# Research on the Physiological Factors of Cardiac Regeneration

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**Abstract.** The heart is one of the most important organs in mammals. When cardiovascular disease occurs, such as myocardial infarction, many cardiac myocytes die due to hypoxia, leading to heart failure. The ability of adult mammalian cardiomyocytes to proliferate gradually decreases during development. Therefore, when the heart is damaged, it cannot be repaired by the natural division and regeneration of cardiomyocytes, which in turn leads to impaired heart function. Therefore, it is particularly important to improve the proliferative capacity of cardiomyocytes during the treatment of cardiac diseases. Here, we summarize the effects of different factors such as environmental oxygen, energy metabolism, extracellular matrix, nervous system and immune response on cardiomyocyte proliferation and cardiac regeneration with the aim of providing a theoretical basis and potential directions for the treatment of heart disease.

## 1. Importance of heart regeneration

Cardiovascular disease (CVD) is the leading cause of death worldwide, accounting for nearly one-third (approximately 18 million) of global deaths each year. [1]Due to high morbidity and mortality, CVD has become one of the most concerning diseases around the world. The adult mammalian heart is difficult to regenerate naturally after injury because mature cardiomyocytes have terminated the cell cycle and have poor regenerative capacity. The dead cardiomyocytes are replaced by fibroblasts, leading to fibrosis formation and myocardial remodeling, which subsequently leads to heart failure. Many treatments have been developed for cardiovascular disease caused by myocardial injury, but the mortality rate for patients with severe heart failure remains high. [2]The basic treatment for end-stage heart failure is heart transplantation. However, the efficacy of this approach is greatly limited by the lack of donors, patient selection criteria, and the risks of the procedure.[3]

Cardiomyocyte plasticity plays a key role in cardiac adaptive responses such as myocardial remodeling and cardiac repair.[4]The heart of mammalian animals is composed of cardiomyocytes, smooth muscle cells, fibroblasts and other cells. when the heart is damaged, fibroblasts secrete collagen and form scars. In addition, fibroblasts can be trans-differentiation (trans-differentiation refers to the process by which a type of differentiated cell is expressed selectively by genes to transform them structurally and functionally into another differentiated cell) to repair cardiomyocytes in the event of heart damage. [5, 6]

Cardiac myocytes account for 75% of the volume of the left ventricle in healthy adults and facilitate the pumping of blood into the circulatory system by coordinating systole and diastole.[4] Because mature cardiomyocytes do not undergo a complete division process, that is, they do not complete cytoplasmic division, cardiomyocytes have polykaryocytes, in which the ratio of polynuclear cells to monocytes can reflect the proliferative ability of cardiomyocytes. [7, 8]When a heart disease occurs (such as myocardial infarction), blood vessels are blocked by various substances such as fat, resulting in abnormal circulation of blood, further leading to the death of related cells (including cardiomyocytes) due to hypoxia.[9] Due to the proliferation ability of mature cardiomyocytes being reduced, they cannot complete cardiac repair through self-dividing. Therefore, how to improve the proliferation ability of cardiomyocytes has become an important issue in the treatment of heart disease.

To date, a series of cell cycle regulators, signal pathways, non-coding RNAs, and other molecules have been shown to be involved in cardiomyocyte proliferation and cardiac repair after injury.[4] In addition, factors such as hypoxia, energy metabolism, extracellular matrix, neural, epicardial factors, and inflammation are also involved in regulating cardiomyocyte proliferation and cardiac repair. Targeting these factors in the infarcted heart becomes a new therapeutic strategy for cardiac regeneration.[4]This literature review summarizes the influence of different factors on cardiomyocyte proliferation and cardiac regeneration, in order to provide some insights for the treatment of heart diseases.

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# 2. Influencing factors of heart regeneration

## 2.1 Heart regeneration ability of different species

Animals at different levels of evolution have different abilities to regenerate their hearts. Lower vertebrates such as zebrafish and newt have a surprising ability to regenerate injured or lost cardiac tissue. [10, 11]When the apex of the zebrafish heart is removed three months after birth, the proportion of collagen at the injured position on the seventh and fourteenth days after the injury is still high, and the heart regeneration ability is poor. Thirty days after the injury, the heart was completely repaired and the proportion of collagen decreased. Through the BrdU staining, the researchers found that the zebrafish cardiomyocytes in the experimental group had a higher proportion of BrdU, especially in the apex area. The above results can show that the cardiomyocyte proliferation ability of zebrafish has also improved significantly in the process of heart regeneration. In order to further explore the source of cardiomyocyte, researchers found that the cardiomyocyte filament structure depolymerizes after heart injury and the stable arrangement disappeared. The above results show that new cardiomyocytes are generated by dedifferentiation of existing cardiomyocytes and re-division into the cell cycle in the zebrafish.[12, 13]

As a model mammal animal, the ability of cardiomyocyte proliferation and heart regeneration ability of mice are gradually reduced during development. In order to explore this problem, the researchers resected the heart apex of mice on the first-day post-birth (P1). Through staining, there was a blood scab on the heart on the first and second day after surgery. By the seventh and twentieth days, the heart slowly recovered. The above results showed that the heart of mice has a high regenerative ability on the first day of life. The ejection fraction and contraction fraction of the heart also shows that the heart function has recovered. In order to further explore the reasons for heart regeneration and repair in mice, researchers can find the cardiomyocytes proliferation ability in the apex area, border area and remote area has been significantly improved. [14]

On the seventh day after the birth of the mouse, a similar heart injury model was made. After staining, it was found that the heart could not be fully repaired at this time. The above results can show that the cardiomyocyte proliferation ability and heart regeneration ability of mice are lost on the seventh day after birth. In order to further study the source of new-born cardiomyocytes, researchers stained cardiomyocytes by genetic modification and removed the apex of the heart. 21 days after the injury, it can be found that the new-born cardiomyocytes are also stained. Combined with the above sarcomere depolymerization experiments, it can be shown that the newly generated cardiomyocytes arise from the division of already existing cardiomyocytes. [15]

## 2.2 The effect of oxygen concentration

By comparing the living environment of the zebrafish and the embryonic mice, it is not difficult to find that zebrafish and embryonic mice live in water with low oxygen concentration. Therefore, oxygen concentration is considered as a candidate regulator of cardiac regeneration. The researchers put three-month-old mice in a low-oxygen environment. They found the area of mitochondrial spines in mouse cardiomyocytes decreased, and the amount of mitochondrial DNA decreased. The above results prove the oxygen released by mitochondria is low, and cardiomyocytes are in a low-oxygen environment. Some studies have shown that the degree of DNA damage in a low-oxygen environment has decreased significantly. [16] In addition, the researchers calculated the change of the heart size of mice by comparing the heart weight to their weight. The results show that in a low-oxygen environment, the heart weight increases significantly. Using WGA to label cell membranes, and measure the volume of cardiomyocytes, it can be found that the cell volume is smaller, so it can be concluded that the number of cells may increase. By isolating cardiomyocytes from the heart and counting the number of cells, it can be seen that the number of cardiomyocytes has indeed increased. The DNA replication, nuclear and cytoplasmic division of cardiomyocytes were characterized by BrdU, pH3 and Aurora B respectively. The results can show that the proliferation ability of cardiomyocytes in the low-oxygen environment is significantly enhanced. On the contrary, after the oxygen-producing drug Diquat is injected into the heart, the degree of DNA damage is significantly increased, and the proliferation ability of cardiomyocytes is reduced. The above results can show that low-oxygen concentration leads to reduced DNA damage and enhanced the proliferation ability of cardiomyocytes.[16]

The hypoxic environment improves the regenerative ability of the heart. The researchers first used mice for two months to make a model of myocardial infarction. After a week, the oxygen concentration in their surroundings was gradually reduced and maintained a low-oxygen concentration to observe changes in the repair ability of the heart. The researchers found that after heart damage, the heart of mice in a hypoxic environment increased the weight and the cell volume became smaller, which also showed that the number of cardiomyocytes increased and the ability of cardiomyocytes increased at this time. BrdU, pH3 and Aurora B are still used to characterize DNA replication, nuclear division and cytoplasmic division of cardiomyocytes, respectively. The results showed that the ability of mouse cardiomyocyte to proliferate has indeed improved. The proportion of heart collagen in a low - oxygen environment decreased and the fibrosis level decreased. By staining the blood vessels in the heart, it can be found that the hypoxic environment can not only promote the proliferation of cardiomyocytes but also promote the regeneration of blood vessels.[17]

To sum up, the low-oxygen environment promotes the proliferation ability of mouse cardiomyocytes, and can also improve the regeneration ability of mice after heart injury.

### 2.3 The effect of energy metabolism

Adult cardiomyocytes get the majority of their energy from oxidative phosphorylation in mitochondria.[16]Increased oxidative stress contributes to cell cycle arrest due to changing energy supply patterns before and after birth. [17]Meanwhile, inhibited glycolysis impairs the regeneration of newborn mouse cardiomyocytes. [18]Recent research has showed that pyruvate dehydrogenase kinase (PDK) regulates post-injury glycolysis and pyruvate metabolism in marginal zone cardiomyocytes of zebrafish. [19]Furthermore, PDK4 loss boosts cardiomyocyte proliferation, improves left ventricular function and decreases remodelling. [20]These data imply that energy metabolism is important in cardiomyocyte post-injury growth.

### 2.4 The effect of extracellular matrix (ECM)

Periostin is an secrete ECM protein that plays an important role in cardiac development. Studies have shown that it is re-expressed after cardiovascular and skeletal muscle injury, but it is unknown whether it will affect the heart regeneration ability after heart injury.[21]

The researchers first studied the effect of Periostin on the proliferation ability of cardiomyocytes in vitro. When cardiomyocytes are cultured in the medium, their shape will change from a short rod shape to an irregular pseudopod shape. After adding Periostin to the medium, the BrdU positive rate of cardiomyocytes was increased, and the elevated level was comparable to that of the positive control group. Using Aurora B to characterize the level of cardiomyocytes cytoplasmic division, they also found that the ratio of Aurora B is increasing. After 3 days, 6 days, 9 days and 12 days of adding Periostin, it can be found that the proportion of DNA replication is high and the proportion of cytoplasmic division is low in 3 days, that is, the level of DNA replication is much faster than that of cytoplasmic division. Through the staining of the nucleus, it is found that the cells undergoing cytoplasmic division are monocytes, and binuclear cells do not have the ability to divide.[22]

Subsequently, the researchers further studied the effect of Periostin on the proliferation ability of cardiomyocytes in vivo. The researchers injected Periostin directly into myocardial tissue. By staining the Periostin, it can be found that the Periostin has penetrated the heart tissue. Moreover, the area and fibrosis level of cardiomyocytes have not changed, indicating that direct injection of Periostin does not cause additional damage to the heart. Subsequently, BrdU, pH3 and Aurora B were used to characterize the proliferation ability of cardiomyocytes. After adding Periostin, the level of DNA replication, cell nuclear division and cytoplasmic division all increased to varying degrees.

In order to explore the effect of Periostin on heart regeneration, researchers buried drugs in the heart which that sustainably release Periostin to the heart. After observation for a while, they found that the Periostin also penetrated into the heart. After cardiac injury in different experimental groups, the ejection fraction and contraction fraction of the heart in mice can be detected, Periostin can significantly improve the heart function after injury. Through staining collagen, it was found that after adding Periostin, the proportion of collagen decreased after heart injury. Through the proportion of heart weight, it can be found that the heart size has not changed, but the volume of cardiomyocyte was decreased.

In order to further explore the changes of the cardiomyocyte's proliferation ability after heart regeneration, the researchers tested the positive ratios of BrdU and pH3 in regenerated cardiomyocytes. The results showed that the DNA replication level and nuclear division ability of cardiomyocytes have increased. Subsequently, VWF and SMA were used to observe vascular regeneration in cardiac tissue. It can be found that Periostin also promotes cardiovascular regeneration.

To sum up, Periostin can improve the proliferation ability of cardiomyocytes at the in vitro level, and the continuous release of Periostin in the body can further promote the heart repair ability after heart injury.[23, 24]

### 2.5 The effect of nervous system

Researchers found that the increase in the number of neurons in the process of regenerative repair after heart injury in zebrafish, this may suggest a link between neuron regeneration and heart regeneration.[25]After overexpressing Sema3aa gene in zebrafish cardiomyocyte, the researchers found that the proliferation ability of zebrafish cardiomyocytes decreased after heart damage, and the proportion of collagen was high. The above results can show that inhibition of nerve growth affects the heart regeneration process of zebrafish.

In order to further explore the role of cholinergic neurons or adrenaline neurons in the process of heart regeneration, the researchers used atropine (cholinergic neuron inhibitor) and Pronel (adrenaline neuron inhibitor) to treat zebrafish and found that after using atropine to inhibit cholinergic neurons, the proliferative ability of cardiomyocyte is weakened, but Pronel has no effect. In order to further explore the role of cholinergic neurons, researchers removed the apex of the heart in mice on the day of birth and used atropine to inhibit nerve growth. The experimental results found that the proportions of pH3 and Aurora B in mouse cardiomyocytes decreased significantly, and the weight of mice also decreased significantly.

Subsequently, the researchers further explored the effect of nerves on heart regeneration by directly removing nerves. First, studies have shown an increase in the expression of M2 protein after excision of neurons, and the expression of Nrg1 and NGF protein decreases. The study also found changes in the expression of related proteins after excision of neurons, and further showed that the excision part was neurons. However, cutting off this part of the nerve has no effect on the function of the heart, and follow-up experiments can be carried out. Next, the researchers set up three groups of experiments, the control group (G1), the myocardial infarction injury group (G2), and the myocardial infarction injury and resection of the nerve group (G3). The results showed that the survival rate of mice in G3 decreased significantly, and contrary to

G3, the ratio of pH3 and Aurora B in G2 increased significantly, and expression of collagen in G3 was high. To sum up, the regeneration ability of the heart is impaired after the removal of the nerve.

Finally, by adding Nrg1, Ngf protein to cardiomyocytes in vitro, the researchers were able to see an enhanced replication capacity of DNA. Subsequently, the researchers used myocardial infarction and nerve amputation as models of cardiac injury, and simultaneously injected mice with Nrg1 and Ngf proteins. Experiments found that the number of pH3 and Aurora B increased significantly and the proportion of collagen decreased significantly 7 days after heart injury. Taken together, Nrg1 and Ngf proteins can promote cardiac repair.

In summary, the researchers used three different methods: transgenic means, pharmacological means and direct denervation to verify the importance of nerves in the process of cardiomyocyte division and heart regeneration, see figure 1. [26]

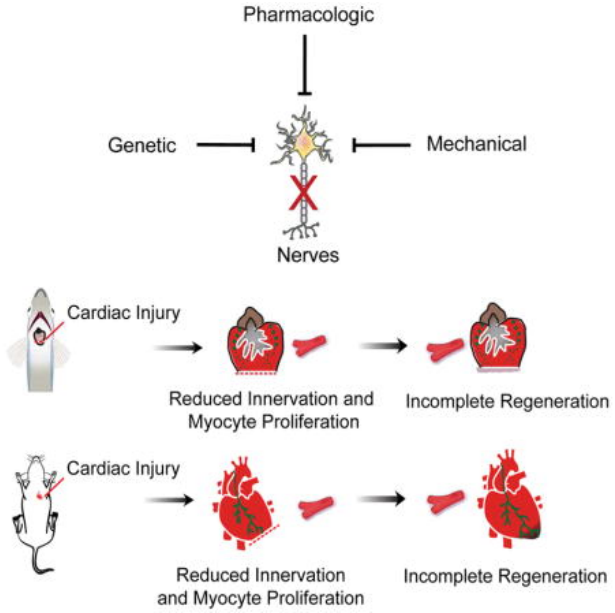


**Figure 1.** Neurons regulate cardiomyocyte proliferation and heart regeneration[26].

### 2.6 The effect of immune response

Adult tissue regeneration is triggered by tissue injury and therefore the immune response and inflammatory response to injury are important influencing factors in the regeneration process. In lower vertebrates and mammals, the consequences of the inflammatory/immune response are remodeling through scar formation in non-regenerating animals and remodeling through cell proliferation in regenerating animals. Infiltration of inflammatory cells in the damaged heart peaks around 3 days after cardiac amputation in zebrafish. [27]In mice, cardiac regeneration requires the involvement of monocytes and macrophages. By comparing the differences in cellular immune responses to myocardial infarction between 1- and 14-day-old mice, they also found that the more developed the immune system, the lower the regenerative capacity.[28] But injury-induced cardiac proliferation is inhibited by the immune system. Cardiac regeneration in neonatal fetal rats requires an acute inflammatory episode, and in the absence of interleukin 6 (IL-6), cardiomyocytes are unable to proliferate in the presence of injury. [29]The above studies illustrate that inflammation can both drive and inhibit regeneration under different conditions.

## 3. Conclusion and Prospect

The heart is one of the most important organs in mammals. The occurrence of heart disease leads to the death of a large number of cardiomyocytes, and the proliferation ability of mammalian cardiomyocytes is gradually reduced during development. Therefore, how to improve the proliferation ability of cardiomyocytes after heart injury has become a key issue in the treatment of heart disease. This article summarizes a variety of factors affecting cardiomyocyte proliferation and heart regeneration. Among them, low oxygen concentration, ECM, nervous system and immune response can promote cardiomyocyte proliferation and heart regeneration. These influences are expected to be applied to the treatment of cardiovascular diseases based on the induction of myocardial regeneration, but many questions remain to be addressed in their practical application.

First of all, we need to improve the regional targeting of the appropriate modulation methods. For example, how to reduce the ambient oxygen concentration, regulate the concentration of periosteal proteins in the ECM, or neuromodulation in a way that only targets the heart and does not affect the normal function of other organs.

Second, further research is needed to determine which of the many influencing factors are dominant in driving the cardiac regeneration process. And it is still a big challenge to translate the existing research findings into clinical drug design and therapies. We need to find the most appropriate direction as well as therapeutic approaches that can maximize the regeneration of the human heart while minimizing the impact on other organs.

Finally, the above experiments have been validated only at the cellular and animal level, but the occurrence of heart disease in humans may be very different from that in animals. It is likely that conclusions drawn in animal experiments cannot be applied to humans. Therefore, we need more experimental support in various model organisms and further validation experiments of the translated therapies in the clinic.

At present, the incidence and mortality of heart disease worldwide is still high, and the cost of treatment places a greater burden on the society economics. We summarize the factors affecting myocardial cell division and heart regeneration, and puts forward opinions on the existing problems, so as to provide some reference opinions for the treatment of heart diseases.

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