entrapment. Based on these results the authors concluded that upon intra-arterial delivery only a small proportion of MSCs integrated into the microvascular wall. This implies, that in order to enhance therapeutic success one needs to avoid micro-embolization, primarily by aiming to retain the original size of MSCs (which is half that of the cells used in this study) during *in vitro* expansion, while preserving their putative ability for active engraftment.

The authors themselves acknowledge that only few stem cells survive and integrate into perivascular niches at 3 days. Thus it is likely that the number of MSCs surviving is far too small to induce a quantifiable angiogenic or regenerative response. In addition, the relative number of integrated MSCs might be overestimated in this case, since the study by Toma et al.<sup>15</sup> does not exclude the possibility that during the fragmentation of MSCs the remaining fluorescent probe, used to label these cells, can be taken up and incorporated into the surrounding phagocytes residing in the microvascular wall. This problem could be addressed if the fate of individual cells were to be followed by real time imaging to ensure cell identity during the observation period. Furthermore, in the aforementioned study by Vulliet et al.<sup>14</sup> and in the present paper by Toma et al.<sup>15</sup> MSCs were injected intra-arterially to perfuse uninjured tissues, i.e. the heart and skeletal muscle of healthy animals. Most likely, due to the larger cell size of MSCs, acute microembolization developed upon intra-arterial injection, leading promptly to tissue ischemia, although in the present instance one needs to consider that the cremaster muscle has a low oxygen consumption and therefore tissue injury may only be slight. It should be emphasized that in the study by Toma et al.<sup>15</sup> clumping of MSCs itself would cause ischemia and injury to the tissue, whereas in a clinical situation it is the already distressed tissue, to which the implanted MSCs will be attracted, to exert their paracrine effects,

eventuating in tissue repair.<sup>2</sup> Various cell-culture conditions to reduce the size of MSCs and thus limit the tendency for microembolization, as suggested by the authors, do not necessarily yield a more efficient cell engraftment in the already ischemic tissue. However, it is possible that smaller MSCs would penetrate deeper into the microcirculatory network, especially, if in presence of a vasospasm, vasodilator agents were co-administered with the cells.<sup>13</sup> On the other hand, one can envision that entrapment of the relatively large-sized MSCs at precapillary level would facilitate their transmigration and integration into tissues. This seems especially important since a large body of evidence indicates that the therapeutic efficacy of MSCs (e. g. preservation of myocardial function) is closely related to the number of *in situ* viable cells implanted into the hostile environment of hypoxic and inflamed tissues.<sup>12</sup> In this context, previous studies elegantly demonstrated that genetic modification of MSCs – for instance, over-expression of the pro-survival gene Akt<sup>12</sup> or the anti-apoptotic gene, Bcl-2<sup>11</sup> – enhances survival of the engrafted MSCs in the heart after acute myocardial infarction, resulting in improved cardiac performance.

Collectively, there appears to be a series of both mechanical and biological events, including those described by Toma et al,<sup>15</sup> that have to be taken into account when investigating the acute and chronic fate of stem cells in tissue repair processes. Importantly, the impact of these factors should be investigated in a setting similar to clinical conditions. Accordingly, the fate of implanted stem cells should be evaluated in injured tissues, in which the microvascular architecture has deteriorated, as in the infarcted myocardium. Real-time detection of the implanted stem cells seems also essential; this however, requires novel imaging techniques, in which intravital microscopy is used to study a preparation that is available for chronic observation. This experimental design would also facilitate evaluation of an angiogenic response and tissue repair initiated by the implanted stem cells.

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