Editorial

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Regenerative Medicine

Rebuilding the infarcted heart with noncellular material

"...injection of collagen mass into the infarcted wall of the left ventricle achieved many of the beneficial effects seen with cellular therapies without the complexities associated with cell therapy..."

Keywords: alginate • biomaterials • cellular retention • collagen • extracellular matrix • hydrogel • left ventricular remodeling wall thickening • myocardial infarction • noncellular material

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Hundreds of studies document attempts to regenerate cardiac tissue following acute myocardial infarction by injecting various types of cells into the vasculature or directly into the wall of infarcted hearts. Injected fetal [1], neonatal [2] or immature cardiomyocytes, derived from embryonic stem cells [3,4] or induced pluripotent stem cells can regenerate functional cardiac myocytes and in some studies electrical connections to host cardiomyocytes have been demonstrated [3,5–6]. These grafts typically thicken the wall of the left ventricle, reduce deleterious left ventricular remodeling including left ventricular dilatation, and improve cardiac function, though efficiency is still a challenge in these regenerative approaches. Other cells, such as mesenchymal stem cells can mediate positive effects on cardiac function after myocardial infarction (MI), but not by regenerating new cardiac muscle [7]. Rather these cells may work at least in part through secreted trophic factors [8] and exosomes, and promotion of angiogenesis [7]. In our studies, injection of bone marrow-derived mesenchymal stem cells improved short-term (4 weeks) but not long-term (6 months) stroke volume and ejection fraction; these cells did enhance angiogenesis; however, they did not generate large sheets of phenotypic cardiomyocytes with cross striations, in contrast to fetal or neonatal cardiomyocytes or immature cardiomyocytes derived from embryonic stem cells. [1–3]. Numerous clinical trials using many different cell sources and doses, delivery methods and timing after MI point to a therapeutic signal, but optimal cell therapy approaches for MI are still evolving [6]. Research in cellular therapies also spawned development of carriers that can enhance cell therapy residence and function [9,10]. Preclinical studies suggest that these 'carriers' can independently provide a therapeutic benefit after MI [11–15].

Experimental (rat) proximal left coronary artery occlusion leads to a paper thin scar after 4–6 weeks, in a large portion of the anterior–apical left ventricle (LV). When the nearby noninfarcted ventricle contracts, the thin collagenous scar bulges outward. That is, the infarct wall is pathologically dyskinetic. Blood that should be going out of the aorta instead is pushed into an aneurysmal sac that does not contract. Consequently, forward cardiac output is reduced leading to cycles of further stretching and thinning of the scar and dilatation of the LV, increasing wall stress and potentially worsening ischemia if other narrowed coronary arteries are present. We postulated that matrix protein injected into an infarct could have a passive mass effect, by beefing up the thin scar. Using the proximal rat coronary occlusion model, we injected collagen or saline directly into the scar 1 week after MI [12]. Six weeks later, histologic analyses showed that the infarcted walls were thicker in the collagen group and infarct expansion index was reduced. There was no significant difference in postmortem LV volumes between groups. Left ventricular angiography showed that collagen improved left ventricular ejection fraction by about

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8%. Importantly, paradoxical systolic bulging was prevented by the collagen injection. Therefore, by thickening the wall of the LV, which eliminated paradoxical systolic bulging, we were able to improve forward cardiac output; in other words, injection of collagen mass into the infarcted wall of the LV achieved many of the beneficial effects seen with cellular therapies without the complexities associated with cell therapy (isolation, expansion, identification and quality issues and need for GMP facilities).

In a second study [9], we showed that collagen injected with immature cardiac cells (vs cells alone) improved cell retention and reduced the numbers of cells leaving the injection site.

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Recently [13], we used extracellular matrix derived from rat hearts by Y Zhang at Wake Forest, injected directly into 1-week-old infarcts in our rat model (vs saline injection). At 6 weeks, the infarcted anterior free wall of the LV was thicker in the extracellular matrix group. Treated rats showed improved LV ejection fraction and, like collagen injection, paradoxical systolic bulging was reduced. In the extracellular matrix group, infarct expansion index was significantly lower than controls and there was a trend toward smaller ventricular volumes. Unlike our cell therapy studies, we did not observe increases in neovascularization with collagen or extracellular matrix injections.

Other groups have reported benefits of noncellular therapies. Studies with hydrogel [14] and alginate [15], for example, have shown similar benefits in experimental MI models. The intracoronary injection of alginate was shown to be safe in humans [16]. The material is injected as a liquid into the coronary arteries, but hardens when it reaches the myocardium, forming a bioabsorbable cardiac scaffold. This alginate-based injectate (IK-5001) is currently undergoing clinical testing for safety and efficacy in high-risk patients after MI (NCT01226563 [17]).

An advantage of noncellular approaches is that they are 'off-the-shelf' – stored until required for use in a patient with acute MI. By contrast, many cell therapies require cells to be collected from the patient on presentation and may require special processing of the cells and a long 'scale-up' process in order to generate sufficient numbers of cells for reinjection. Other issues with some cellular therapies include potential for contamination during culture expansion, immune recognition of allogeneic cells, potential for a proarrhythmic effect, the concern that teratomas may form and the unknown effect that these cells may have if they relocate to other organs in the body. Disadvantages of noncellular therapy include the fact that these substances only passively add mass and thicken the wall of the left ventricle. They do not contribute to active contraction of the heart; they only prevent a thin, infarcted collagenous wall from pathological dyskinesis. Nevertheless, this is enough to reduce wall stress and improve forward cardiac output. More complex extracellular matrix preparations, however, may be able to protect cardiac muscle: injected porcine-derived hydrogel improves global cardiac function after MI, and more cardiac muscle is preserved in extracellular matrix-injected pigs than in controls [10]. The potency of this matrix compares favorably to many cellular therapies, and a clinical trial using the porcine-derived hydrogel has recently opened (NCT02305602 [18]). So an interesting natural experiment is underway in which the potency of cellular therapies (with their inherent advantages and disadvantages) will be compared with newer noncellular therapies, also with their advantages and disadvantages. Presumably, combination therapies will be developed using noncellular adjuncts to improve cellular therapies.

Our observation that retention of mesenchymal stem cells in a myocardial infarct was improved by combining the cells with collagen [9] points to the potential utility of such combined therapies. Others have shown similar results: Panda *et al.* [19] showed that retention of mesenchymal stem cells injected into the border zone of infarcts was improved when the cells were suspended in alginate versus saline. In this study, the alginate-suspended mesenchymal stem cells had improved electrical impulse conduction. Roche *et al.* [20] also reported that two injectable biomaterials: alginate and chitosan/β-glycerophosphate and two epicardial patches: alginate and collagen resulted in better retention of mesenchymal stem cells compared with delivering the cells with saline into the infarcted border zone of experimental MIs. In rats receiving saline, retention rates of mesenchymal stem cells were only 10% at 24 h; while coupling cell therapy with the four types of biomaterials improved cell retention rates to about 50–60%. Other investigators have also shown that graft (patches) combining stem cells and various matrices is another promising approach [21]. Recent studies suggest that noncellular material may elicit an immune response directed at the extracellular matrix material but not necessarily against cells. This issue will need to be taken into account, but might be useful if the matrix only needs to be present for a finite period of time [22]. Stem cell therapy for MI is likely to become an important therapeutic approach but only after the many cur-

rent clinical trials are reviewed and compared, so that the optimal approaches emerge. However, even if cell therapies alone do not find a place in standard clinical care, noncellular therapies should be pursued as a means to passively thicken the infarcted wall, prevent the infarcted wall from bulging the wrong way during systole, lessen infarct expansion, improve left ventricular ejection fraction and ultimately reduce adverse left ventricular remodeling. Certainly, combined therapies deserve clinical evaluation as well.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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