wrong could be debated, but one fact remains: thromboembolic complications have been an unusual cause of early post-operative morbidity or mortality in our experience, as shown by the 0.5% incidence that we reported in the CPB group. In an article by Clagett and associates,¹ there was no mention of specific prophylaxis for cardiac surgery. Nevertheless, we do agree on the importance of prophylaxis in OPCAB surgery. So far, we have not found any negative side effects associated with it. Since we started applying this policy to all our OPCAB patients, we have seen an obvious decrease in the incidence of thromboembolism without any increased bleeding complications or transfusion needs.

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Single fiber skeletal muscle transplantation or purified myoblast engraftment?

To the Editor:

We read with interest the article of Suzuki and colleagues,¹ "Single Fibers of Skeletal Muscle as a Novel Graft for Cell Transplantation to the Heart." They investigated the feasibility of single skeletal muscle fibers as a vehicle to deliver putative myoblasts-myotubes into the myocardium in rat models. They further demonstrated the improvement of cardiac function and histologic evidence of cell proliferation and survival after single muscle fiber engraftment into the infarcted myocardium.

This is an important study in skeletal myoblast transplantation for cardiac repair, because mechanisms for improvement of cardiac function after myoblast transplantation have not been clearly identified. Although clinical application of myoblast transplantation for cardiac repair is already

being assessed in phase I human studies,² many questions regarding the optimization of the transplantation conditions and procedure remain unanswered.³

It appears logical to consider that the number of myoblasts that repopulate the infarcted myocardium must be important. In all other experimental studies, a significantly larger number of cells were implanted. Improvement in myocardial function has been directly related to the number of cells injected which in rodents is 3 to 6 million cells and large animals as many as 1 billion cells. Suzuki and colleagues¹ in their study used only 4 single fibers per heart for injection. According to their findings, each single fiber retained 20 myoblasts, as assessed by immunostaining for M-cadherin, meaning that only 80 myoblasts were transplanted. Therefore myoblasts had to undergo extensive proliferation after transplantation to achieve an optimum cell count for beneficial effect. It seems unreasonable to expect that as few as 4 single skeletal fibers (80 myoblasts) would bring about significant improvement of heart function. From the figures shown, the extent of repopulation of the myocardium with cells is unclear, and only a few myotubules have been formed, which would be unlikely to improve cardiac function significantly in a severely damaged heart.

The disadvantages pointed out by Suzuki and colleagues¹ of using expanded

Figure 1. Xenotransplanted human skeletal myoblasts carrying *lacZ* reporter gene with nuclear localization signal in porcine heart model of chronic ischemia at 6 weeks after transplantation. Tissue sections were stained with X-gal according to standard protocol. Arrows represent donor human myoblast nuclei.

cultures of myoblast as opposed to single fibers are only theoretic. The problem of embolism from myoblast injection has never been reported, even when they are injected intra-arterially or intracoronarily.^{4,5} Similarly, in clinical perspective, delaying an elective operation on a patient in stable condition to expand the myoblast number is not a major problem. Our experience and the current literature do not indicate that repeated passaging of myoblasts leads to significant deterioration or problems.

One of the important findings in this article is the development of multinucleate branching myotubes. Because the grafted myofibers were postmitotic, it was deduced that skeletal myoblasts were the source of the newly formed fibers. In a porcine model of chronic ischemia with transient immunosuppression, we implanted *lacZ*-positive human myoblasts of greater than 95% purity. At 6 weeks we demonstrated similar formation of new branching centrally located multinucleate myotubules. Most of the nuclei stained positively for β -galactosidase (Figure 1). We believe that after myoblast transplantation neomyogenesis results in fibers with phenotypic features similar to skeletal and cardiac muscles and that this is the mechanism of improvement in cardiac function.

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Reply to the Editor:

We appreciate the interest of Sim and colleagues in our article describing the implantation of single, isolated skeletal muscle fibers into the heart. We agree that many important questions need to be answered before further clinical trials of myoblast transplantation into the heart.

A major issue is the mechanism by which skeletal myoblast transplantation can improve cardiac function. Sim and colleagues' suggestion that the improvement in myocardial function is directly related to the number of myoblasts implanted has not been firmly established. We consider the quality of cells implanted to be as important as, or perhaps even more important than, cell number.¹ In addition, only one recent study with donor cardiac myocytes has attempted to adequately quantify graft cell survival with quantitative real-time polymerase chain reaction, and no direct correlation between numbers of surviving donor-derived cells and functional improvement was made in that study.²

It should be noted that the issues regarding the adverse effects of prolonged in vitro culture on skeletal myoblasts are far from theoretical, as suggested by Sim and colleagues. There is clear evidence for the susceptibility to infection, as well as altered immunogenicity and adverse effects on myogenicity.³ With respect to

the risk of coronary embolism after cell transplantation, we have reported that infusion of more than 2 million myoblasts in a volume of 1 mL into the coronary circulation of the rat heart results in embolization, with edema and cardiac arrest.⁴

Sim and colleagues suggest that the functional benefit of myoblast transplantation is due, at least in an ischemic heart failure model, to the formation of new muscle ("neomyogenesis") with "phenotypic features similar to skeletal and cardiac muscle." This statement is not clear, and such a mechanism would require extensive functional, electrophysiological, and molecular characterization.

The relevance of the discordant human-to-pig xenogenic model cited by Sim and colleagues is not clear, and it is unlikely that a significant degree of myoblast survival would be seen after grafting with only transient immunosuppression—although the protocol for this is not detailed. We find interpretation of the staining in the figure difficult. With respect to labeling, the promoter driving reporter *lacZ* expression is not stated; thus one cannot be absolutely certain as to the identity of the stained cells in the presented figure. The varied distribution of the X-gal stain is difficult to explain, and counterstaining with skeletal myosin-specific antibody, as reported in our own data, would be informative; an additional nuclear marker would confirm the specificity of the stain.

The key findings in our study are that myogenic precursor cells derived from the parent fibers appeared to survive, proliferate, and differentiate within the cardiac microenvironment. Further, this treatment improved cardiac function in ischemic and dilated cardiomyopathy models to a degree that was far beyond what one might expect for the amount of new muscle formed. We have proposed at least two possible causes for this remarkable effect. One is a paracrine effect resulting in neovascularization, as shown in our article; the other is the endogenous protection afforded to the resident satellite cells by the parent fiber. This latter effect may be important in enhancing graft cell survival in the most hazardous acute phase after grafting and may also deliver satellite cells that have some important qualitative differences. We consider the skeletal muscle fiber system to be

promising, although further studies are needed to investigate the effects that we have proposed.

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Large cell neuroendocrine carcinoma of the lung: What we have to do

To the Editor:

I read with interest the excellent article of Takei and colleagues¹ about large cell neuroendocrine carcinoma (LCNC) of the lung. This series represents the largest ever published in English language literature.

The family of neuroendocrine tumors of the lung has recently been the object of study by several pathologists and surgeons. The recent World Health Organization classification criteria² consider neuroendocrine tumors of the lung as a distinct subset of neoplasm with specific morphologic, ultrastructural, immunohistochemical, and molecular characteristics. Travis and associates³ distinguish between typical and atypical carcinoid, LCNC, and small cell neuroendocrine carcinoma of the lung. Of these tumors, LCNCs appear to have an intermediate biologic behavior between atypical carcinoid and small cell carcinoma.³ LCNCs have a tendency to spread