



## AMERICAN JOURNAL OF PHARMTECH RESEARCH

### Stay Your Heart Young Using Regenerative Strategy

**Dalvi Apeksha M\*, Patel Salim G, Hande Swapnali D.**

*Vishal Institute Of Pharmaceutical Education And Research Ale. Pin 4124111, Tal-Junnar Pune.  
Savitribai Phule Pune University, India.*

#### ABSTRACT

Adult mammals undergo minimal regeneration having cardiac injury or ongoing burden of heart failure. Recent studies have established the importance of several evolutionary conserved mechanism of heart renovation by cyclic division of differentiated cardiomyocyte, cardiomyocyte proliferation, angiogenesis, extracellular matrix and heart regeneration, tissue engineering, transcription profile of cardiomyocytein development and regeneration, stem cell derived cardiomyocyte, and there transplantation. The c-kit-expressing cardiomyocyte stem cell also provide. The DNA translation is also occurs in regeneration process. In the review is part of special issue related to continuous renovation on cardiomyocyte and there related mechanism involved and gives future concept about novel biotechnology and tissue engineering.

**Keywords:** Cardio cell cardiomyocyte, differentiation and proliferation, regeneration, transplantation, tissue engineering.

\*Corresponding Author Email: [apekshamdalvi@gmail.com](mailto:apekshamdalvi@gmail.com)

Received 10 April 2019, Accepted 25 April 2019

Please cite this article as: Dalvi AM *et al.*, Stay Your Heart Young Using Regenerative Strategy. American Journal of PharmTech Research 2019.

## INTRODUCTION

Most studies agree that the adult heart continues to renew cardiocytes even after neonatal period . cardiocytes can be generated by self-duplication and by cardiac stem cells. Both modes of cardiomyocyte renewal have been proposed at different ages and even after cardiac injury. However, the magnitude of myocytes turnover in homeostasis and disease has been heavily debated (1).

In this review, we will provide renewal of cardiomyocyte, with focus human cardiac system, cardiac regeneration approach, basic mechanism differentiation and proliferation. Cardiomyocytes in the adult human heart appear to turn over at a very low rate, estimated at 0.5-1% per year.

### **Cardiac Cardiomyocyte turnover-**

The human heart has the regenerative capacity to renew completely within 5 years or even more rapidly after cardiac infarction(2).

One argument for the high turnover of myocyte has been the detection of apoptotic and necrotic myocyte(3).

Indeed, cell death has been found in cardiac pathologies and in healthy myocardium(4).

The TUNEL technique ,which detect the apoptosis by identifying DNA nicks, is not solely specific for programmed cell death and MGH also label cells undergoing DNA repair(5).

Now ,a days cardiomyocyte renewal is easy to establish. The most common tool to study proliferation have been immune histochemical marker of proliferation such as Ki67 or the mitotic marker phosphate histone 3 (pH 3) .However, because only a short glimpse of the proliferating cells can be achieved, established turnover dynamics in cell populations is problematic .Whether only a small subpopulations of cells renews and the majority of cells are remains unknown. This would cause a dramatic overestimation of the overall cell turnover. Another shortcoming of this strategy is that cardiomyocytes derived from a stem cell population would not be detected or the number would be under estimated because only cycling myocytes that already expressed a myocyte lineage commitment would be identified. A birth marker that is incorporated into CSCs or duplicating cardiomyocytes is required to chase newborn myocyte and establish their number and survival in the human heart(6).

Human myocytes shows extensive polyploidy during growth and in disease. This process complicates the use of IdU labelling when ploidy levels are not taken into consideration(7).

<sup>14</sup>C retrospective dating is a new technology to overcome limitation in measuring cardiomyocyte renewal in human hearts(8).

This strategy is based on the incorporation of nuclear test bomb-derived  $^{14}\text{C}$  into genomic DNA ; therefore ,it provide a cumulative measure for cellular turnover that is different from immunohistochemical strategies (9).

To determine the myocardial turnover, the correct identification of cardiomyocytes and/or cardiomyocyte nuclei is critical and has been challenging(10).Because most archived heart tissue is only available frozen ,an isolation strategy based on the cellular level is not feasible.

### **Ploidy and Multinucleation of Cardiomyocytes**

A stringent analysis of myocyte renewal that accounts for the establishing the magnitude of myocyte proliferation, particularly when studying diseased hearts. Because  $^{14}\text{C}$  birth dating of diploid cardiomyocytes or by mathematical correction for measured ploidy, it is also possible to investigate renewal in heart diseases, in which the ploidy levels are higher than in healthy hearts(11).

### **CSCs as a source of Adult Cardiomyocytes-**

One decade has passed since Beltrami and co-workers provided evidence for the existence for c-kit-expressing cardiac stem cells (CSCs)(12).

The presence of cells that express c-kit ,stem cells factor receptor, in the absence of any hematopoietic lineage markers, may allowed for the generation of all major lineages in the heart, including cardiomyocytes, endothelial cells and mesenchymal cells in vitro and in vivo(13) .

Several other putative CSCs in adult heart have been report(14).

The proliferation and survival of hematopoietic stem cells, germs cells and other lineages is dependent on c-kit activation by binding to its ligand the stem cell factor (SCF).The role of residing c-kit-expressing cells in the postnatal and adult heart is, however, much more controversial.

In adult hearts, the number of c-kit-expressing cells decreases dramatically, and EGFP (c-kit) expression is restricted to endothelial cells and smooth muscles cells, suggesting that they have a role as vascular progenitor cells(15).

A recent study, however, suggested that c-kit-positive cells in the adult heart were capable of regenerating cardiomyocytes after diffuse myocardial damage with isoproterenol(16).

The novel aspect in this was direct evidence with a fate mapping strategy that c-kit-positive cells sow in vivo stem cell potential.

Patients with post-infaraction left ventricular dysfunction received autologous CSCs through their coronary arteries. Preliminary Data suggest improved clinical parameters and reduced scar size.

Paracrine effects on myocyte survival and angiogenesis and a direct contribution to the endothelial

lineage should be considered, similar to what has been proposed, as a mode of action in the bone marrow mononuclear cell infusion trials(17).

#### **Adult cardiomyocyte regeneration-**

General high regenerative competence within its adult individuals, has been studied at the single cell level. Culturing isolated cardiomyocytes revealed that only 1/3 of the cardiomyocytes enter the cell cycle and mitosis, suggesting that even in a highly regenerative species, such as the new, the myocardium is heterogeneous(18).

#### **Mechanism-**

If cardiomyocytes are the source of adult regeneration, then the mechanism for cell cycle re-entry is unknown. The molecular profiling of macrophages suggested that secretion of pro-angiogenic cytokines may be responsible for their important role in cardiac regeneration (19).

#### **Angiogenesis and Heart Regeneration -**

Gene expression and genetic analyses have established regulators of angiogenesis that are essential for the response of cardiac injury. FGF receptor expression in the epicardium and FGF ligand expression in the myocardium appear to be required for the formation of new vasculature and this process is thought to regulate epithelial to mesenchymal transition (EMT) of epicardium in order to form coronary vasculature (20).

#### **Extracellular Matrix and Heart Regeneration-**

During the normal cardiac development, signaling from the ECM provides structure and guidance for cellular migration, proliferation and differentiation.

ECM components secreted from embryonic fibroblasts include fibronectin, collagen, heparin binding EGF-like growth factor, and these factors can promote cardiomyocytes proliferation in a paracrine fashion (21).

Therefore, it is possible that postnatal changes to ECM composition alter the proliferative capacity of cardiomyocytes.

Controversy arises in the field when genetic manipulation of periostin did not alter cell-cycle activity, cardiomyocyte content, or cardiac repair (22).

#### **Stem cell-derived cardiomyocyte and their transplantation-**

Stem cell derived cardiomyocyte mature to a limited extent in culture. When transplanted, they lack the anatomy and physiology of adult ventricular cells, and thus they must grow and mature quickly in vivo upon leaving the culture dish (23,24).

#### **Future concepts-**

The main issue of current pharmacological , interventional or operative therapies is their disability to compensate the irreversible loss of functional cardiomyocytes.

Hence, the future challenge of cardiovascular therapies will be the functional regeneration of myocardial contractility by novel concepts, such as cell based therapies, tissue engineering or reprogramming of scar fibroblast.

Novel cells sources for cardiac regeneration with “True” regenerative properties. Embryonic stem cells are undifferentiated cells obtained from the inner cells mass of blastocyst revealing unlimited self-renewal capacity and pluripotency (25). They have the potential to develop into derivatives of all three germ layers-endoderm, mesoderm, ectoderm (26). These properties made ESCs particularly interesting for cardiac regeneration. Transplantation of cells with “true” regenerative potential.

The efficacy of ESC transplantation after myocardial infarction has early been demonstrated in studies. So far, ESCs have not been used clinically since, aside from ethical and political concerns, it would be immunologically incompatible to the patient and reduce risk (27) .

#### **Direct reprogramming-**

Direct reprogramming approaches may vary due to the different choice of mouse models and evaluations methods of cardiac phenotypes (28).Hence, all approaches had significant issues with efficacy that have to be overcome before a therapeutically application becomes feasible.

#### **Tissue engineering-**

Tissue engineering is actually an extension of cell transplantation in combination with a variety of scaffolds as one is more and more aware that the three dimensional micro equivalent plays an important role in cell differentiation and especially maturation(29).

Current tissue engineering techniques have mainly been tested for functional improvements in animal models, but whether these approaches can be transferred to large animal models or even to human patients remain elusive (30).

#### **Stimulation of endogenous cardiac repair-**

It has been demonstrated that the neonatal murine heart holds a remarkable regenerative capacity(31).Apical excision resulted in a total recovery of removed tissue by myocyte proliferation without scarring(32).The stimulation of cell-cycle reentry of pre existing cardiomyocyte might therefore be an approach for cardiac regeneration .Pharmacological agents like a p38 MAP kinase inhibitor, the growth factor neuregulin-1 or the extracellular matrix signaling protein periostin showed encouraging results in vitro but they failed to replicate their beneficial effects to the full extent in vivo(33-35).

## DISCUSSION

Multiple studies have demonstrated that adult mammals renew cardiomyocyte at a very low rate from a cellular source of pre-existing cardiomyocyte. Endogenous progenitor cells may play a role in therapeutic approach. The release of cell cycle blocks in adult cardiomyocytes and progenitor cell activation can facilitate an increase in cell cycle activity. An emerging line of research seeks to define endogenous mechanism of cardiomyocyte proliferation in the context of post natal development and in response to neonatal injury. Cardiomyocyte cell cycle activity should be measured with multiple ,direct, and independent methods on the same sample .A conclusion that cardiomyocyte proliferation occurred should be supported by direct quantitative evidence. Stereology is considered by many as the gold standard for qualifying cardiomyocytes in intact hearts .Cell cycle outcomes should be determined i.e., multinucleation, ploidy, and division.

## CONCLUSION

New research in heart regeneration in different experimental models in revealing that many mechanism may be shared by organism that are able to regenerate their hearts. These mechanism include stimulation of an essential immune response ,a role for nerves, and a critical contribution for cardiomyocyte division. We still do not understand the barriers to heart regeneration that lead to extensive scarring and eventual heart failure in humans who have major cardiac injuries. The imperative for the field is to learn from the development biology of the hear and define the regenerative pathways. Currently, after blood flow is stored in heart attack patients, we largely watch and see how extensive the injury becomes. In this critical time window, there is an opportunities to identify which patients may develop heart failure in future and treat them with a regenerative therapy. If successful, these studies may indeed uncover a development of blueprint for cardiac regeneration.

## ACKNOWLEDGMENT

We want thank to Mr. Gaikwad.D.,& Mr.Patel.S. for comments on manuscript & for continuous support. The author apologize for any references, which were not include in this review. We apologize to our colleagues in the field for omitted citations of original reports due to restriction on space and number of references.

## REFERENCES

1. Adler,CP.,1991.The development and regenerative potential of cardiac muscle .In: Oberpriller,J.O., Oberpriller.J.C., Mauro .A(Eds).,The Development and Regenerative Potential of cardiac muscle.HAP,London,pp.227-252.

2. Kajstura, J., Gurusamy, O., Ogorek, B., Goichberg, P., Clavo Randon, C., Hosoda, T., Anversa, P., 2010a. Myocyte turnover in the aging human heart. *Circ. Res.* 107 (11), 1374-1386. <http://dx.doi.org/10.1161/CIRCRESAHA.110.231498>.
3. Anversa, P., Kajstura, J., Rota, M., Leri, A., 2013. Regenerating new heart with stem cells. *J. Clin. Invest.* 123(1), 62-70. <http://dx.doi.org/10.1172/JCI63068>.
4. Mallat, Z., Fornes, P., Costagliola, R., Esposito, B., Belmin, J., Lecomte, D., Tedgui, A., 2001. Age and gender effects on cardiomyocyte apoptosis in the normal human heart. *J. Gerontol. A: Biol. Med. Sci.* 56, M719-M723.
5. Kanoh, M., Takemura, G., Misao, J., Hayakawa, Y., Aoyama, T., Nishigaki, K., Fujiwara, H., 1999. Significance of myocyte with positive DNA in situ nick end-labelling (TUNEL) in hearts with dilated cardiomyopathy: not apoptosis but DNA repair. *Circulation* 99, 2757-2764. <http://dx.doi.org/10.1161/01.CIR.99.21.2757>.
6. Porrello, E.R., Mahmoud, A.L., Simpson, E., Johnson, B.A., Grinsfelder, D., Canseco, D., Sadek, H.A., 2013. Regulation of neonatal and adult Mammalia heart regeneration by the miR-15 family. *Proc. Natl. Acad. Sci. U.S.A.* 110, 187-192. <http://dx.doi.org/10.1126/science.077857>.
7. Bergmann, O., Zdunek, S., Alkass, K., Druid, H., Bernard, S., Frisen, J., 2011. Identification of cardiomyocyte nuclei and assessment of ploidy for the analysis of cell turnover. *Exp. Cell Res.* 327 (2), 188-194.
8. Bergmann, O., Bhardwaj, R., Bernard, S., Zdunek, S., Barnabe-Heider, F., Walsh, S., Frisen, J., 2009. Evidence for cardiomyocyte renewal in humans. *Science* 324 (5923), 98-102. <http://dx.doi.org/10.1126/science.1164680>.
9. Spalding, K.L., Bhardwaj, R., Buchholz, B.A., Druid, H., Frisen, J., 2005. Retrospective birth dating of cells in humans. *Cell* 122, 133-143.
10. Ang, K.L., Shenje, L.T., Reuter, S., Soonpaa, M.H., Rubart, M., Field, L.J., Galnanes, M., 2010. Limitations of conventional approaches to identify myocyte nuclei in histologic sections of the heart [Comparative Study]. *Am. J. Physiol. Cell Physiol.* 298 (6), C1603-C1609. <http://dx.doi.org/10.1152/ajpcell.00435.2009>.
11. Bergmann, O., Liebl, J., Bernard, S., Alkass, K., Yeung, M.S.Y., Steir, P., Frisen, J., 2012. The age of olfactory bulb neurons in humans. *Neuron* 74 (4), 634-639. <http://dx.doi.org/10.1016/j.neuron.2012.03.030>.

12. Beltrami, A.P., Barlucchi, L., Torella, D., Baker, M., Limana, F., Chimenti, S., Anversa, P., 2003. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 114 (6), 763-776.
13. Kajstura, J., Urbanek, K., Perl, S., Hosoda, T., Zheng, H., Ogorek, B., Anversa, P., 2010b. Cardio myogenesis in adult human heart. *Circ. Res.* 107 (2), 305-315.
14. Stamm, C., Choi, Y.-H., Nasser, B., Hetzer, R., 2009. A heart full of stem cells: the spectrum of myocardial progenitor cells in the postnatal heart. *Thromb. Haemostasis* 109 (3), 215-229.
15. Jesty, S.A., Steffey, M.A., Lee, F.K., Breitbach, M., Hesse, M., Reining, S., Kotlikoff, M.I., 2011. c-kit<sup>+</sup> precursor support postinfarction myogenesis in the neonatal, but not adult, heart. *Proc. Natl. Acad. Sci. U.S.A.* 109, 13380-13385. <http://dx.doi.org/10.1073/pnas.1208114109>.
16. Fant, X., Srsen, V., Espigat-Georger, A., Merdes, A., 2009. Nuclei of non-muscle cells bind centrosome protein upon fusion with differentiating myoblast. *Plos One* 4, e8303.
17. Yoon, C.H., Koyanagi, M., Iekushi, K., Seeger, F., Urbich, C., Zeiher, A.M., Dimmeler, S., 2010. Mechanism of improved cardiac function after bone marrow mononuclear cell therapy. Role of cardiovascular lineage commitment. *Circulation* 121 (18), 2001-2011.
18. Bettencourt-Dias, M., Mitnacht, S., Brookes, J.P., 2003. Heterogeneous proliferative potential in regenerative adult newt cardiomyocyte. *J. Cell Sci.* 116, 4001-4009. <http://dx.doi.org/10.1242/jcs.00698>.
19. Smart, N., Bollini, S., Dube, K.N., et al. De novo cardiomyocyte from within the activated adult heart after injury. *Nature* 2011; 474: 640-644.
20. M. A. Laflamme, C.E. Murry, *Nature* 473, 326-335 (2011).
21. S. Martin-Puig, Z. Wang, K.R. Chien. *Cell Stem Cell* 2, 320-331 (2008).
22. Bernstein, H.S., Srivastava, D. Stem cell therapy for cardiac disease. *Pediatr Res* 2012; 71: 491-499.
23. Templin, C., Luscher, T.F., Landmesser, U. Cell based cardiovascular repair and regeneration in acute myocardial infarction and chronic ischemic cardiomyopathy-current status and future developments. *Int J Dev Biol* 2011; 55: 407-417.
24. Puliafico, S.B., Penn, M.S., Silver, K.H. Stem cell therapy for heart disease. *J Gen Intern Med* 2013; 28: 1353-1363.
25. Ieda, M., Fu, J.D., Delgado-Olguin, P., et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 2010; 142: 375-386.

26. Zhang D, Shadrin IY, Lam J, et al. Tissue-engineered cardiac patch for advanced functional maturation of human ESC-derived cardiomyocytes. *Biomaterials* 2013;34:5813-5820.
27. Eschegagen T, Eder A, Vollert I, et al. Physiological aspects of cardiac tissue engineering. *Am J Physiol Heart Circ Physiol* 2012;303:H133-143.
28. Laflamme MA, Murry CE. Heart regeneration. *Nature* 2011;473:326-335.
29. Bergmann O, Bhardwaj RD, Bernard S, et al. Evidence for cardiomyocyte renewal in humans. *Science* 2009;324:98-102.
30. Senyo SE, Steinhauser ML, Pizzimenti CL, et al. Mammalian heart renewal by pre-existing cardiomyocyte. *Nature* 2013;493:433-436.
31. Bersell K, Arab S, Haring B, et al. Neuregulin 1/ErbB4 signaling induces cardiomyocyte proliferation and repair of heart injury. *Cell* 2009;138:257-270.
32. Kuhn B, Del Monte F, Hajjar RJ, et al. Periostin induces proliferation of differentiated cardiomyocytes and promotes cardiac repair. *Nat Med* 2007;13:962-969.

***AJPTR is***

- Peer-reviewed
- bimonthly
- Rapid publication

Submit your manuscript at: [editor@ajptr.com](mailto:editor@ajptr.com)

