Stem Cell Tracking in Preclinical Experiments of Regenerative Cardiology

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RESEARCH ARTICLE

High-resolution X-ray microtomography for three-dimensional imaging of cardiac progenitor cell homing in infarcted rat hearts

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

Recent published reports have contributed to identifying possible cellular therapy approaches to generate new myocardium, involving transcoronary and intramyocardial injection of progenitor cells.

However, one of the limiting factors in the overall interpretation of clinical results obtained by cell therapy is represented by the lack of three-dimensional (3D) high-resolution methods for the visualization of the injected cells and their fate within the myocardium.

This work shows that X-ray computed microtomography may offer the unique possibility of detecting, with high definition and resolution and in ex vivo conditions, the 3D spatial distribution of rat cardiac progenitor cells, labelled with iron oxide nanoparticles, inside the infarcted rat heart early after injection.

Myocardial infarction was produced in the rats and 3 weeks later implantation of 5×10^5 rat clonogenic CPCs (MI-FeCell group) was performed. The cells were labelled with Feridex nanoparticles and supplemented with hepatocyte growth factor (HGF; PeproTech)

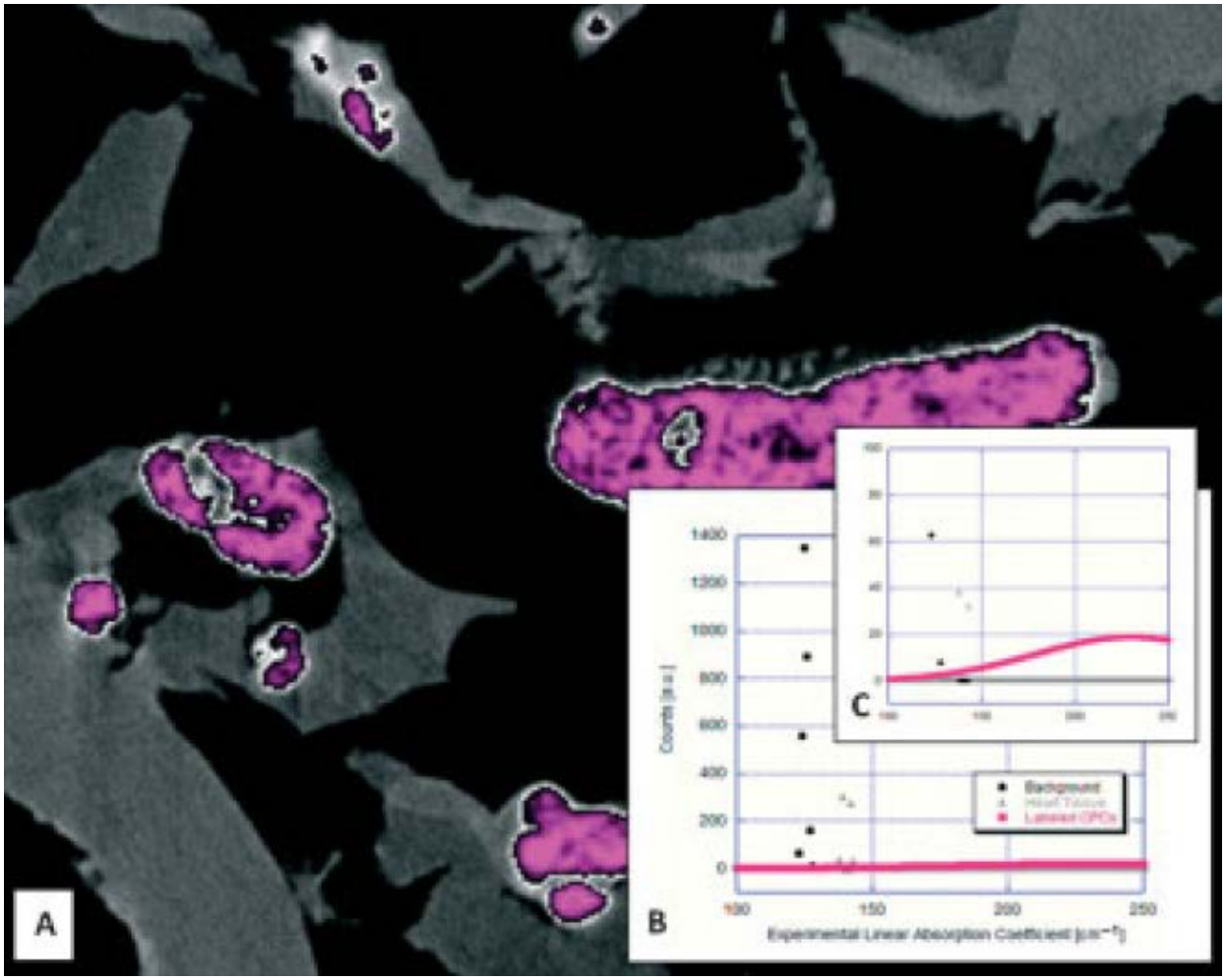
A microCT system [Beamline BM05, at the European SynchrotronRadiation Facility (ESRF), Grenoble, France] was used.

The MicroCT experiment was performed in two modes: with a 15 keV monochromatic X-ray beam and a sample-to detector distance of 25 mm for the absorption-contrast, and 500 mm for the phase-contrast.

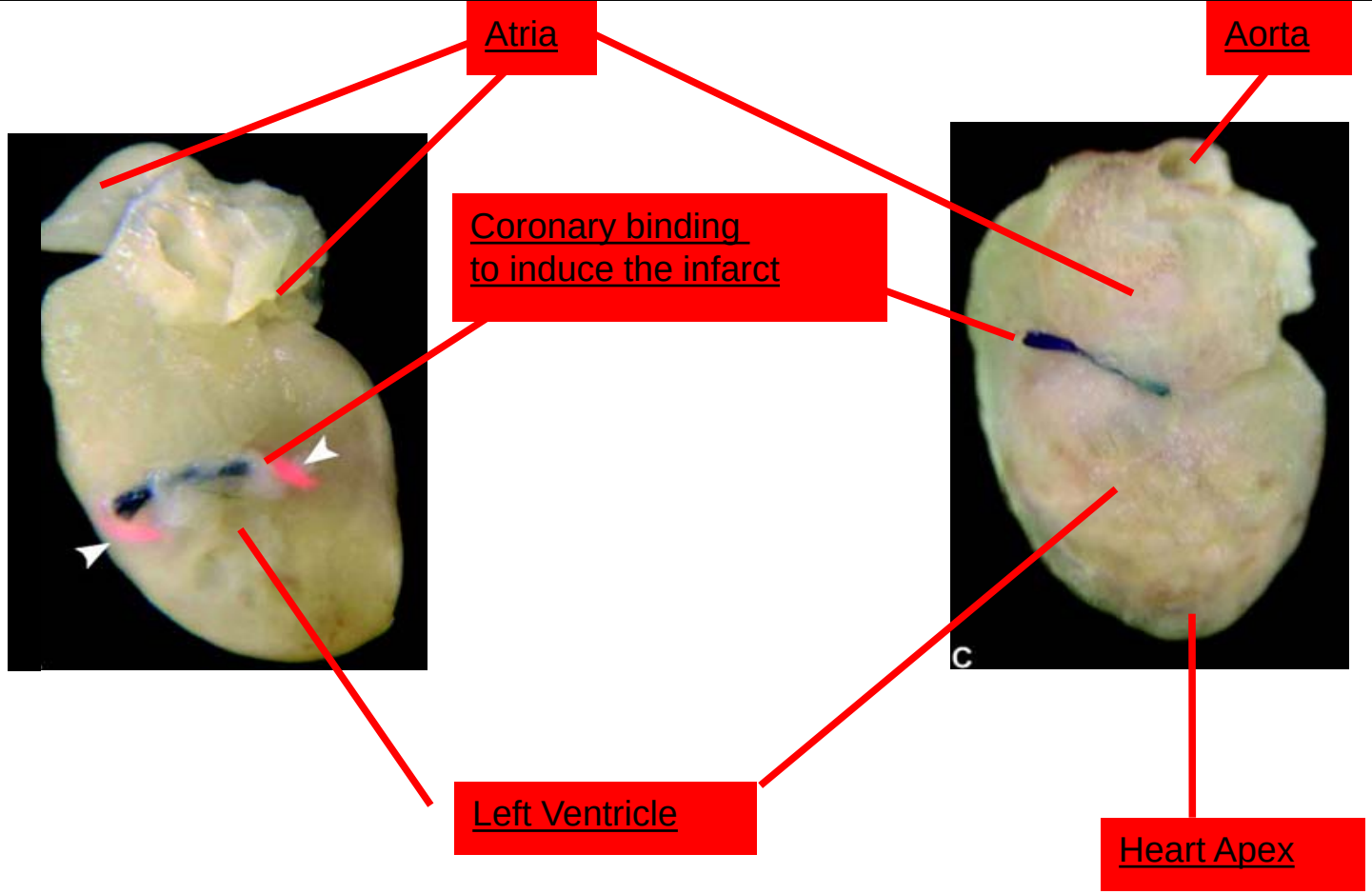
1500 projections and a step of 0.12° were considered for each sample, with an exposure time of 1 *s*/projection.

Absorption and phase-contrast imaging

$$n = 1 - \delta + i\beta$$



(A) Portion of a reconstructed 2D slice. The X-ray attenuation produced by the labelled cells is higher than attenuation referred to the other tissues of the injected hearts, allowing their visualization as bright spots in the 2D images (magenta-coloured spots). **(B)** Histogram of the grey level scale corresponding to the different detected phases. **(C)** Magnification of the portion of the histogram shown in **(B)**, referred to the peak corresponding to the labelled cells' grey level



Infarcted rat heart was injected with $5X10^5$ rat clonogenic cells after loading with iron oxide nanoparticles.

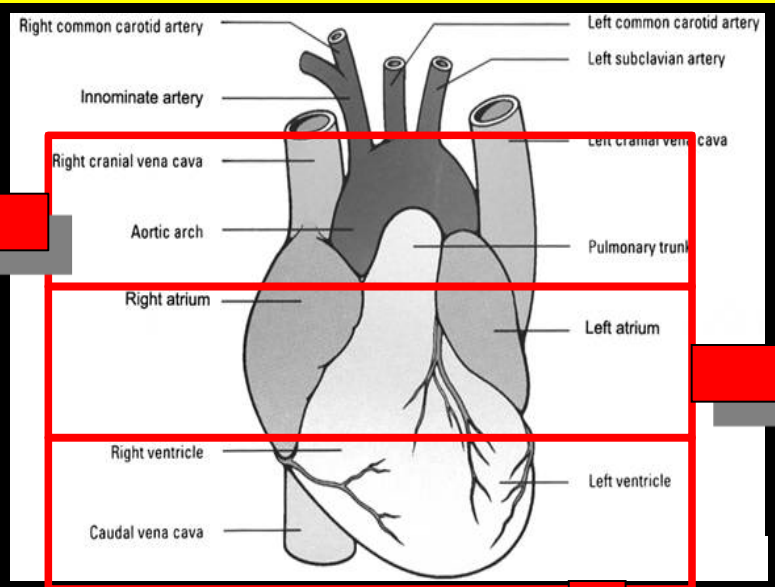
Results obtained

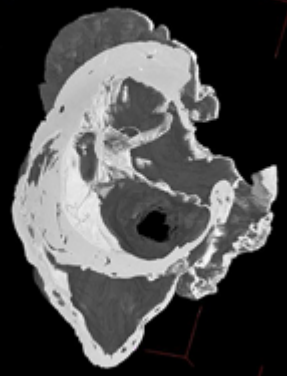
in Absorption Conditions

(spatial resolution: 5.42x5.42x5.42 μm^3)

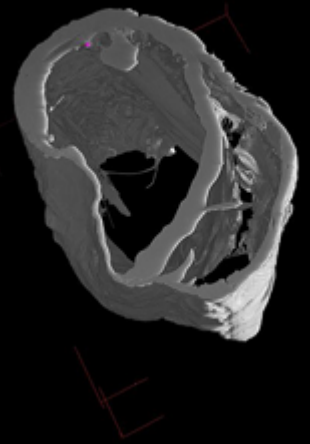
Infarcted Heart analyzed 1 week after injection with $5X10^5$ Rat Clonogenic Progenitor Cells

(previously labeled with Feridex nanoparticles)

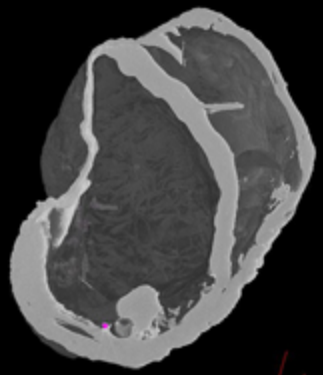




BASE

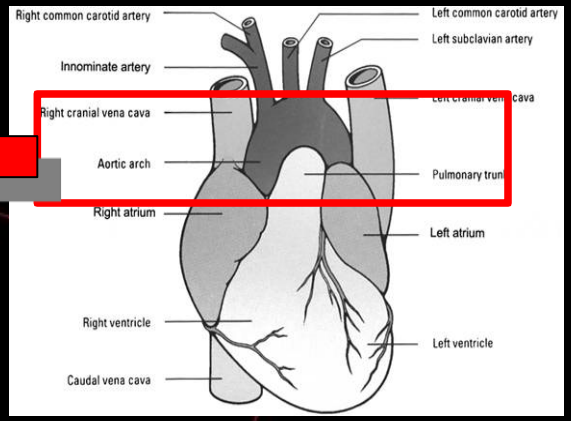


EQUATORIAL PORTION OF THE Heart

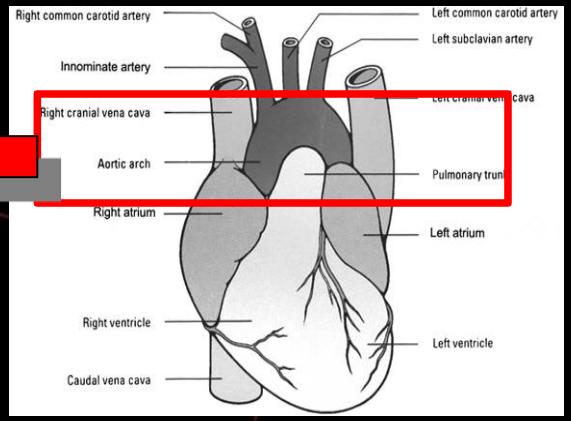


APEX

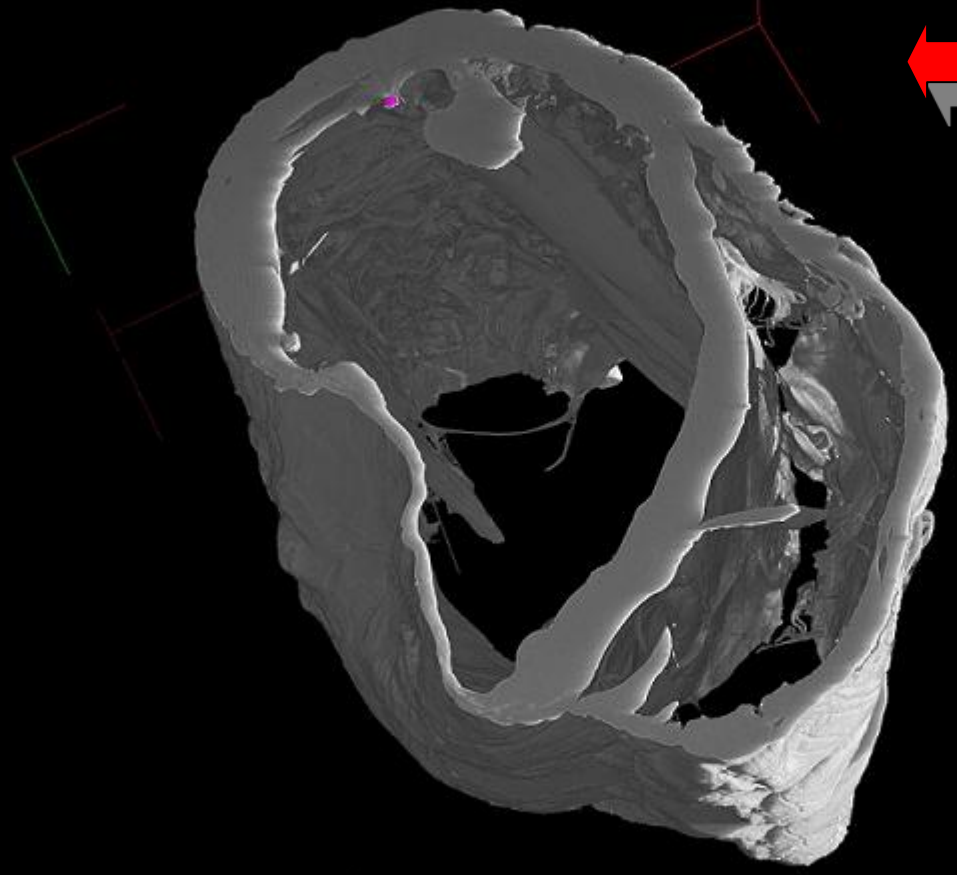
1st Third of the Heart

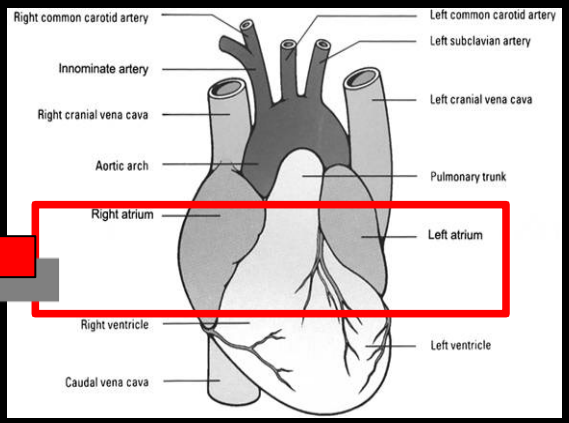


1st Third of the Heart
(just marked cells are visible)

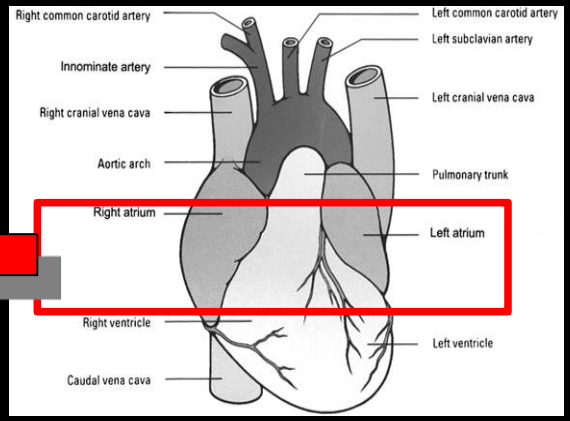


2nd Third of the Heart

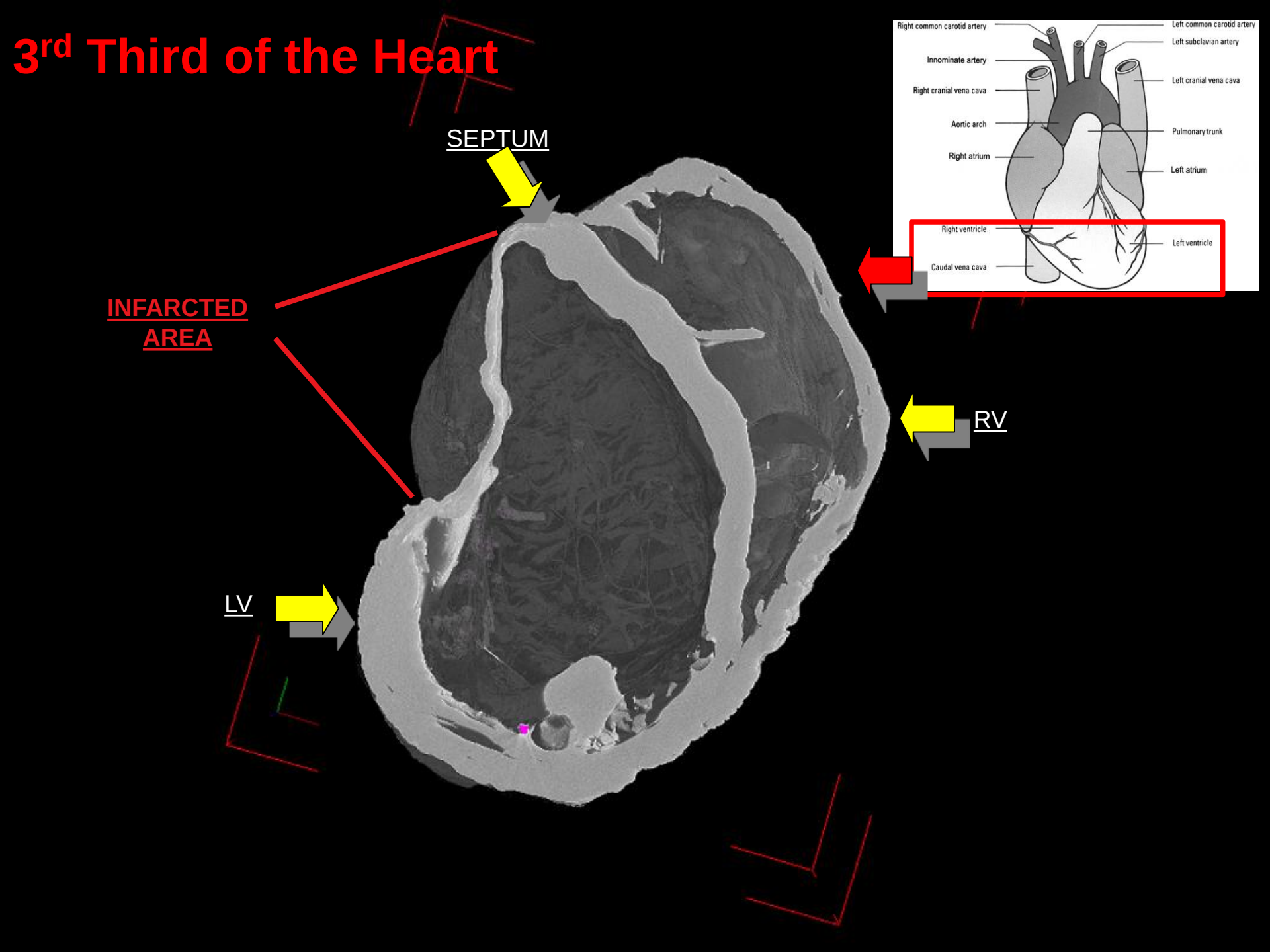




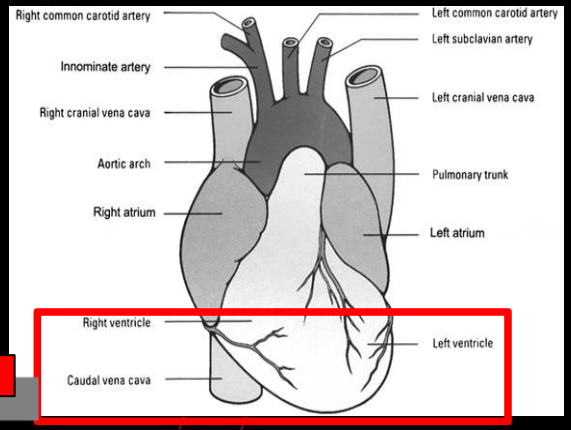
2nd Third of the Heart
(just marked cells are visible)



3rd Third of the Heart



3rd Third of the Heart
(just marked cells are visible)



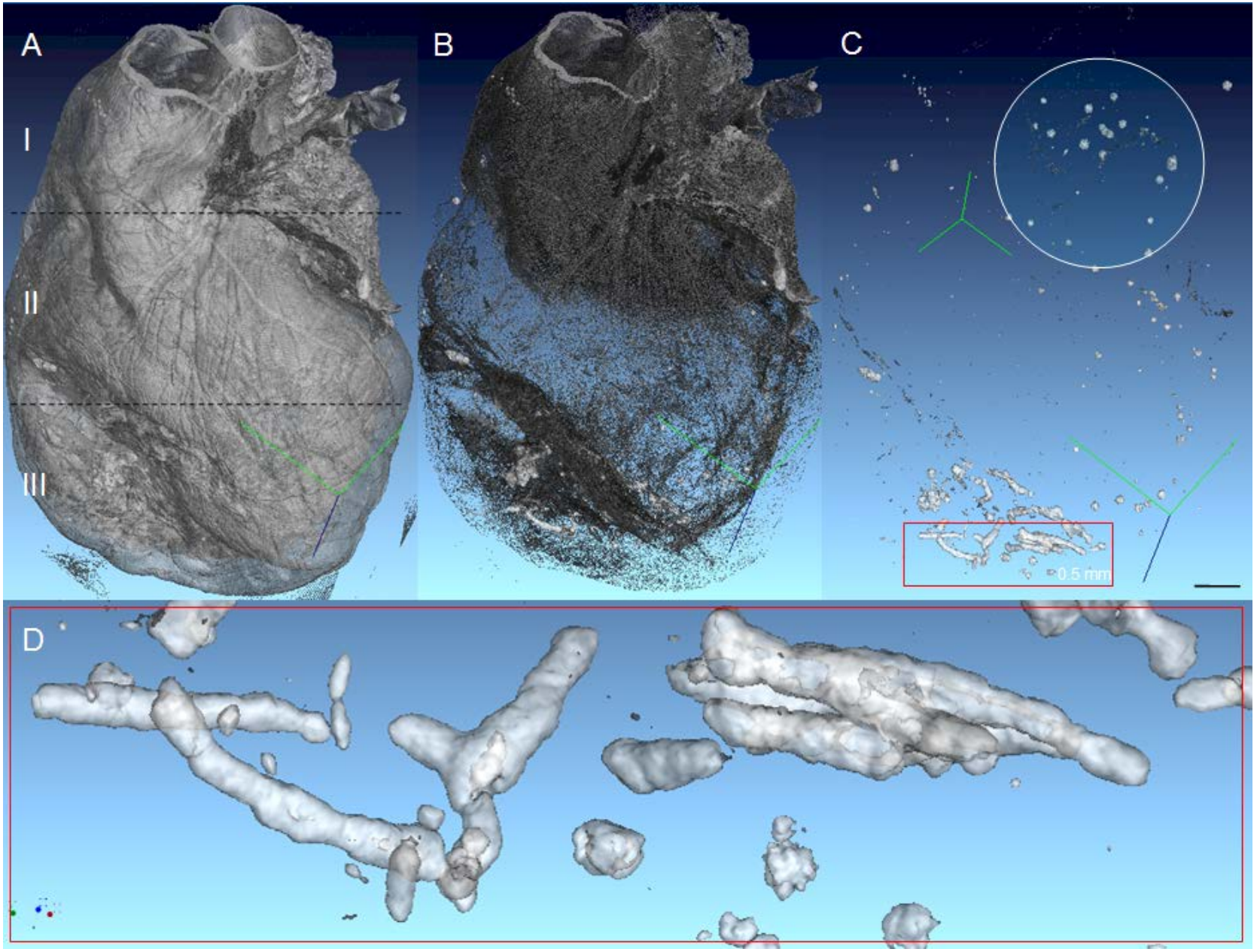
Infarcted Area

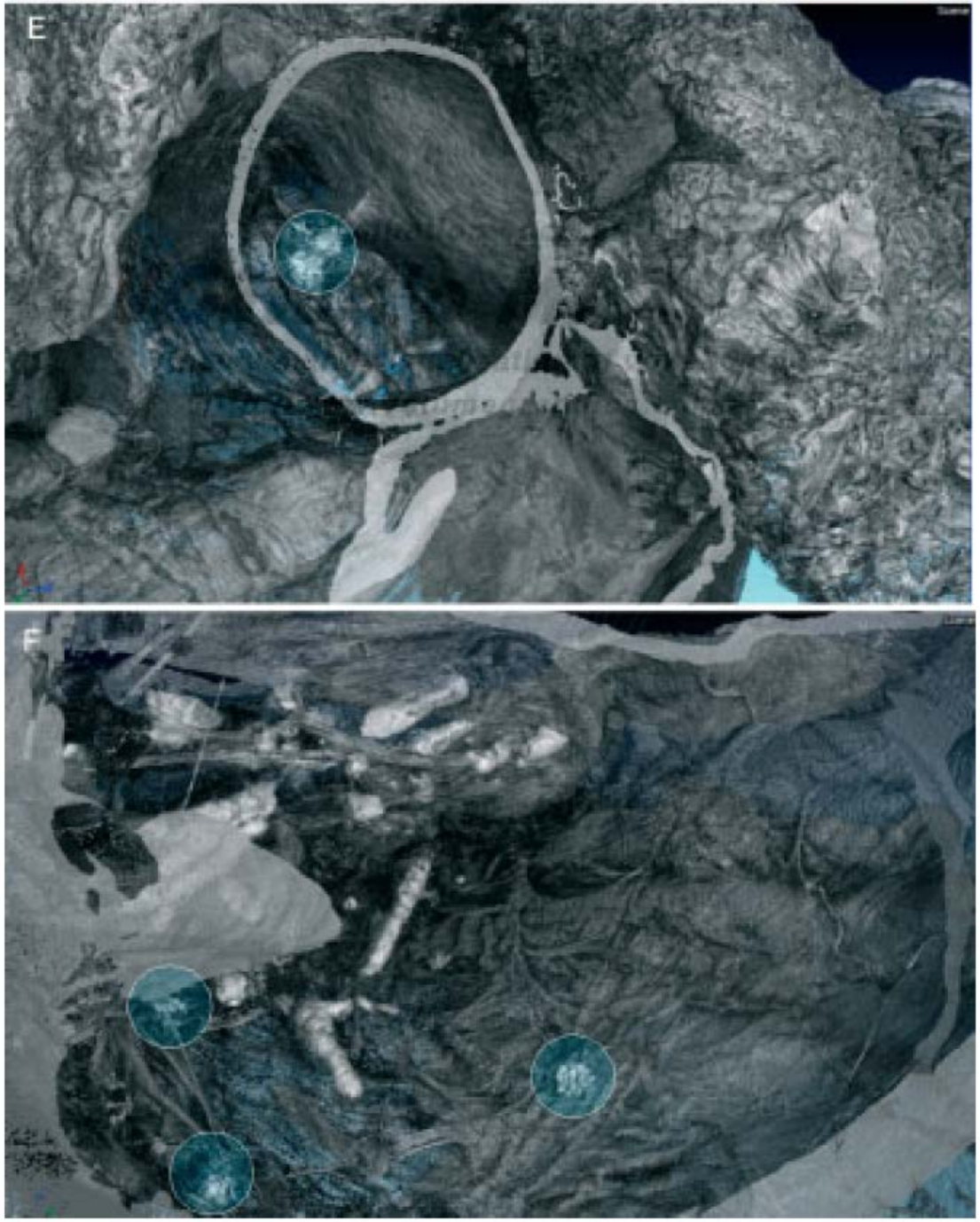
Results obtained combining Absorption and Phase Contrast Data

(spatial resolution: 5.42x5.42x5.42 μm^3)

Refractive index n (X-rays):

$$n = 1 - \delta + i\,\beta$$


A
I
II
III
B
C
0.5 mm
D


E
F

Conclusion

The obtained 3D images represent very innovative progress as compared to the usual 2D histological images, which do not provide the overall 3D distribution of rat clonogenic cells and their progeny within the heart.

The present investigation showed that the distribution of CPCs injected into the infarcted rat heart can be visualized using microCT, providing biological insights into the early processes of cell migration.

This is also potentially interesting for future research on determining the fate of transplanted stem cells in vivo in different cell-based therapies, related to brain pathologies and tumours.

Bacco …

STEM CELLS TRANSLATIONAL MEDICINE

TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Three Years After Transplants in Human Mandibles, Histological and In-Line Holotomography Revealed That Stem Cells Regenerated a Compact Rather Than a Spongy Bone: Biological and Clinical Implications

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Key Words. Clinical trials • Differentiation • Stem cell transplantation • Tissue regeneration • Bone

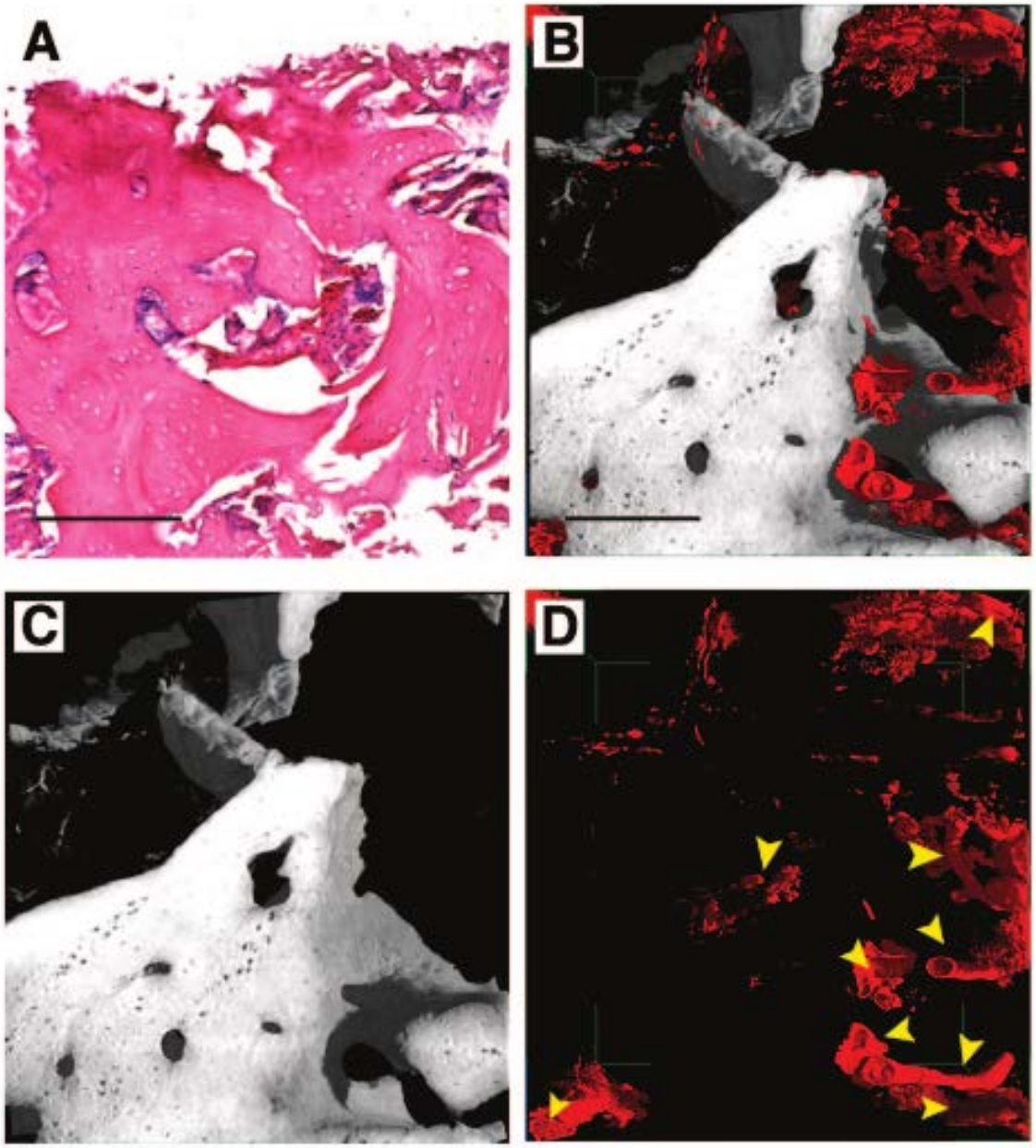
ABSTRACT

Mesenchymal stem cells deriving from dental pulp differentiate into osteoblasts capable of producing bone. In previous studies, we extensively demonstrated that, when seeded on collagen I scaffolds, these cells can be conveniently used for the repair of human mandible defects. Here, we assess the stability and quality of the regenerated bone and vessel network 3 years after the grafting intervention, with conventional procedures and in-line holotomography, an advanced phase-imaging method using synchrotron radiation that offers improved sensitivity toward low-absorbing structures. We found that the regenerated tissue from the graft sites was composed of a fully compact bone with a higher matrix density than control human alveolar spongy bone from the same patient. Thus, the regenerated bone, being entirely compact, is completely different from normal alveolar bone. Although the bone regenerated at the graft sites is not of the proper type found in the mandible, it does seem to have a positive clinical impact. In fact, it creates steadier mandibles, may well increase implant stability, and, additionally, may improve resistance to mechanical, physical, chemical, and pharmacological agents. STEM CELLS TRANSLATIONAL MEDICINE *2013;2:316–324*

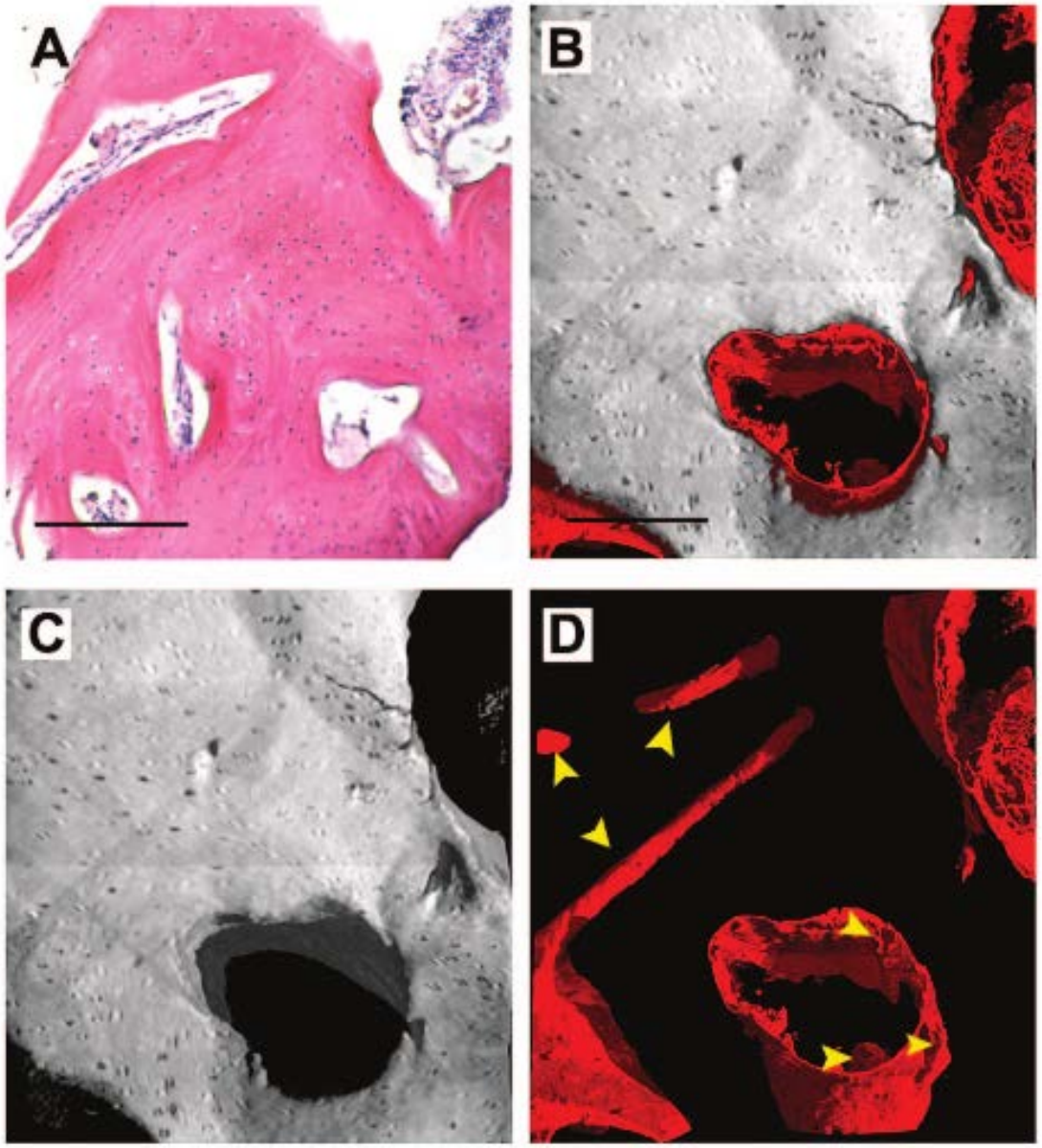
INTRODUCTION

vitro and in vivo in experiments using stem cells and biomaterials [1, 3, 9–11].

Human not-treated mandible



Human in-vivo DPSC-treated mandible



THANK YOU

FOR YOUR ATTENTION