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Journal of Clinical Cardiology Commentary

Cardiac Stem Cell Therapy, Quo Vadis

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Received date: March 08, 2021, **Accepted date:** May 25, 2021

Cardiovascular disease causes 30% of global mortality and is still the number one cause of death worldwide [1]. A main patho-physiological process is the coronary disease leading to malperfusion and ischemic cardiac disease as well as cardiac infarction. Despite the many improvements of cardiovascular therapies such as coronary stents, percutaneous transluminal coronary angioplasty (developed in 1977 [2]) or bypass surgery, scared heart tissue can still not be repaired. It seems, that finding of new therapeutic options are necessary. Hereby, cell therapy is a great hope. It is almost 30 years ago when cardiomyoplasty was proposed, i.e. culturing cardiac cells derived from stem cells and implanting them back into scar tissue. In 1991 Hescheler and Wobus [3] could show for the first time the physiological properties in cardiomyocytes developed within embryoid bodies, i.e. the first functional *in vitro* differentiation system of embryonic stem cells.

Unfortunately, up-to-now, the stem cell research on cardiovascular disease is still on the level of small animal experiments [4]. It has become rather clear that initial ideas were too simple and techniques that are more sophisticated must be found as well as basic research such as cell differentiation, understanding repair mechanism are not fully understood. This commentary is based on our previous review article summarizing the state of the art of cardiac therapies with a special emphasis on pluripotent stem cell derived cardiac cells for regeneration [5]. Here we want to expand these ideas proposing the next steps to be done with emphasis on new imaging techniques.

What are the problems and how can they be solved? In principle, there are two major ideas to bring living stem cells into the scared tissue. The first principle idea is undifferentiated adult stem cells, e.g., MSCs. The hope was that they would differentiate in the right niche. Unfortunately, the idea was not working for adult stem cells due to niche factors. Nevertheless, this approach allowed clinical applications demonstrating the safety of the method of cell integration into the diseased myocardium [6,7]. The second principle idea is to differentiate pluripotent stem cells *in vitro* by respective techniques and to get these differentiated cells into the cardiac tissue. Unfortunately, the integration of cardiomyocytes derived from pluripotent stem cells was proved to be not efficient. Our own workgroup preferentially combines physiological methods with integration studies of stem cells [8]. This work demonstrated that there is a tremendous loss of cardiomyocytes from stem cells, probably due to immunological and inflammatory responses [9]. It is also shown that the integration process works much longer than just the time after a few hours of integration. By using slice techniques and looking at different times after integration of cardiomyocytes into the heart with electrophysiological means it could be demonstrated that there is a slow maturation that takes around 6 days [10]. However, this simple experiment showed that cellular mechanisms are even more difficult to be controlled and major problems with obtaining cardiac cells integrated are on the level of maturation of cardiomyocytes and development of functional cardiac tissue that will integrate in a proper manner. Hence, several developments need to be emphasized:

- New and better understanding of maturation processes of cardiomyocytes within mass-culture techniques.
- It is still not clear which genetic factors will develop functional ventricular cardiac tissue. In the state-of-the-art, development of cardiomyocytes from stem cells will still reveal cardiomyocytes of an early phenotype that have a potential of arrhythmogenicity

after integrated in the diseased cardiac tissue and it needs to get better factors of maturation.

- Cardiac cells should be integrated within the right environment. Therefore, a better understanding of the niche is necessary. This is not only the biochemical and cytokine composition but also the physical properties of the matrix. In another study of our group, it was demonstrated that the elasticity of cardiomyocytes plays an essential role, and the mechanical environment of cardiomyocytes should be regarded [11]. To investigate the development of cardiomyocytes after integration into the diseased muscle there should be more transcription analysis based on DNA array methods as well as other 'omics techniques. Which genes are upregulated after integration? Do the cells express the same genes as found in real ventricular myocytes or do they differentiate into another direction?

- This should also concern the safety studies that must be much better understood. What is the developmental potential of cells integrated in the diseased tissue and whether maldevelopments are possible? Meanwhile the lineage selection is quite well developed and so the development of teratoma from pluripotent cells is probably no longer a problem, but there still might be maldevelopment of cardiomyocytes after tissue integration. This is particularly important because to get a large number of cardiomyocytes, developed mass culture techniques have to be improved and the development of cells should have a higher yield. It is also important to better understand the inflammatory effects and the cells injected in the tissue after cell damage after the myocardial infarction. What is the effect of inflammatory, immunological cells integrating and these things? There is not much understanding on how stem cells will be removed by macrophages and monocytes and how these cells will let the integrated cells survive. More understanding of the interactive role of the immunological inflammatory system and the integrated cells must also be gained. There might be regular oxygen radicals released leading to damage of the cells.

- The probably most important step before clinical translation is experiments with large animals. There are still not many performed, and it must be much better understood how cells would integrate in a large animal and how stable they will stay in the tissue. Why are large animal experiments essential? This is because we must learn how to handle of the different tissue and anatomical conditions of large organs as well as the transplantation of the larger numbers of cells. Unfortunately, the initial work which has already been reported on large animals still relies on relatively simple ideas of cardiac cells to be transplanted but this work needs to be combined with a better understanding of cell environment to be integrated.

- Another important issue of the translational process is the understanding of what happened with the injected cells. Recent advances in small animal imaging, MRI or hybrid PET/MRI, will be a new perspective for cardiovascular applications with cardiac stem cells.

- MRI: Already, MR imaging is widely used in the diagnosis of cardiovascular disease. Functional MR imaging and dynamic perfusion from transit of intravascular contrast medium can be helpful for identifying areas of low myocardial perfusion in terms of tissue viability. On the other hand, late contrast enhancement will be a hint for scar tissue, the location where repairment is needed. Additionally, MRI can be used for quantifying cardiac output function like ejection fraction in terms of evaluation function restorations. We believe that functional MR imaging will become important for translational medicine in terms of quantifying the repairment of scar tissue by stem cells and be helpful for surrogate endpoints of the post-therapeutic outcome of stem cell therapy.

- Cell detection: There are different ideas for cell detection. MRI can detect cells with SPIOs coated transfection agents, which allowed SPIOs stably maintenance in cellular endosomes [12]. Another way is the reporter gene imaging on positron emission tomography (PET.) Hereby, reporter genes (vectors) are transduced in the cells, followed by translation of mRNA, and encoding to reporter protein, which processes specific affinity to imaging of radioactive tracers on PET. An active uptake of radioactive tracers into the cell is a sign of cell viability. In addition, there are *ex-vivo* radioactive labeling strategies for cell detection such as ^{18}F-fluorodeoxyglucose (^{18}F-FDG), which will take up through the glucose transporter type 1 (Glut1) into cells, as well as other radioactive tracers as 99mTc-HMPAO, which detects cells by passive diffusion into the cell. Elhami et al. [9] labeled adipose-derived stem cells of male rats with super-paramagnetic iron oxide (SPIO) and ^{18}F-FDG. The tail vein injection resulted in 1.2% of total injection cell retention were in the heart, respectively 14% by direct myocardial injection. In the animals treated 1 week after the LAD occlusion, rate of cell retention in the heart was only 4.5%. We think that PET will become a helpful tool for non-invasive visualization and tracking of stem cells in repairment mechanism.

- Visualization of tissue metabolism: Moreover, PET images can *in-vivo* quantify and visualize tissue metabolism. Nose et al. [13] showed a metabolic substrate shift from glucose (tracer ^{18}F-2-fluoro-2-deoxy-d-glucose - short FDG) to fatty

acid (tracer 125I-β-methyl-iodophenyl-pentadecanoic acid -short 125I-BMIPP) after cardiac differentiation of human introduced pluripotent stem cells. Expression of fatty acid transport and binding proteins on hiPSC-CM were confirmed with immunostaining. New tracers for evaluating cardiac innervation, angiogenesis, and reporter genes are coming up.

In conclusion, small and large animal imaging, MRI, and hybrid PET/MR imaging, will become of interest in further research. PET can be helpful for *in-vivo* cell detection by reporter gene tracking or MRI for cell detection with SPIOs coated transfection agents in terms of monitoring the cardiac stem cells in tissue repairment. Secondly, PET can *in-vivo* quantify and visualize tissue metabolism, and third MRI, a non-invasive *in-vivo* imaging, will gain of interest in terms of surrogate endpoints of the post-therapeutic outcome. Moreover, future PET imaging may involve reporter genes for cell tracking and functionally identifying the physiological state of transplanted stem cell derived organotypic cells, e.g. cardiomyocytes. In particularly receptor PET-imaging may be a helpful tool to better understand mechanism of integration/repair of stem cells in scar heart tissue. In 2012, Yamanaka got the NobelPrize for pioneering work on induced pluripotent stem (iPS) cells. The availability of patient's identical iPS cells together with some already performed clinical studies on pluripotent stem cells for transplantation [14] gives hope that there is the potential of organ repair and regenerative medicine also for the heart. The next steps will keep in future very fascinating.

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