ESRF	**Experiment title:** High-resolution X-ray microCT for three-dimensional visualization of embryonic stem cells (ESC)-derived cardiomyocytes after injection into infarcted mice hearts	**Experiment number**: MD-326
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Report:

Objectives

The recent introduction of stem cells in cardiology provides new tools in understanding the regenerative processes of the normal and pathologic heart and has opened the search of new therapeutic strategies.

Recent published reports have contributed to identify the possible approaches of cellular therapy to generate new myocardium involving systemic and local mobilization of progenitor cells. Moreover, we have provided the first unequivocal documentation of the existence in the adult human heart of primitive cells able to generate all the different component structures of the myocardium. The possibility to rebuild muscle, arteries and capillaries is the necessary requirement to obtain successful approaches in cardiac regeneration. Formation or implantation of a single cellular component will inevitably fail to repair the damaged organ. So, the aim of this study is to obtain 3D visualization of spatial distribution of injected rat clonogenic progenitor cells (CPCs, labelled cells) inside infarcted rat heart, to understand what and how they are able to regenerate damaged myocardium.

Achievements

The feasibility of adult autologous cellular therapy of acute myocardial infarction has been demonstrated in animal models and in humans. However, many unresolved questions to link experimental with clinical observations remain for the present research concerning:

-The long-term fate of transplanted stem cells in the recipient tissue.
-The ability of transplanted stem cells to find the adequate myocardial environment.
-The potency of stem cells to transdifferentiate into cardiac cells.
-The complete functional integration of the regenerated myocardium with the spared tissue.
-The angiogenic background needed for transplanted cells in an ischemic tissue.
-The capability of the host tissue to allow the differentiation of engrafted cells.

-Specific tracing of engrafted cells or cell populations detectable by imaging techniques.

The microCT was used to image and characterize 3D distribution of injected rat clonogenic CPCs inside the heart tissue of infracted mice (Fig. 1a). 3D visualization of the spatial distribution of the grafted cells in respect with the host myocardium, veins and capillary system was obtained (Fig. 1b). In particular, the X-ray absorption of the labeled cells by magnetic iron oxide nanoparticles was higher than the other heart tissues, allowing their visualization as bright spots in the 2D images (Fig. 1c). These slice images were compiled and analyzed to render 3D images and to obtain a better visualization of cell distribution within the samples. 3D visualization of the spatial distribution of the grafted cells is shown on Fig. 1d.

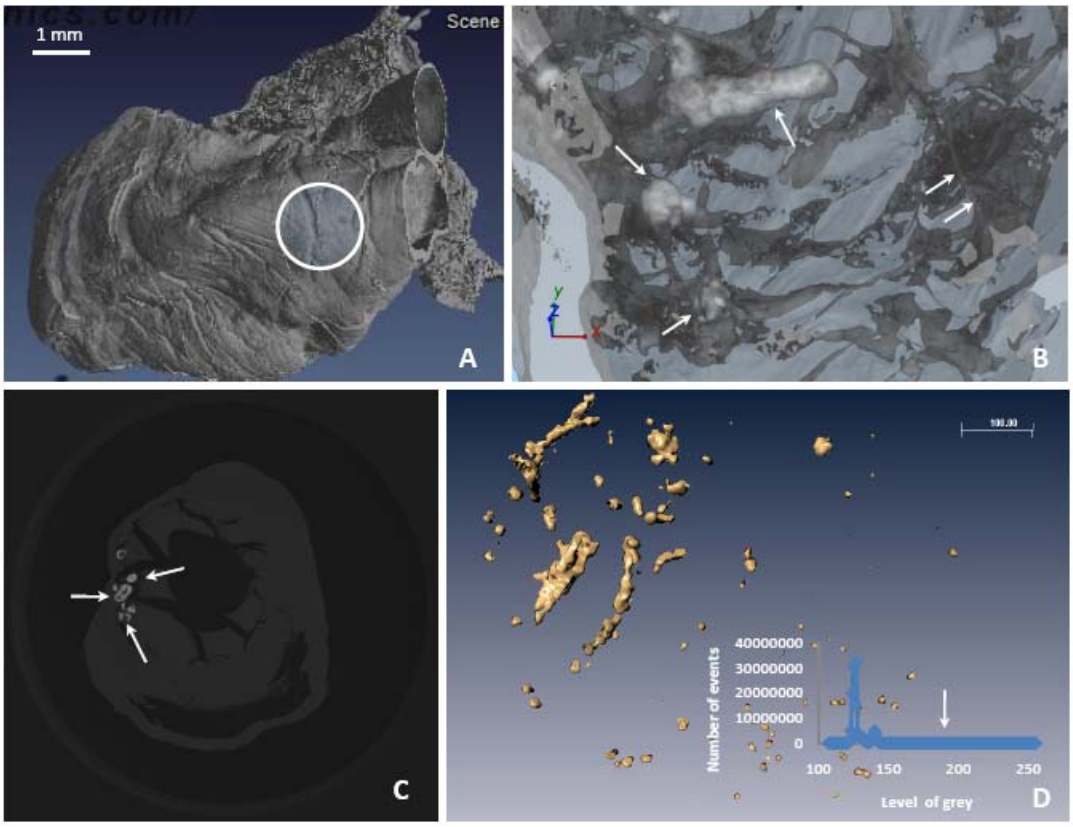

Fig. 1. MicroCT image of the infracted heart (a). 3D image of the spatial distribution of the grafted cells in respect with the host myocardium, veins and capillary system (b). 2D original slice (c). 3D visualization of the spatial distribution of the grafted cells (d).

The 3D images so obtained constituted a very innovative progress, as compared to the usual 2D histological images, which do not provide the correct position of the rat clonogenic cells within the heart. Moreover, this is potentially interesting for future research on determining time variable (early and late differentiation stage), because through the microCT we hope to be able to observe in 3D the migration of cells with respect to the cardiac vessel, with important structural details not observable by the conventional bidimensional imaging techniques. Together with these particular structures, for their dimensions probably cell clusters, we observed also single smaller units, in all parts of the heart, as right ventricle (Fig. 2). This is a very important and new data: in particular it is a confirm that these cells can migrate through the myocardium by biological mechanism still unknown.

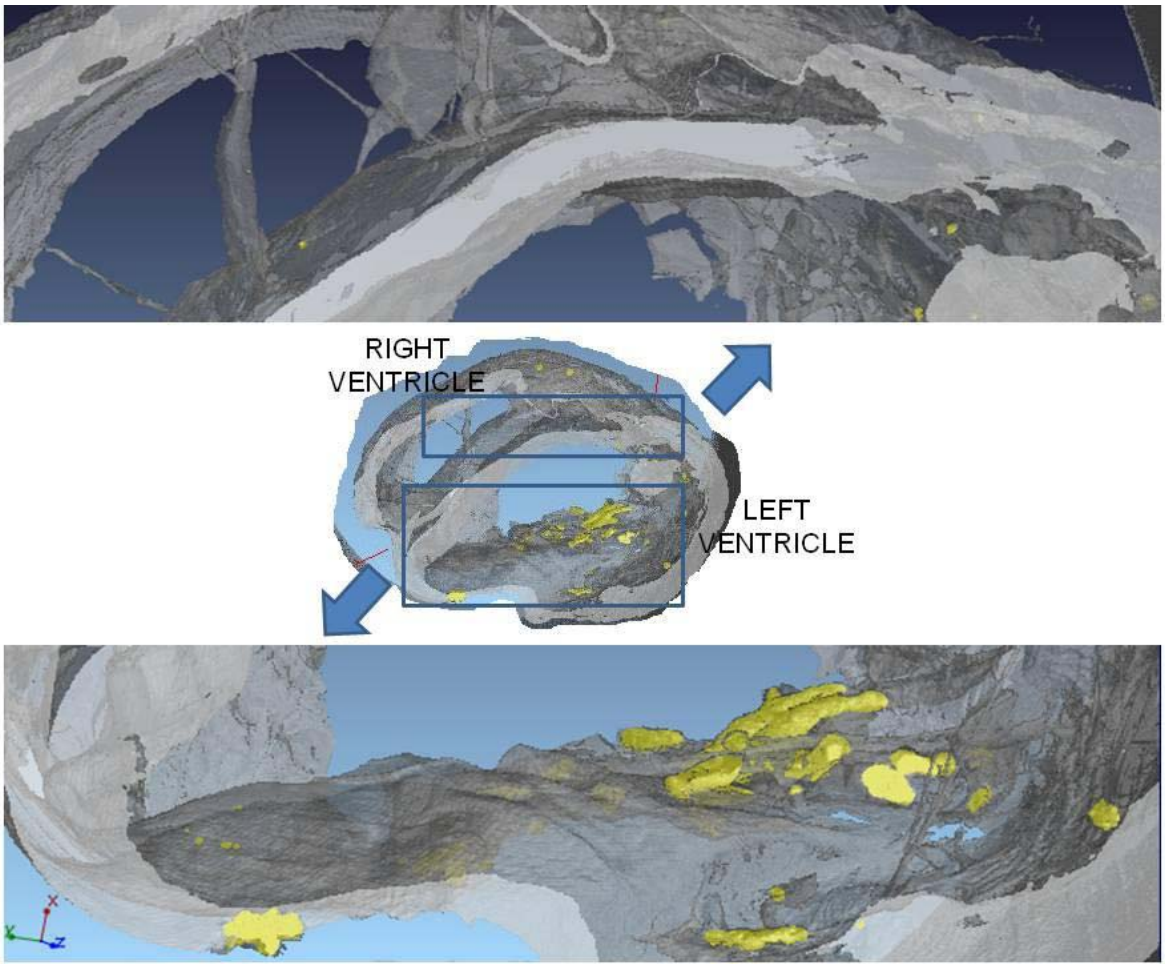

Fig. 2. MicroCT image of the infarcted heart. The yellow finger-like structure corresponds to a high X-Ray absorption coefficient, related to the presence of iron oxide particles. They are interpreted as cell clusters. Single cells are visible in right and left ventricle.

A time dependent analysis could explain this point, to understand the fate of the cells after the therapeutic injection. However, we should note that due to a complicate nature of the investigated object the data treatment is still in progress.

Please note below the references of all papers published during the past 18 months as a result of measurements which have done at the ESRF.

1. Papadimitropoulos A., Mastrogiacomo M., Peyrin F., Molinari E., Komlev V.S., Rustichelli F., Cancedda R. Kinetics of in vivo bone deposition by bone marrow stromal cells within a resorbable porous calcium phosphate scaffold: a X-ray computed microtomography study // Biotechnology and Bioengineering. 2007. V. 98(1). P. 271-281.
2. Cedola A., Mastrogiacomo M., Lagomarsino S., Cancedda R., Giannini C., Guagliardi A., Ladisa M., Burghammer M., Rustichelli F., Komlev V. Orientation of mineral crystals by collagen fibers during in vivo bone engineering: an X-ray diffraction imaging study // Spectra Acta Part B. 2007. V. 62. P. 642-647.
3. Komlev V.S., Mastrogiacomo M., Peyrin F., Cancedda R., Rustichelli F. X-ray synchrotron radiation pseudo-holotomography as a new imaging technique to investigate angio- and microvasculogenesis with no usage of contrast agents // Tissue Engineering. (ahead of print).
4. Albertini G., Giuliani A., Komlev V., Moroncini F., Pugnaloni A., Pennesi G., Belicchi M., Rubini C., Rustichelli F., Tasso R., Torrente Y. Organization of extracellular matrix fibers within PGA/PLLA scaffolds analyzed by X-ray synchrotron radiation phase contrast microtomography // Tissue Engineering. (ahead of print).