International Society for Cellular Therapy
ISCT

Cytotherapy (2005) Vol. 7, No. 1, 3–15

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# BM stem cells and cardiac repair: where do we stand in 2004?

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*Adult BM stem cells are being investigated for their potential to regenerate injured tissues by a process referred to as plasticity or transdifferentiation. Although data supporting stem cell plasticity is extensive, a controversy has emerged based on findings that propose cell–cell fusion as a more appropriate interpretation for this phenomenon. A major focus of this controversy is the claim that acutely infarcted myocardium in adult hearts can be regenerated by BM stem cells. Many researchers consider the adult heart to be a post-mitotic organ, whereas others believe that a low level of cardiomyocyte renewal occurs throughout life. If renewal occurs, it may be in response to cardiac stem cell activity or to stem cells that migrate from distant tissues. Post-mortem microscopic analysis of experimentally induced myocardial infarctions in several rodent models suggests that cardiomyocyte renewal is achieved by stem cells that infiltrate the damaged tissue. For a better understanding of the possible involvement of stem cells in myocardial regeneration, it is important to develop appropriate technologies to monitor myocardial repair over time with an emphasis on large animal models. Studies on non-human primate, swine and canine models of acute myocardial infarctions would enable investigators to utilize clinical quality cell-delivery devices, track labeled donor cells after precision transplantation and utilize non-invasive imaging for functional assays over time with clinical accuracy. In addition, if stem cell plasticity is to reach the next level of acceptance, it is important to identify the environmental cues needed for stem cell trafficking and to define the genetic and cellular mechanisms that initiate transdifferentiation. Only then will it be possible to determine if, and to what extent, BM stem cells are involved in myocardial regeneration and to begin to regulate precisely tissue repair.*

**Keywords**

*infarction, ischemia, myocardial regeneration, plasticity, stem cell.*

## Goals for stem cell therapy

The anticipated goals for stem cell therapy are lofty; they purport to regulate the regeneration of tissues and organs. In the normal course of events, many tissues undergo constant regeneration and researchers have, with partial success, identified the stem cells that form the basis for this regeneration. Perhaps the best-described hierarchy of cell types related to tissue renewal comes from investigations of hematopoiesis. In fetal and adult BM, stem cells have been identified that give rise to progenitor cells and blast cells that transit through multiple levels of cell maturation and proliferation, leading to the formation of red and white blood cells and platelets. These hematopoietic stem cells (HSC) are present in BM at a ratio of approximately 1:10 000 cells. Their isolation has enabled researchers and clinicians alike to achieve a degree of control over hematopoietic tissue regeneration. Advances such as these have provided insight into normally occurring regenerative processes. Now stem cells are being investigated for a newly proposed attribute, referred to as stem cell plasticity or transdifferentiation. According to this hypothesis, stem cells from one specific tissue may differentiate into cells of a different tissue, even one whose origin is from another embryonic germ layer. The concept of stem cell plasticity has provoked an enormous amount of interest and promise, matched by a large dose of skepticism. This review is an attempt to delineate what has been achieved thus far and what needs to be accomplished in future studies to establish stem cell plasticity as a basic component of today's science and medicine.

## The controversy: stem cell plasticity versus cell fusion?

The early papers that described stem cell plasticity were received with great enthusiasm and initiated a surge in

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DOI: 10.1080/14653240510018028

research reports claiming success in generating diverse cell types from BM transplants, including skeletal myocytes [1–4], hepatocytes [5], pulmonary [6], gastrointestinal [6] and renal [7] epithelium and even cells long considered to be non-renewable, such as neurons [8,9] and cardiomyocytes [10,11]. Several of these investigations presumed to utilize the Y chromosome as a definitive marker for transdifferentiation when male donor cells were transplanted into female recipients. This was eventually deemed an inconclusive marker of plasticity because of evidence that emerged demonstrating *in vitro* fusion of female-derived embryonic stem (ES) cells with adult male BM mononuclear cells [12] and neural stem cells (NSC) [13]. The level of cell–cell fusion was less than 0.001%, with 10 clones forming per $1 \times 10^6$ BM cells, but the scientific evidence for fusion based on karyotyping and DNA content was nevertheless compelling. Monitoring for fusion and clonogenicity revealed tetraploidy, three X chromosomes and one Y chromosome, and enhanced (4 N) DNA content in individual cells within the resulting clones [12]. Subsequently, cell fusion was demonstrated *in vivo* using Cre/lox engineered β-galactosidase-positive (β-gal$^+$) donor cells to reconstitute ablated BM in transgenic mice that expressed enhanced green fluorescent protein (EGFP) [14]. β-gal$^+$ EGFP$^+$ hepatocytes, Purkinje neurons and cardiomyocytes were observed at a frequency of approximately 1:1000 cells. These initial cell-fusion investigations triggered a series of studies aimed at establishing the BM cell type involved in fusion [15,16]. Several investigators using a Cre/lox-based strategy or β-gal expression suggested a role for mature donor macrophages in cell–cell fusion with fumarylacetoacetate hydrolase-deficient (FAH$^{-/-}$) host hepatocytes [17,18]. Reports such as these challenged the concept of plasticity and prompted a re-evaluation of stem cell plasticity as an approach to regenerative medicine.

Although the controversy continues, several reports at the end of 2004 presented compelling evidence in support of stem cell plasticity [19,20]. In an *in vitro* study involving EGFP$^+$ mouse (m) NSC and human (h) endothelial cells (EC), the mNSC that normally produce neurons and glial cells were coaxed into relinquishing their role in neurogenesis while adopting a development fate leading to the formation of mEC [20]. This unexpected pattern of differentiation occurred when mNSC were co-cultured in the presence of fresh or 0.5% paraformaldehyde-fixed hEC, suggesting that cell surface contact, not fusion, was the cellular mechanism driving transdifferentiation. The mNSC-derived mEC displayed a typical mouse karyotype, EC surface antigens, EC-specific Weibel–Palade bodies and absence of neural cell markers, suggesting diminished or total loss of capacity for neural differentiation. These findings not only reaffirmed the concept of (neural) stem cell plasticity, they further challenged the theory that there is no cellular crossover of the boundaries that exist in tissues derived from different embryonic germ layers.

## Does myocardium regenerate?

The long-held dogma that defines myocardium as a static tissue lacking the capacity to renew itself is no longer considered absolute. Several reports now suggest that adult cardiomyocytes are produced throughout the lifetime of an individual [21,22], albeit at a low frequency when compared with the rate of proliferation in rapidly renewing tissues such as epithelium and BM. However, even a low rate of cardiomyocyte proliferation may account for a significant level of tissue renewal during the lifetime of an individual. Because of an intense desire by cardiologists to develop a method for repair of injured myocardium in patients with ischemic heart disease and heart failure, basic research and clinical trials are underway to identify a population of stem cells with regenerative potential.

## Skeletal myoblasts and myocytes for myocardial repair

Following injury, new myocytes arise within skeletal muscle. The cells responsible for this renewal are the satellite cells that reside on the surface of pre-existing, large, multinucleated myocytes (Figure 1). Because satellite cells are programmed to develop into myocytes, they are also referred to as skeletal muscle stem cells or myoblasts. Although cardiac satellite cells do not exist, it was reasoned that if skeletal satellite cells were transplanted into cryoinjured myocardium they might respond to local cues directing their differentiation into cardiomyocytes. However, when the fate of β-gal$^+$ canine satellite cells was determined histologically at 4–6 weeks post-transplantation, they were seen to give rise to skeletal not cardiac myocytes [23]. Although little or no evidence has been published in support of this early observation [24], it nevertheless established the concept of cellular cardiomyoplasty as a possible cure for cardiac disease. In