

## ADULT STEM CELL: A NEW THERAPY TO TREAT HEART FAILURE

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## ABSTRACT

The objective of this review article is to summarize recent data leading to and resulting from current clinical application of cellular treatment for acute myocardial infarct (heart attack) and congestive heart failure. We precisely concentrate on use of adult stem cells and match and differentiate bone marrow and adipose tissue; two unlike sources from which stem cells can be collected in significant numbers with restricted morbidity. Cellular treatment is the latest in a series of approaches applied in an effort to inhibit or diminish the progressive and otherwise permanent loss of cardiac function that often follows a heart attack. By comparing, demonstration of the capability of adult stem cells to undergo cardiomyocyte variation both in vitro and in vivo advises a potential for reformative remedy. This potential is being inspected in initial clinical readings. Usually, stem cells can be classified into embryonic or adult forms. human adult stem cells are ethically appealing and have already been used in clinical trials in a variety of disease states. Bone marrow derived stem cells, skeletal myoblasts and resident adult cardiac stem cells are being explored as potential cell types for heart failure treatment. There are a variety of various issues that need to be discussed before this technology can be applied safely, including government regulations to ensure the clinical safety and effectiveness of the procedures, as well as the prevention of improper handling or the use of contaminated tissues.

**Keywords:** Stem cells, Heart failure, Adult stem cells, Cell therapy, Bone marrow.

## INTRODUCTION

The concept of term "stem cell" arise from the that these cells have properties analogous to those of the stem of a plant. In plants, the stem may growth produce more stem, that is, more of itself, or different structures such as leaves or flowers. This elegantly illustrates the two key properties that define stem cells. Firstly, they have the capability to renovate themselves for extended phases through cell division. Secondly, under specific conditions they can differentiate into a spectrum of different cell types (Fig 1.).

Stem cells having a hierarchy capability in terms of distinguish into other cell types. This ability of stem cell is termed their differentiation "potential" (Fig 2.). In nature, the greatest ability of stem cell to differentiate into various different cell types is the zygote—the first cell of human life, a sperm cell fused with an egg cell. This cell is termed Fi "totipotent" as all cell types of the body arise from it and additionally the extra embryonic structures such as the placenta. Arises of an embryonic stem cell from subsequent division of a zygote, is termed "pluripotent" In all adult mammals adult stem cells are present are termed

"multipotent" given their ability to differentiate into different tissue types<sup>1</sup>.

Finally, the committed progenitor cell is termed "unipotent" as it is destined only to become one cell type. The different types of stem cells are classified in constantly evolving process. Broadly, primarily stem cells can be classified into embryonic or non-embryonic with the non embryonic category being subsequently divide into adult stem cells and cord blood stem cells. The advantages human embryonic stem cells haveing pluripotent and highly expandable (i.e., easily grown in ex vivo conditions), though their disadvantages include ethical objections, difficulty in isolating them, risk current therapies address this process at two main points:

- the duration of ischemia reducing by surgically or pharmacologically removing the vascular blockage; and
- by using drug therapy (ACE-inhibitors and beta blockers) to mitigate ventricular remodeling<sup>1,2</sup>.

While these approaches have had a substantial impact in the management of heart attacks there remains a considerable amount of room for improvement.

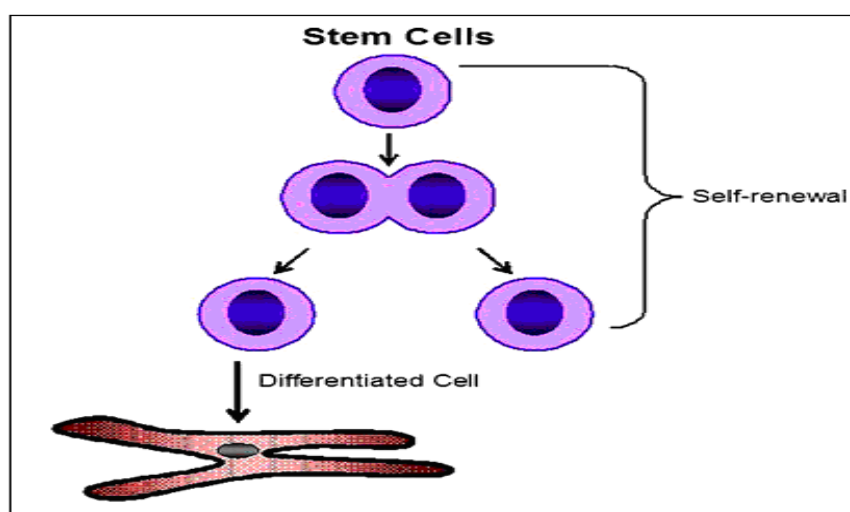


Fig. 1: Two key defining properties of stem cells<sup>1</sup>

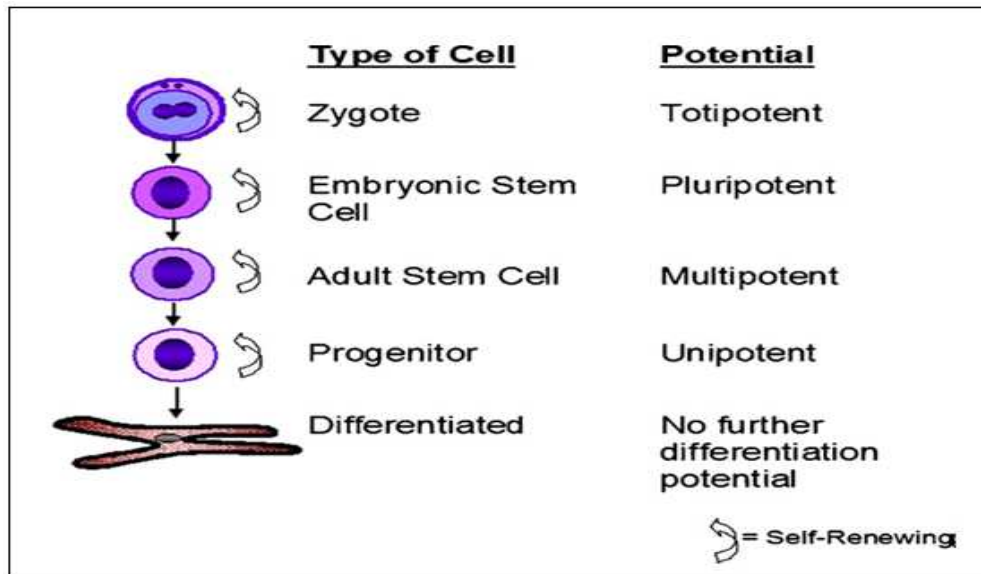


Fig. 2: Hierarchy of stem cell potency.<sup>1</sup>

The ideal therapy would have the following activities; it would minimize loss of cardiomyocytes by reducing cell death, promote return of stunned and hibernating myocardium to normal purpose, stimulate revascularization of the ischemic region by enhancing angiogenesis, and rejuvenate viable cardiomyocytes to change those lost to the primary ischemia thereby preserving contractile occupation and reducing the opportunity for scarring. Recent advances in biotechnology and in the understanding of tissue regeneration have allowed development of novel therapeutics with the potential to approximate this ideal. Recently, methods have been established for the in vitro culture of stem cells, offering exceptional prospects for learning and understanding human embryology<sup>2</sup>.

#### Stem cell research

Stem cells, regardless of origin, have the remarkable potential to extend into many dissimilar cell types in the body. When a stem cell splits, every new cell has the probable to either persist a stem cell or become another kind of cell with a extra specific task, such as the beating cells of the heart. Stem cells have potential in several regions of medical research. One probable application is to make new cells and tissues for medical therapies. Such as, donated organs and tissues are frequently used to exchange those that are diseased or destroyed. Inappropriately, the number of individuals suffering from diseases that might benefit from stem cell therapy is much greater than the number of organs and tissues available for transplantation. Stem cells suggest the probability of renewable sources of auxiliary cells and new tissues to treat many kinds of diseases, conditions, and disabilities<sup>3</sup>.

#### Cellular therapies: potential treatment for heart disease

Recent discoveries in cellular therapy research present new opportunities for cellular products to be used in disease areas with critical, unmet medical needs. In the USA, the Food and Drug Administration (FDA) regulates cellular therapies to ensure that they are safe and effective, and that persons enrolled in clinical trials using cellular products are protected from undue risk. Cellular products hold great potential for use in treating damaged or diseased tissues of the body. Cellular products come from different sources, for example stem cells from bone marrow and peripheral blood, and myoblasts from skeletal muscle cells<sup>3</sup>. There is still much to learn about how cellular products work, how to administer them safely, and whether, over time, the cells will continue to work properly in the body without harmful side effects. The FDA is committed to supporting cellular therapy research and development, and future licensure of cellular products, and wants the public in the USA to understand both the promise and the challenges presented by these exciting new modes of therapy.

#### Bone marrow derived stem cells

One of the first discovered, and certainly the best understood, types of adult human stem cells are those found in the bone marrow. These stem cells, also known as haemopoietic stem cells, were found to be liable for the continuous production and replenishment of RBCs, WBCs and platelets. Within the population of cells found in bone marrow, the cell type that has generated most interest, in terms of application to cardiovascular disease, are haemopoietic stem cells which have been found to be precursors of endothelial cells<sup>4</sup>.

It is thought these cells originate in the bone marrow and are subsequently released into the bloodstream. Increased blood levels of the cells have been found following acute myocardial infarction<sup>5-7</sup>. Strategies to augment numbers or function of EPCs have been targeted at mobilizing greater numbers from the bone marrow using agents for example granulocyte colony exciting factor (G-CSF)<sup>8</sup> granulocyte-macrophage colony exciting factor (GM-CSF)<sup>9</sup> erythropoietin (EPO)<sup>10</sup> and vascular endothelial growth factor (VEGF)<sup>11</sup>. In terms of improving EPC function, other than statins, p38 mitogen-activated protein (MAP) kinase inhibitors and nitric oxide enhancers have been propose<sup>12</sup>. Another type of human adult stem cell found in bone marrow is the mesenchymal precursor cell (MPC). This cell is, as suggested by its name, the precursor cell of mesenchymal stem cells. MPCs exist in the bone marrow in extremely low numbers (around less than 0.01% of all bone marrow cells), however, once isolated they can be grown and stored easily<sup>13</sup>. These cells, being stem cells of mesenchymal tissue, can differentiate into bone, cartilage, fat or muscle cells depending on their environmental stimuli. At least one research group has published data to indicate they were able to direct MPCs to differentiate into cardiomyocytes.

#### Cloning and therapeutic potentials

Recently, it has become possible to successfully culture embryonic cells from 5 to 6 days old human pre implantation embryos (blastocysts) and primordial germ cells from 5 to 9 weeks old human embryos in suitable nutritional media<sup>14</sup>. Embryonic stem (ES) cells and primordial germ (PG) cells remain diploid and conserve their embryonic and proliferative features over several cell divisions in culture. ES cells are resulting from the inner cell mass (ICM) of cultured human blastocysts formed by in vitro fertilization for clinical needs in aided reproduction and donated by patients. Therapeutic cloning may be performed by utilizing donated pre implantation embryos from in vitro fertilization (IVF) and intra cytoplasm in sperm injection (ICSI) programs (Fig 3.) or by creating embryos via nuclear transfer from patients' somatic cells into enucleated oocytes (Fig 4.).



Fig. 3: Preimplantation-stage embryos from IVF and ICSI programs in assisted reproductive technologies (ART)<sup>16</sup>

Preimplantation-stage embryos from IVF and ICSI programs in assisted reproductive technologies (ART) may be used for isolation, selection, and expansion of embryonic stem cells resulting from the inner cell mass (ICM) of blastocysts cultured in vitro and that may serve for heterologous transplantation therapy (Fig.3). When human ES cells were transplanted into immune-suppressed SCID-mice, they developed teratomas that contained a variety of tissue derivations from all three germ layers. These teratomas included gut epithelium, cartilage, bone, smooth and striated muscle, neural and stratified squamous epithelium, and enclosed clusters of undifferentiated stem cells, so-called

embryonal carcinoma (EC) cells. In mice, teratomas are composed of a comparable variety of differentiated tissues and may also include EC cells. When these EC cells were transplanted individually into recipient mouse blastocysts, they populated clonally the developing embryos and participated normally to fetal growth giving rise to healthy chimeric mice<sup>15</sup>. Biopsied tissue from patients may be cultured in vitro for somatic donor cells used in nuclear transfers into enucleated recipient oocytes. The resulting cloned blastocysts genetically derived from the respective nuclear cell donor may be taken for embryonic stem cell culture and autologous transplantation therapy (Fig.4).

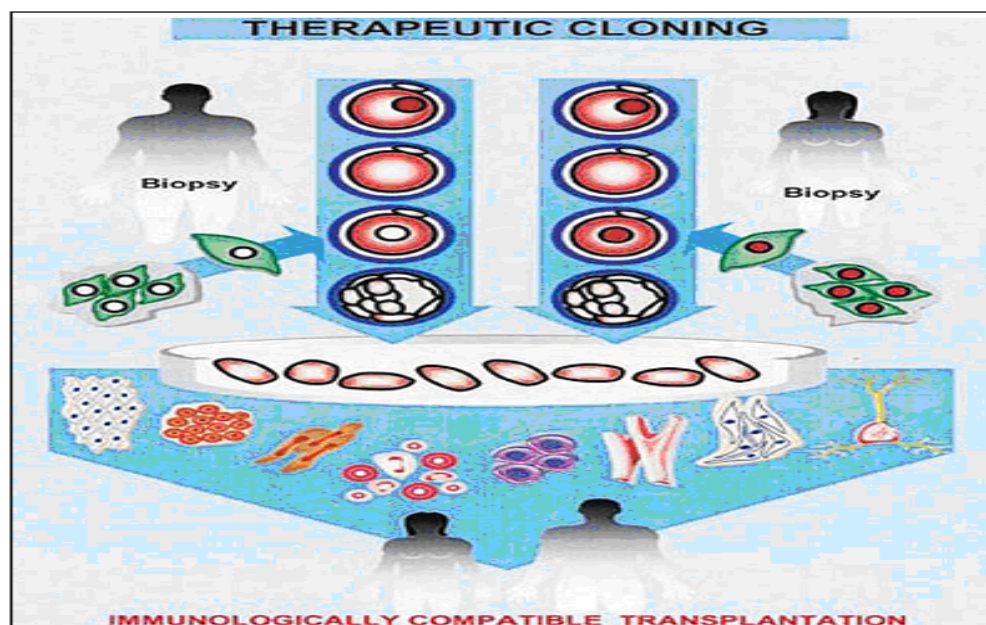


Fig. 4: Immunologically compatible transplantation of biopsied tissue.

#### Skeletal myoblasts<sup>16</sup>

Skeletal myoblasts, also known as satellite cells, are precursor cells of skeletal muscle. They are found between the basement membrane and sarcolemma of skeletal muscle fibres. Skeletal myoblasts can be easily obtained by muscle biopsy from adults, however, take some

weeks to grow to sufficient quantities for therapeutic use<sup>16</sup>. Skeletal myoblasts have been used in humans to treat ischaemic cardiomyopathy in a number of clinical trials<sup>17,18,19</sup>.

A major concern that emerged during these studies was an increased incidence of ventricular tachy arrhythmias including

episodes of ventricular fibrillation. The problem of arrhythmias is thought to be related to the inherently different electrical properties of skeletal myoblasts compared to cardiac muscle cells<sup>20,21</sup>. The safety concern of arrhythmias with skeletal myoblast treatment resulted in investigators of the most recent trial of skeletal myoblast treatment requiring all trial patients to have implantable cardioverterdefibrillators (ICDs) and strongly recommending prophylactic amiodarone therapy starting at the time of muscle biopsy and continuing for 3 months after skeletal myoblast treatment<sup>22</sup>.

### Cellular treatment to treat heart disease

Although many latest advances in medical therapy and interventional techniques, ischemic heart disease and congestive heart failure (CHF) remain the major causes of morbidity and mortality in the United States<sup>23</sup>. Cellular therapy for treating these and other heart conditions is a growing field of clinical investigation. Potential cell managements for patients with congestive heart failure (CHF) and ischemic heart disease are of great interest to medical researchers and treating physicians. Research to date has involved cells from autologous (donors who are also the recipients of the cellular therapy) skeletal muscle (myoplasts), hematopoietic stem cells from autologous peripheral blood, and unspecialized mesenchymal or hematopoietic stem cells from bone marrow. They have been administered through catheters into the coronary arteries, transcatheterially through injection catheters into the left ventricular myocardium, or transeptically through a needle during coronary artery bypass graft<sup>23</sup>.

### Adult stem cell therapy

A number of studies have suggested that the plasticity of adult stem cells is such that they can differentiate into cardiomyocytes. Thus, Orlic's group have published a number of studies showing expression of cardiomyocyte genes in donor bone marrow stem cell-derived cells in animal models of myocardial infarct<sup>24-28</sup>. In these studies, the investigators evaluated the distribution and phenotype of male bone marrow-derived mouse cells expressing green fluorescent protein (GFP) after injection into infarcted female mouse hearts. Nine days after treatment, roughly 50% of myocytes, endothelial cells, and smooth muscle cells developing within the infarcted portion of the heart were donor derived, as evidenced by GFP staining and detection of the Y chromosome localized within the same cells as cardiac myosin heavy chain or sarcomeric actin, factor VIII, and smooth muscle respectively. Cardiomyocyte function was confirmed by connexin-43 staining, which was localized to the cytoplasm and surface of adjacently aligned cells. Similar data with bone marrow cells has been obtained by<sup>29</sup> in representing the skill of a fraction of cells greatly improved for hematopoietic stem cells to engraft injured heart and to express cardiomyocytic markers. Other investigators have demonstrated the ability of bone marrow-derived mesenchymal stem cells (MSC) to acquire a cardiomyocyte phenotype<sup>30,31</sup>.

Four days after treatment, MSC were evenly dispersed throughout the left ventricle with retention of approximately 0.44% of the total cell dose (0.5×10<sup>6</sup>) cells. Galactosidase producing cells were morphologically similar to cardiomyocytes and had aligned with host cardiomyocytes by 14 days after treatment. Between 14 and 60 days post-treatment, donor cells expressed several markers of cardiomyocytes, as evidenced by co-detection of galactosidase with cardiac troponin, and phospholamban, a regulator of cardiac sarcoplasmic reticulum Ca<sup>2+</sup> ATPase.

A number of investigators have sought to address these issues by *ex vivo* expansion of bone marrow derived stem cells applied in either the autologous or allogeneic setting. Use of autologous culture-derived cells requires delayed administration of cells in order to grow a sufficient quantity of cells<sup>32,33</sup> while allogeneic cells are associated with risk of rejection and transmission of infectious disease. Interestingly, marrow-derived stem cells appear to enjoy a degree of immune privilege and their use may require minimal need for immunosuppressive drugs<sup>34,35</sup>. Use of cultured cells is also associated with significantly altered capacity for homing<sup>36</sup>. However, homing defects can be addressed by direct injection of cells into injured myocardium and, although this may be associated with

increased risk of perforation<sup>37</sup> this risk may be mitigated by technique and mechanism of delivery<sup>38</sup>.

### Alternate adult stem cell sources

While bone marrow is the most widely studied stem cell source, it is by no means the only source, as multipotent cells with extensive self-renewal capacity have been described in adipose tissue<sup>39</sup> skin<sup>40</sup> blood vessels<sup>41</sup> and muscle and brain<sup>42</sup>. Adipose tissue is the only one of these alternatives that allows extraction of a large volume of tissue with limited morbidity. For this reason, we have focused our attention on this source.

### Adipose tissue-derived stem cells

In 2001, investigators from the University of California at Los Angeles and the University of Pittsburgh demonstrated that a population of cells derived from collagenase-digested human adipose tissue could be induced to differentiate into multiple cell lineages including adipose, cartilage, and bone. Subsequent work by members of this group and others have confirmed and extended this work demonstrating a capacity for myogenic<sup>43,44</sup> neuronal<sup>45</sup>, and even cardiomyocytic<sup>46</sup> differentiation. Most importantly, the Hedrick laboratory has used clonal analysis to demonstrate that individual adipose tissue-derived cells possess multilineage potential and extensive self-renewal capacity<sup>47</sup>. We have now extracted adipose tissue-derived stem cells from multiple species, including rats, rabbits, cats, dogs, pigs and, as described above, from humans. While the common pattern of mesenchymal lineage distinction of adipose and marrow-derived cells is very similar, some differences have been observed. Both cell populations expressed definite for collagen type I, osteocalcin, osteonectin, osteopontin, BMP-2, parathyroid hormone receptor, retinoic acid receptor  $\alpha$  (RXR), Vitamin D and CBFA-1, a transcription factor that regulates multiple osteogenic genes<sup>47</sup>. Results in our laboratories also indicate very similar *in vitro* chondrogenesis from both adipose and marrow-derived cells with micromass culture of cells from both depositing a proteoglycan rich, collagen type II containing extracellular matrix with chondroitin-4-sulfate and keratin sulfate, two predominant glycosaminoglycans expressed in cartilage proteoglycan<sup>48</sup>. However, Winter et al. have published data suggesting that *in vitro* differentiation of adipose tissue-derived cells in three dimensional matrices is less than that observed with marrow-derived cells<sup>49</sup>.

### Modes of stem cell delivery to the heart

A variety of methods of stem cell delivery to the heart have been used in clinical studies to date. The earliest human work most frequently used direct intra-myocardial injection during coronary artery bypass grafting operations<sup>50</sup>. Subsequently, less invasive approaches evolved and have been favored. Such approaches include intracoronary delivery via coronary artery catheters<sup>51</sup> retrograde coronary sinus delivery<sup>52</sup> and intra-myocardial delivery via electromechanical mapping catheters modified with a stiletto needle<sup>53</sup>. An altogether different strategy has been to organize stem cells from the bone marrow inside the patient. This is achieved by the administration of subcutaneous granulocyte colony stimulating factor (G-CSF)<sup>54</sup>.

### Clinical trials of adult stem cell for the treatment of myocardial infarction in humans

Randomized control trials have been performed using bone marrow-derived stem cells as a therapy post myocardial infarction. Initially, these trials again showed consistently favorable results, with regard to left ventricular function, however, more recent trials have produced conflicting data. Of note, in the positive studies, irrespective of cell type or delivery method, a similar magnitude of improvement in left ventricular ejection fraction (LVEF) was seen. This improvement is in the order of a 4–5% increase in LVEF. In meta-analyses, this benefit of stem cell therapy holds, except for G-CSF<sup>55</sup>, even when negative trials are included<sup>56</sup>. Important caveats with respect to the meta-analyses are the multiple differences between the individual trials in terms of (a) the stem cell type, quantity and viability, (b) the route of stem cell delivery, (c) the timing of stem cell delivery and (d) the clinical trial design. The benefits seen in human clinical trials of adult stem cell therapy for

myocardial infarction are consistent with an improvement in cardiac function equal to that of current pharmacological therapies<sup>57-59</sup>. As discussed earlier in this paper, the ultimate goal of stem cell therapy is to achieve tissue regeneration and repair. Both the relatively small magnitude of improvement in LVEF, and the lack of restoration to normal cardiac function, indicate that this goal is not being achieved at present.

#### Problems with adult stem cell therapy for heart failure

In any new therapy, the benefits must always be weighed against the potential risks. To date, adult stem cell treatment for the management of heart failure has had a good safety record with most studies reporting no difference in adverse event rates between the stem cell treatment groups and controls. However, as mentioned earlier, a notable exception to this was the increased incidence of ventricular tachyarrhythmias in clinical trials using skeletal myoblasts. Problems with the effectiveness of stem cell delivery techniques have also become evident. One of the first papers documenting early distribution of bone marrow mononuclear cells after intra-coronary delivery demonstrated that, at 18 h post-delivery, only 1% of the injected stem cells remained in the heart. Ninety-nine percent of the stem cells had passed through the coronary vascular bed and back out into the systemic circulation with most of them finally ending up in the spleen<sup>60</sup>. Clearly an optimal response to stem cell treatment cannot be expected if the delivered cells do not reach their targeted site of intended action. Consequently, many stem cell research groups are developing methods to track stem cells once delivered. Labeling of stem cells with iron has allowed magnetic resonance imaging of their location in vivo following stem cell treatment<sup>61</sup>. Results of similar future research will be pivotal in understanding and fine-tuning stem cell delivery techniques. There was a quick release of large dose followed by non-uniform release<sup>62</sup>.

#### CONCLUSION

There are a number of facts in terms of the use of adult stem cells to treat heart failure. Adult stem cells do exist and are able to be isolated and grown. Multiple clinical trials have demonstrated the feasibility of adult stem cell treatment for impaired left ventricular function. Furthermore, stem cell treatment in this setting has generally had a promising safety profile. Efficacy has been found to be additive to, and of similar magnitude to, current drug therapy. Ongoing randomized control trials are in progress and assure further results for critical evaluation and determining future research directions. What remains fiction at present is the complete regeneration and repair of diseased coronary artery and/or myocardial tissue. In order to make the shift from fiction to fact, a more comprehensive understanding of the fundamental processes of regenerative physiology at the "benchside" will be paramount combined with ongoing investigations at the bedside.

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#### Declaration of interest

The authors report no financial or non financial conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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