

## Myocardial Regeneration in Health and Disease

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Myocardial infarction (MI) is one of the leading causes of death in modern world and its incidence is rising throughout the world. About 900,000 people have MI each year in USA.<sup>1</sup> In UK, mortality rate from MI is around 200 per 100,000 deaths per year.<sup>2</sup> In Pakistan one study indicated a prevalence of 1.5%.<sup>3</sup> Ischemic heart disease has become a modern epidemic.<sup>4</sup>

The purpose of this review is to give an introduction to the modern views about cardiac regeneration, different modalities available for that and their future prospective at clinical levels.

It is certain that for a specie to have a long life span some features are essentials, one of which is the renewal of the body tissues in response to the insults that occur with time.<sup>5</sup> Until recently, heart was considered as terminally differentiated organ, but now it has been proven that regeneration and renewal of myocardium does occur. First, because there exists a basal rate of disappearance of cardiac myocytes (through apoptosis) and it has been calculated that even at the basal rates the whole myocardium can disappear in a few decades, which in reality does not occur.<sup>6</sup> Secondly, an increase in size of heart as a person grows cannot merely be explained on the basis of cellular hypertrophy, an increase in the number of cardiac myocytes also occurs.<sup>7</sup> Evidences from different studies have shown that myocardial regeneration is not only a response to a pathological insult but it also occurs in normal hearts; rather there exists a homeostasis between cell death and renewal in the myocardium.<sup>8</sup>

### Myocardial Regeneration and Renewal in Normal Hearts

As it has been mentioned before that even at basal rates of cardiac myocytes apoptosis whole myocardium would have disappeared in a few decades in a normal heart and in a few years in a failing heart unless regeneration and renewal co-exists to replace the lost cells. Now we have many evidences form various studies about the existence of regenerating cardiomyocytes. Ki-67, a marker of cell division,<sup>9</sup> has been identified in some cardiac myocytes of normal hearts.<sup>10</sup> Mitotic indices have been calculated in

normal and pathological hearts. In normal heart there exists a mitotic index of 14 per  $10^6$  cardiomyocytes.<sup>11</sup> In a normal adult male there exist almost  $5.8 \times 10^9$  cells in left ventricle suggesting a total of 8120 cells dividing in the entire ventricle at a time. Most cells complete mitosis in an hour time, indicating generation of  $0.7110^9$  cardiac myocytes in a normal ventricle in a year<sup>11</sup>. It has been found through various studies that there exists a balance or homeostasis between cardiac myocytes death and renewal. Evidence of myocardial regenerative ability has been further augmented by the identification of a population of cells in the adult human brain which has the ability to regenerate and replace the neurons,<sup>12</sup> which were also considered a post-mitotic organ. Question arises that if cardiomyocytes possess the regenerative ability then why do people die of cardiac failure and MI? This can be explained on the basis of the critical importance of the organ, regeneration is a slow process but continuity of blood flow to the body is essential for life. Furthermore regenerative capacity becomes weak in failing and aged hearts.

### Myocardial Regeneration in Diseased Hearts

A study conducted by Bertrami et al 2001 demonstrated that under stress of ischemia or heart failure, the proliferation rate of cardiac myocytes increases tremendously.<sup>13</sup> Rewarding or not an augmented proliferation does occur in heart following MI.<sup>14</sup> Immunohistochemical studies were done for the presence of Ki-67 protein in the nucleoli of hearts of patients who died few days after a major MI and compared with hearts of persons dying from non-cardiac causes (taken as controls). It was found that cells exhibiting Ki-67 protein were 84 times more in number in hearts of patients of MI as compared to those of normal hearts. Moreover numbers of visible dividing cells were 70 times more in the myocardium around the infarcted area. Many studies demonstrated that along with hypertrophy, hyperplasia of cardiac myocytes also play role in ventricular remodeling in ischemic and failing hearts.<sup>14,20</sup> An absolute increase in the number of myocytes has been documented in the hearts with

pressure or volume overload.<sup>21</sup>

## **Origin of Cells Responsible for Myocardial Renewal in Normal and Diseased Hearts**

Having many hopeful evidences that mitotically active cells are present in myocardium with an augmented response in a pathological state, the next question of concern was about the origin of these cells and this is still a matter of controversy. If we imagine the possible sources of these cells then these can be of either cardiac origin or non-cardiac origin. It means that there may be a reserve of cardiac stem-like cells in the heart itself or cardiac myocytes reenter into cell cycle,<sup>5</sup> or these cells may come from non cardiac source i.e. bone marrow (BM).<sup>22</sup> Experiments have also shown that stem-like cells taken from other organs if injected into the infarct, reconstitute the myocardium to some extent.<sup>23,24,25</sup> Present day studies are focused on three types of cells, skeletal myoblasts (SkM); bone marrow derived hematopoietic stem cells (BMHSC) and stem cells of cardiac origin.

### **1. Skeletal Myoblast Cells (SkM)**

Following injury to muscle, these cells enter into cell cycle, proliferate and repair the damage by producing adult muscle cells.<sup>26</sup> It was found through many trials that SkM if injected into the infarcted area along with coronary artery bypass graft surgery (CABG) would improve the ventricular output considerably if compared to output that comes from CABG alone.<sup>27,28</sup> Ejection fraction (EF) improvement was 23.8–32.1 % more than usual. Later autopsy studies from these patients showed that myocytes derived from injected SkM cells showed some features similar to cardiac myocytes like expression of slow isomer of myosin heavy chain (MHC) in around 65 % of these cells.<sup>29</sup> Slow isomer of MHC is characteristic to cardiac muscle while fast isomer is characteristic to skeletal muscle. However immunohistochemistry revealed that they were negative for cardiac specific connexin-43, desmosomes and cadherins and there were no connections between them and the rest of the myocardium so they were not electrochemically coupled, contracting on their own in the fibrous tissue of infarct.<sup>30</sup>

### **2. Bone Marrow Derived Hematopoietic Stem Cells (BMHSC)**

BMHSC have extensive capability of self renewal as well as differentiating into many lineages.<sup>31</sup> Indeed BMHSC have the ability to re-synthesize active

marrow after it has been completely destroyed through irradiation. Many studies have indicated that mobilization of BMHSC occurs following MI to the region of infarct.<sup>22,32,34</sup> Patrick et al<sup>35</sup> (2002) showed the existence of Y-chromosome positive (Y-Ch+) cardiomyocytes in a male who was transplanted with a female heart (sex mismatched transplantation), he said probably the Y-Ch+ cardiomyocytes in female heart came from the BMHSC of the recipient bone marrow. It was questioned that these cells might also derive from the remnants of tissues at atria and major blood vessels where heart was fused. Arjum et al confirmed that these were derived from bone marrow<sup>32</sup>. They confirmed the existence of Y-Ch+ cardiomyocytes in a female patient who was given a sex mismatched bone marrow transplantation after a complete marrow destruction. Fluorescence in situ hybridization (FISH) technique was used to identify Y-chromosome and  $\alpha$ -sarcomeric actin to specify that they were cardiac myocytes.<sup>36</sup>

Having confirmed that BMHSC play a vital role in myocardial regeneration; the next question was to identify the subtype of BMHSC which was the center of all the game. BMHSC have various subpopulations like bone marrow mononuclear cells (BMMNC), mesenchymal stem cells, c-kit+ cells, sac+1 cells, endothelial progenitor cells, etc. Of all of them; the transplantation of BMHSC yielded significantly rapid and better improvement of left ventricular output.<sup>37</sup>

### **3. Cardiac Stem-Like Cells**

Some studies have shown that adult myocardium possesses a population of cells that have the ability to differentiate into mature cardiac myocytes, called cardiac stem cells.<sup>38,39</sup> It was found that certain growth factor such as hepatocyte growth factor (HGF) and insulin like growth factor 1 (IGF-1) can stimulate their proliferation, differentiation and mobilization to the site of infarct.

Having discussed various types of cells which are documented to take part in myocardial regeneration it may be emphasized that not a single type of cell rather all these cells and others that are still not discovered may be playing a role simultaneously.

## **Current Clinical Trials on Myocardial Regeneration**

Currently two types of cells have been tried clinically; they are SkM cells and BMHSC.<sup>40</sup> Usage of SkM cells in patients following MI was first done by Marelli et al (1992) in a dog heart.<sup>41</sup> Further studies confirmed improvement of ejection fraction (EF) and cardiac

output (COP) with this procedure.<sup>42</sup> Menasche et al (2003) in their study reported first case in which SkM cells were injected in a human along with CABG with fruitful outcomes<sup>43</sup>.

Although autologous SkM transplantation is advantageous in the way that problems of availability, immunosuppression and graft rejection are not there but SkM has some drawbacks;

- (i) Duration of few weeks is required before the cells can be injected because of the fact that SkM cells taken through biopsy are first cultured in the laboratory to increase their number.<sup>40</sup>
- (ii) They have to be injected at the site of infarct intramyocardially while in contrast BMHSC can be given intracoronary and even intravenously.
- (iii) The muscle tissue that develops from the injected SkM cells lacks the electro-mechanical connection with the rest of the myocardium through gap junctions and therefore contract independently.
- (iv) Capillary density remains low as SkM cells can only transform into muscle cells, in contrast BMHSC have the ability to transform into endothelial cells as well yielding good capillary density.
- (v) There is an initial phase of rapid and extensive loss of injected SkM cells because of poor blood supply of the affected area and this at

time results into failure of transplantation.

The differentiating capacity of BMHSC is much more than SkM cells and they also possess many other advantages, e.g. they have the ability to differentiate into cardiomyocytes, they fuse with the surrounding myocardium and also improve angiogenesis due to their ability to differentiate into endothelial cells.<sup>44, 45, 46</sup>

They have an added advantage that they can be administered locally through injection<sup>47, 48</sup> as well as intracoronary or intravenously along with administration of their specific stimulating factors.<sup>47</sup>

### Future Prospective and Conclusion

The aim of stem cell therapy in MI patients is to reduce morbidity as well as mortality. As we have discussed earlier that localized injection of stem cells into the infarcted area is not always fruitful in contrast to intracoronary administration of BMHSC together with the infusion of specific growth and differentiation factors. One of the critical aspect is the availability of stem cells in sufficient number so that they should be available as early a possible following MI. this can be accomplished through establishing stem cells banks and clinics and storing a culture of stem cells there for every person.

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