A role of myocardial stiffness in cell-based cardiac repair: a hypothesis

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Abstract

Determining which time point is optimal for bone marrow–derived cell (BMC) transplantation for acute myocardial infarction (AMI) has attracted a great deal of attention. Studies have verified the interaction between cell treatment effect and transfer timing and have suggested that the optimal time frame for BMC therapy is day 4 to day 7 after AMI. However, the potential mechanism underlying the time-dependent therapeutic response remains unclear. Recently, a growing body of *in vitro* evidence has suggested that stem cells are able to feel and respond to the stiffness of their microenvironment to commit to a relevant lineage, indicating that soft matrices that mimic brain are neurogenic, stiffer matrices that mimic muscle are myogenic and comparatively rigid matrices that mimic collagenous bone prove osteogenic. Simultaneously, considering the fact that the myocardium post-infarction experiences a time-dependent stiffness change from flexible to rigid as a result of myocardial remodelling following tissue necrosis and massive extracellular matrix deposition, we presume that the myocardial stiffness within a certain time frame (possibly day 4–7) post-AMI might provide a more favourable physical microenvironment for the phenotypic plasticity and functional specification of engrafted BMCs committed to some cell lineages, such as endothelial cells, vascular smooth muscle cells or cardiomyocytes. The beneficial effect facilitates angiogenesis and myocardiogenesis in the infarcted heart, and subsequently leads to more amelioration of cardiac functions. If the present hypothesis were true, it would be of great help to understand the mechanism underlying the optimal timing for BMC transplantation and to establish a direction for the time selection of cell therapy.

Keywords: stiffness • acute myocardial infarction • cell therapy • timing • hypothesis

The net loss of cardiomyocytes during myocardial infarction is a key factor in the resulting remodelling and in the impairment of cardiac-pump function [1]. Prompt reperfusion of the infarct-related coronary artery has considerably salvaged the ischaemic myocardium and limited the infarct size [2]. Nevertheless, heart failure that develops after infarction remains a leading cause of morbidity and mortality [3].

The bone marrow harbours stem cells and progenitor cells that may be capable of solid-organ repair [4]. Experimental studies have suggested that bone marrow–derived cell (BMC) transfer can enhance functional recovery after acute myocardial infarction (AMI) [5, 6]. Based on these data, stem cells and progenitor cells derived from bone marrow have been proposed for use in the repair of cardiac tissue after infarction in patients [7–9]. However, the reported benefits of cell therapy are very different among these studies. Of the various reasons for the different results, the timing of cell administration might be one of the most important factors affecting therapeutic efficacy. Recently this issue—what time point is optimal for the cell transplantation for AMI—has attracted a great deal of attention. An experimental study addressing the impacts of timing of transplantation on cardiac function post the infarction demonstrated that BMC therapy at 1 week after AMI was superior to transplantation within 1 hr and at 2 weeks [10]. Similarly, data from the largest randomized trial (REPAIR-AMI study [7]) of cell therapy for AMI to date verified the interaction between BMC treatment effect and transfer timing, indicating that BMC transfer on day 5 post-AMI or later resulted in a significant increase of left ventricular ejection fraction by 5.1%, whereas no benefit was observed in patients treated within day 4. The other

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Table 1 Therapeutic efficacy and the time point of transplantation of bone marrow-derived cells

■ Randomized controlled trials						
Study	**Publication**	**Sample size**	**Cell type injected**	**Time to cell transfer (days)**	**Follow-up (months)**	**Primary endpoints (control/treatment)**
Within 24 hrs after AMI						
Janssens S *et al.* [9]	*Lancet* 2006	67	BMMNC	<1.0	4	LVEF: Δ2.2%/Δ3.4%; $P = 0.36$
At day 4–7 after AMI						
REPAIR-AMI [7]	*N Engl J Med* 2006	204	BMMNC	4.3	4	LVEF: Δ3.0%/Δ5.5%; $P = 0.01$
BOOST [8]	*Lancet* 2004	65	BMMNC	4.8	6	LVEF: Δ3.0%/Δ5.5%; $P = 0.01$
Suarez de Lezo J *et al.* [26]	*Rev Esp Cardiol* 2007	20	BMMNC	7.0	3	LVEF: Δ0.7%/Δ6.7%; $P = 0.0026$
More than 2 months after AMI						
Yao K *et al.* [27]	*Heart* 2008	47	BMMNC	390	6	LVEF: Δ1.6%/Δ2.4%, $P = 0.52$; Infarct area: Δ-1.6%/ Δ-2.3%, $P = 0.35$
Subgroup analysis in RCTs						
REPAIR-AMI[7]	*N Engl J Med* 2006	204	BMMNC	4.3	4	LVEF: Δ3.9%/Δ4.5%; $P = 0.62$ (within 4 days post-AMI); LVEF: Δ1.9%/Δ7.0%; $P = 0.004$ (at day 5–7 post-AMI) P value for interaction = 0.03
■ Experimental studies						
Study	**Publication**	**Animal model**	**Cell type**	**Transfer timing**	**Follow-up (weeks)**	**Main results***
Hu X *et al.* [10]	*Eur J Cardiothorac Surg* 2007	SD rats	BMMSC	1 hr, 1 week and 2 weeks	2	LVEF: 1 hr/1 week/2 weeks: 41.4%/48.1%/44.4%, $P < 0.05$; Infarct area: 1 hr/1 week/2 weeks: 41.4%/32.8%/37.1%, $P < 0.05$

*Comparison of each treatment group with the control group. Δ Changes from the baseline values.
AMI, acute myocardial infarction; BMMNC, bone marrow mononuclear cell; BMMSC, bone marrow mesenchymal stem cell; LVEF, left ventricular ejection fraction; RCTs, randomized controlled trials.

randomized controlled trial conducted by Janssens *et al.* [9] showed that cell transfer within 24 hrs post-AMI failed to improve left ventricular contractile function. Based on these preliminary results, the optimal time frame for cell therapy for AMI seems to be within the period from day 4 to day 7 after the infarction (Table 1). However, the mechanism underlying time-dependent therapeutic efficacy remains unclear.

Current researches on this scientific issue tend to decipher it by time course of the production of inflammatory cytokines and growth factors after AMI, which were involved in survival and differentiation of the engrafted cells [6, 11, 12]. Experimentally, the early inflammatory process in infarcted myocardium, which might adversely affect the biological and functional behaviours of the engrafted cells, subdued at 1 week post-AMI [13], and meanwhile some beneficial factors (*e.g.* vascular endothelial growth factor [VEGF], hepatocyte growth factor [HGF]) are at their peak concentrations [14]. In this period, the biochemical microenvironment within the ischaemically injured myocardium might be suitable for the regeneration of functional myocardium and neovascularization in the broken heart associated with cell-replacement therapy [15–17]. However, it is noteworthy that the biochemical response within the myocardium after AMI is an exceedingly complex network. Although some inflammatory factors and cytokines may benefit the engrafted cells, the majority are believed to be deleterious for survival and differentiation of the stem cells [18]. Even the same cytokines often have

paradoxical effects or counteract each other [19, 20]. Obviously, it seems to be irrational to elucidate the mechanism of the time-dependent therapeutic effects of BMC delivery for AMI by several beneficial cytokines alone. On the basis of the data, there may exist other factors responsible for the fate of engrafted cells and subsequently impacting cell-based cardiac repair beyond the biochemical factors.

Recently, a growing body of evidence has also shown an effect of physical characteristics of the microenvironment around the engrafted cells on their differentiation, suggesting that the stiffness of matrix corresponding to specific tissues could promote tissue-mimetic differentiation of naive BMCs *in vitro* [21–23]. The cellular phenotype and behaviour post-differentiation induced by deformable matrix with varied stiffness may more closely mimic that of the cells in their normal host tissue. Concretely, soft matrices (elastic modulus [*E*, a material property that describes its stiffness or elasticity] of 0.1–1.0 kPa) that mimic brain favoured differentiation of BMCs into neuronal-like cells, moderate elasticity ($E \sim 11$ kPa) that mimics muscle-promoted myogenic differentiation, and a rigid matrix ($E \sim 34$ kPa) that mimics collagenous bone–stimulated osteogenic differentiation [21]. That is to say, stem cells are able to feel and respond to the stiffness of their microenvironment to commit to a relevant cell phenotype.

It is natural to associate these findings with the fact that the myocardium post-infarction experiences a time-dependent stiffness change from flexible to rigid. Pathologically and anatomically, the injured cardiomyocytes no longer stay intact in its early stage of infarction as tissue necrosis and inflammatory edema follow. Young scar formation begins about 1 week after the infarction. Scar maturation begins at 2 weeks and completes at 4 weeks after AMI [24]. The cardiac remodelling process following myocardial infarction is mainly induced by myocardial fibrosis starting with massive extracellular matrix deposition, which in combination with the tissue necrosis stiffens the heart muscle. Berry *et al.* experimentally found that the elastic modulus for the non-infarcted myocardium of rats was about 18 kPa, whereas the 2-week infarcted myocardium is threefold stiffer than the normal myocardium ($E \sim 55$ kPa) [25]. However, whether the time-related stiffness change in infarcted myocardium is associated with engrafted cells' fate remains unclear.

Taken together, there exist three facts. First, the therapeutic effect of BMC transplantation for AMI is associated with the transfer timing. Second, the myocardium post-infarction experiences a time-dependent stiffness change. Third, matrix stiffness directs stem cell lineage specification. On the basis of these scientific findings, we presumed that myocardial stiffness within a certain time frame (possibly at day 4 to day 7) post-AMI might be more suitable for the phenotypic plasticity and functional specification of the engrafted BMC along some cell lineages, such as endothelial cells, smooth muscle cells or cardiomyocytes, than that at others time points, which facilitated angiogenesis and perhaps myocardiogenesis and, therefore, resulted in cardiac repair and amelioration of cardiac functions. The defined time domains will be regarded as the optimal time frame for the BMC administration for AMI.

To test our hypothesis, we performed preliminary *in vitro* experiments and observed that murine bone marrow mononuclear cells cultured in the medium with a matrix stiffness ($E \sim 31$ kPa) similar to the elasticity of infarcted myocardium at day 7 had a greater ability to differentiate into endothelial lineage cells, whereas those grown in the medium with a relatively soft matrix (E 4–17 kPa) that mimics stiffness of infarcted myocardium between 1 hr and 24 hrs after AMI showed minimal differentiation.

If the present hypothesis is true, it will contribute greatly toward understanding the mechanism underlying the optimal timing for BMC transplantation and to establishing a direction for the time selection of cell therapy. Importantly, these patients with missed opportunity for cell transplantation will still be able to benefit from cell-replacement therapy by attenuating cardiac remodelling and consequently changing myocardial stiffness post the infarction. Cell transplantation in combination with anti-remodelling treatment might be more beneficial for patients on cardiac repair than the procedure used alone.

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