Editorial Comment

Stem cell therapy for failing hearts: there is something else beyond the cells

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Heart failure (HF) affects a rapidly growing population of patients. Despite improvements in the understanding and therapy of many stages of cardiovascular disease, there has been little progress in treating HF. In late-stage disease, current options are cardiac transplantation and mechanical support-options that are limited to a small patient collective. The ischemically injured failing heart lacks contractile myocardium, functional vasculature, and electrical integrity, which has made treatment of the underlying injury untenable in the past. Restoring all of these components at once seems to be an overwhelming challenge.

Of the various cell types being investigated for this purpose, skeletal myoblasts are an attractive option, because they are readily available from muscle biopsies and, if autologous cells are used, immunosuppression is not required and ethical issues are avoided. Several studies have shown that the cells can survive and differentiate after transplantation, and promising clinical results have been reported. Encouraging results in terms of increased left ventricular ejection fraction, decreased left ventricular diastolic diameter, improved perfusion, and increased metabolic activity1,6 have been recently reported in humans using endovascular or surgical implantation of autologous skeletal myoblast implantation. Lately, some concerns such as the impact of delivery route, associated vasculogenesis, matrix environment, and muscle denervation on skeletal muscle cell survival and differentiation, have begun to be addressed.5,10 In this issue of the Journal of Geriatric Cardiology, the article by Ye et al.11 can be considered as a good step toward a more comprehensive approach to skeletal myoblast implantation for cardiovascular purposes; the real promise of a stem cell-based approach for cardiac regeneration and repair lies in the promotion of myogenesis and angiogenesis at the site of the cell graft to achieve both structural and functional benefits. Moreover, the article by Ye et al. suggests two fundamental considerations: First, the advantages and disadvantages of skeletal myoblast as a preferred cellular line with the related problem of denervation, and second, the importance of the matrix including proper vascular environment.

Advantages of skeletal myoblasts as a cellular line

The critical point of cellular transplantation is how useful non-muscle stem cells, especially those derived from adult tissues, may be for de novo myocardium formation in vivo. Successful transplantation of skeletal or fetal cardiac muscle cells into the hearts has been done using different cell types,12-18 but the small size of the animal model (generally rodents, with few exceptions7) is an obstacle to extending conclusions to humans (see below).

Given the time limit for repair after acute myocardial infarction, the delivery of pre-differentiated cells (cardiomyocyte and vascular cells, possibly derived from stem cells) appears desirable, although the approach means either prohibitively high costs of cell banking or the limitations imposed by the time it takes to expand the clones. Local delivery of these cells results in direct seeding of the damaged zone, but we need to understand more about how the microenvironment promotes cell differentiation in order to exploit this possibility. It is claimed that local delivery is improved, if such cells are engineered into three-dimensional grafts on appropriate matrix/biomaterials, but this approach is fraught with problems related to functional revascularization of the bioengineered prosthesis. Systemic delivery of stem cells is relatively noninvasive and remains an attractive option. This approach obviously relies on the ability of cells to home in on damaged tissue, but at present little is known about the factors which are responsible for such specific tissue targeting. Furthermore, the stem cell population would have to expand and differentiate into functional cardiomyocytes, and local conditions that could direct such phenomena still need to be elucidated. It is still early, and in the absence of solid in vivo data, it seems premature to extend such tests to the clinical setting.

While it is difficult to obtain sufficient quantities of cardiomyogenic cells that are in vivo, in order to achieve measurable benefits, skeletal muscle precursors (myoblasts) can be derived from satellite cells (reserve cells located beneath the basal lamina on the surface of mature myofibers)
or from cells lying beyond the myofiber in adult muscles, i.e., from interstitial connective tissue inside and outside of blood vessels or bone marrow. Both of these latter cells may have stem-like properties. Among the growing list of options, dermal fibroblasts appear promising as a realistic alternative source of exogenous myoblasts for transplantation purposes, only when the differentiation program can be restricted to the required loci, thus eliminating any oncogenic risk.

To date the only safe option is the use of adult satellite cell-derived autologous myoblasts. In the acute treatment of an infarcted area of the myocardium, satellite cells from a muscle biopsy are the only source available a few hours or days after the infarct.

Recently an autopsy at 17.5 months after the implantation of autologous skeletal myoblast around the post-infarction scar, has shown that the derived myotube-like skeletal muscle fibers are viable, and longitudinally co-aligned with the adjacent myocardium. This observation confirms the long-term positive effect on myofiber survival of the passive stretching exerted by the beating heart during myoblast proliferation and fusion. The atrophic myofibers expressed either fast (35%) or slow (32%), or co-expressed both slow and fast (33%), myosin isoforms. Proof of efficacy of skeletal myoblasts compared to other cellular lines have been recently published.

As a disadvantage, it should be remembered that the regenerated myofibers in the myocardium have no chance of being innervated by motorneurons. We would like to debate as to whether this is a prohibitively difficult biological constraint, which will hinder clinical long-term effectiveness of any skeletal myoblast injection into the myocardium, or if in the light of existing knowledge there is some hope.

Normal structural and functional properties of muscle fibers are strictly dependent upon motorneuron innervation. If denervated, skeletal muscle undergoes a rapid loss of both mass and contractile force, accompanied by a series of structural, biochemical, and physiological changes. Atrophy is especially severe when the injury involves lower motorneurons, since this atrophy is complicated by muscle fibrosis and fat substitution of dead myofibers which occurs several months after irreversible denervation.

Such mechanisms are also likely to be activated in the skeletal myoblasts implanted in the damaged heart, and could explain the non-optimal results of skeletal myoblast implantation in human hearts.

However, other researchers have shown in rat models that even after years of denervation, myogenic events continue to occur in permanent denervated muscles. Of course, if this occurs even after proliferation and differentiation of myoblasts into myocardium, the hope for long-term clinical effects would considerably increase.

Importance of the matrix
Skeletal muscle regeneration is a powerful, naturally-occurring process of tissue reconstruction following myofiber damage secondary to myotoxic injury, which does not normally affect tissue circulation and scaffold. However, many reports in the literature show that when myogenic cells are delivered after the implantation of acellular scaffolds at the site of a large portion of harvested muscle (larger than a few mm³), the final outcome is poor. This is also the case in myocardial post-ischemic scarving, which is the result of a race between fibroplasias and myogenesis, in which the blood vessels and the fibroblasts win, and the cardiomyogenic cells lose, since the cardiomyogenic cells have a very poor chance of regeneration at best.

In a recent experimental rat study, three weeks after resection of the central third of the rectus abdominis (2×8×24 mm³, thick, wide, long) and implantation of an acellular matrix seeded with autologous myoblasts, the patch could be seen to have filled with young, centrally nucleated myofibers intermixed with myotubes still expressing embryonic myosin; this occurred when both the implants and the surrounding muscles were injected with a myotoxic agent (bupivacaine), which, extending muscle damage, provides a generous source of autologous myoblasts a few days after the implants. Even an acellular homologous matrix showed a higher rate of myofiber regeneration when the surrounding muscles were injected with bupivacaine. Skeletal muscle regeneration seems to be the result of successive waves of migration of angioblasts, and then satellite cell-derived myoblasts from the muscles surrounding the implant, suggesting that in post-ischemic myocardial guided-healing, implantation of skeletal myoblast transferred with angiogenic factors, as suggested by Ye et al., has a real chance to be effective.

Conclusion
Despite the fact that further studies are required to assess the problems that were outlined above, the article by Ye et al. should be considered meritorious step forward by explaining the complex phenomena at work behind the implanted cells, such as angiogenesis, and creation of a proper matrix environment.

References