Zebrafish Heart Regeneration Process:
Histo-Physiological Basis & Perspectives in Regenerative Medicine

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INTRODUCTION

Zebrafish (*Danio rerio*) is a teleost from *Cyprinidae* family. Teleosts, as urodele amphibians, have the remarkable capacity to regenerate several organs and structures of their body after an injury. **Every 43 seconds, someone in the United States has a myocardial infarction.** The limited regenerative capacity of humans complicates the recovery after an injury or disease (**Fig. 1**).

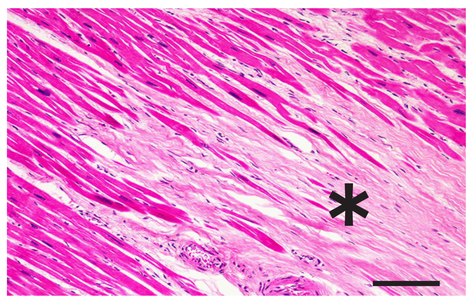


Fig. 1 - Healed human myocardium infarction. Myocardium loss is replaced by fibrous tissue (asterisk). The scar is not contractile and decreases cardiac output, eventually leading to heart failure, arrhythmia and free wall rupture. *Scalebar: 100 µm.* [1]

HEART HISTOLOGY

Despite some differences in the anatomy, there are the same cell types and stratification (**Fig. 2**) in atria and ventricle of zebrafish and human hearts.

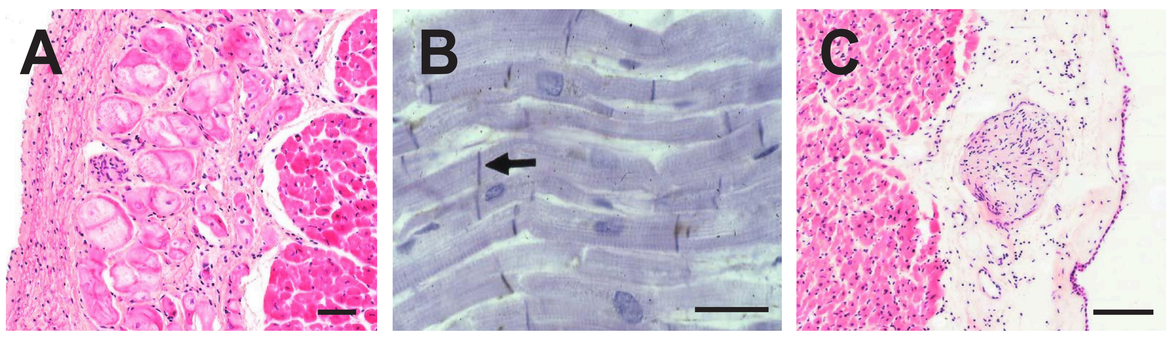


Fig. 2 - Heart wall histology, human samples. **A)** Endocardium. [2] **B)** Myocardium. [3] **C)** Epicardium. [2] *Scalebar in A and B: 50 µm; in C: 100 µm*

ZEBRAFISH HEART REGENERATION PROCESS

WIDE-ORGAN REACTION

After the injury, a wide-organ reaction is triggered (**Fig. 3**) starting in the endocardium and following in the epicardium. Epicardium cells undergo an epithelial-mesenchymal transition (EMT) and finally proliferate over the wound.

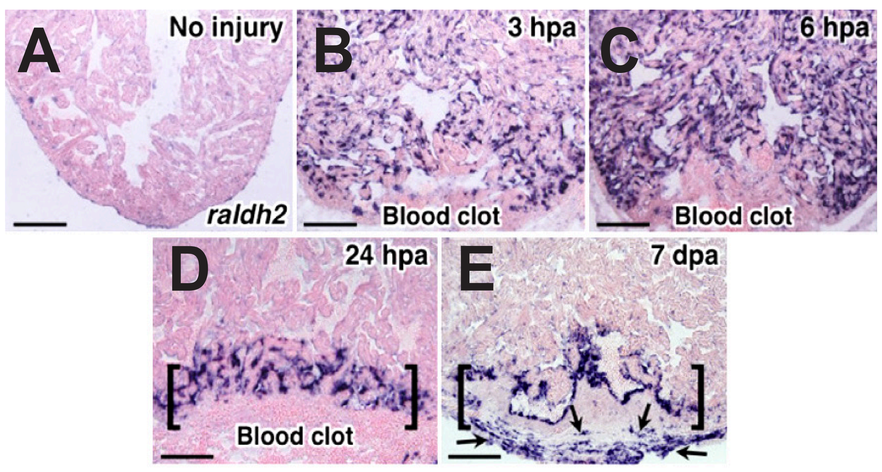


Fig. 3 - Wide organ reaction can be followed through an in situ hybridization of some embryonic markers, such as *raldh2.* [4] *Scalebar: 100 µm.*

NICHE FOR REGENERATION

The wound covering epicardium becomes a niche for regeneration and orchestrates the process (**Fig. 4**).

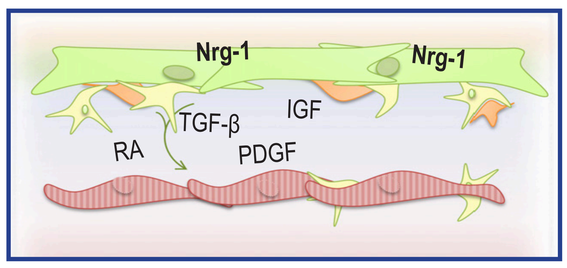
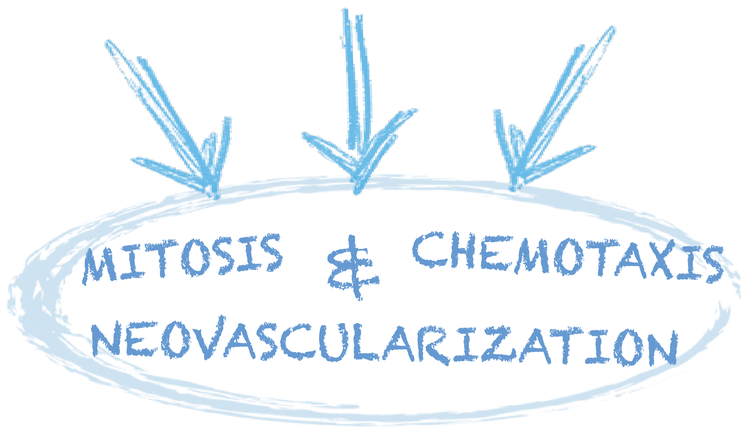


Fig. 4 - Epicardium paracrine signaling acts on injury-adjoining cardiomyocytes. [5]



CARDIOMYOCYTE DEDIFFERENTIATION

CreER recombinase-based fate-mapping study (**Fig. 5**) demonstrate that new cardiomyocytes come from preexisting injury-adjoining cardiomyocytes through dedifferentiation and proliferation.

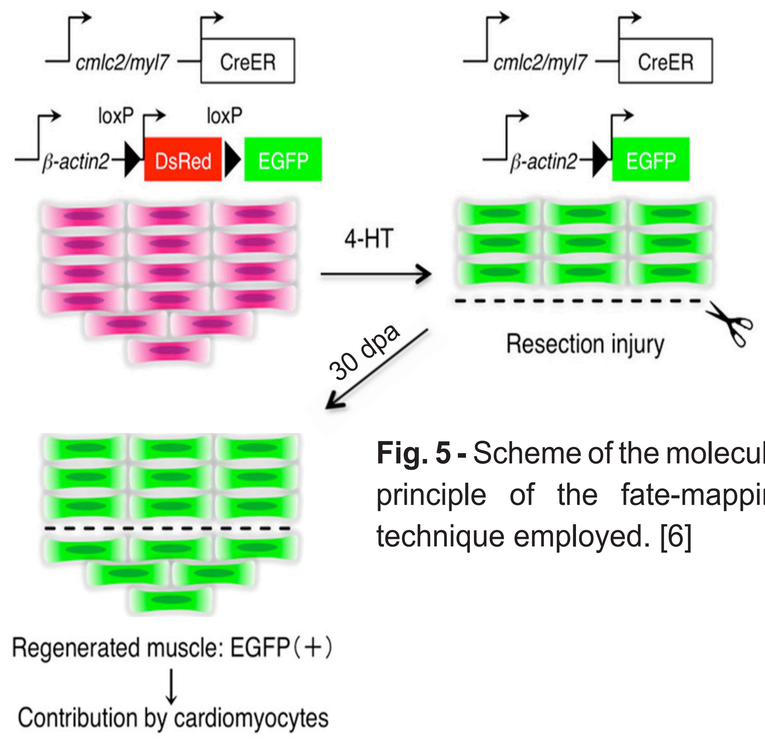
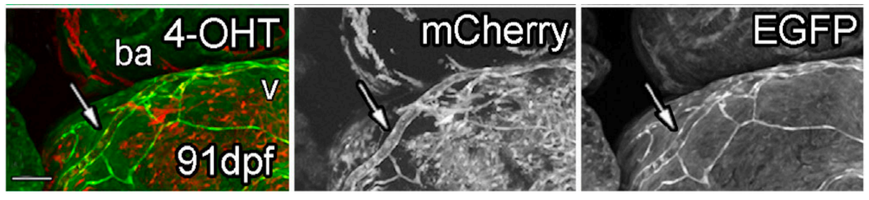


Fig. 5 - Scheme of the molecular principle of the fate-mapping technique employed. [6]

NEOVASCULARIZATION

Neovascularization process occurs through the reactivation of developmental patterns. Coronary vessels derived from activated endocardium (**Fig. 6**), while epicardium contributes to perivascular elements and orchestrates the process (**Fig. 7**).



*ubb:LoxP-**EGFP**-LoxP-**mCherry***
*fli1a:**EGFP***
fli1a:CreER

Fig. 6 - Fate-mapping study results. *Ex vivo* tagged hearts treated with tamoxifen (4-OHT), before coronary vessels formation, shows mCherry and EGFP colocalization in coronary vessels. [7] *Scalebar: 50 µm.*

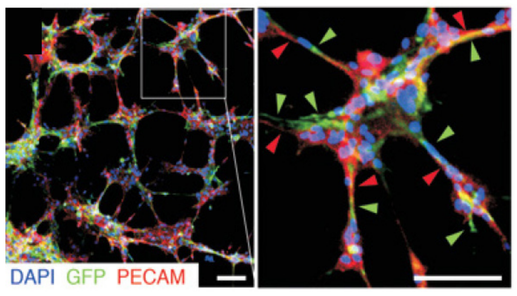


Fig. 7 - Coculture of endothelial cells with epicardial-derived cells results in a robust vessel formation *in vitro*. [8] *Scalebar: 10 µm.*

PERSPECTIVES

THE MAMMALIAN HEART

Mouse heart is able to regenerate ventricular injury during the first week after birth, through the same known dynamics on zebrafish. Some miR-15 family miRNAs seem to be implicated in mouse cardiomyocyte mitotic arrest. Few clinical reports describe cases of **human new-born massive infarction** that **absolutely recovers** in a few days.

KNOWLEDGE ABOUT REGENERATIVE ABILITIES OF ZEBRAFISH WILL GIVE US THE CLUES TO UNDERSTAND REGENERATION AND FINALLY TREAT HEART FAILURE.

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