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PAPER

Phase-contrast microtomography: are the tracers necessary for stem cell tracking in infarcted hearts?

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Abstract

Recent literature has identified innovative approaches of cellular therapy to generate new myocardium involving transcoronary and intramyocardial injection of cardiac progenitor cells (CPCs). One of the limiting factors in the overall interpretation of these preclinical results is the lack of reliable methods for 3D imaging and quantification of the injected cells and for the assessment of their fate within the myocardium. Here, for the first time to the authors' knowledge, we support by demonstrative experiments the hypothesis that phase-contrast microtomography (PhC-microCT) could offer an efficient 3D imaging approach to track the injected cells within the myocardium, without the need of any cell tracer. This deduction has been validated by several observations: (i) a strong phase-contrast signal was observed in infarcted hearts injected with unlabeled cells; (ii) the PhC-microCT 3D reconstructions of hearts injected with only vehicle saline solution and rhodamine particles, i.e. without CPCs, did not show any contrast; (iii) in the 3D PhC-microCT reconstructions of non infarcted hearts, injected with unlabeled CPCs, the contrast signal of the cells was present but differently distributed; and (iv) the contrast signal of injected cells diminished over time apparently following the same timing of cell engraftment and differentiation, as confirmed in literature by histology and fluorescence analysis. The chance to avoid cell tracers is of paramount interest in determining the fate of transplanted stem cells because the quantification of the signal will not be any more dependent on injected dose, concentration of the tracer, cell proliferation and tracer uptake kinetics.

1. Introduction

Cardiovascular diseases, in particular myocardial infarction (MI), are the leading causes of death in western world. Heart failure (HF), which is very often a direct consequence of MI (Roger 2013), represents nowadays a major health problem (Mozaffarian *et al* 2015, Benjamin *et al* 2017).

Promising innovative approaches, reported in recent literature, are based on cellular therapy aiming to generate new myocardium by transcoronary and intramyocardial injection of cells.

Different cells have been employed, mainly of bone marrow (Strauer and Steinhoff 2011) and skeletal muscles (Menasché 2011) origin.

Unfortunately, systematic reviews of clinical trials on a large sample described contradictory results, sometimes reporting moderate or even no clinical benefits. Thus, the scientific community is in agreement with the contention that these results require further preclinical research.

In the last 10 years, increasing attention has also been paid to resident cardiac progenitor cells (CPCs), as alternative cell sources for the therapy of ischemic cardiomyopathies and HF. The approach is based on the rationale that the heart contains progenitor cells that, being programmed to generate myocardium, are better equipped to reconstruct the tissue lost with MI (Chamuleau *et al* 2009, Frati *et al* 2011, Leri *et al* 2011, Li *et al* 2012, Nadal-Ginard *et al* 2014, Savi *et al* 2016).

A special class of stem cells resident in the heart are cardiac mesenchymal stem cells (MSCs) (Leite *et al* 2015), which have been referred to in the literature as cardiac mesenchymal stem-like cells (Ryzhov *et al* 2014) and cardiac mesenchymal-like stromal cells (Rossini *et al* 2011, Vecellio *et al* 2012). These MSCs were recently identified in the cardiac stroma (Chong *et al* 2011).

However, regardless of the cell origin, a critical limiting factor that hampers a reliable interpretation of the retrieved results is the lack of efficient methods for the 3D imaging and quantification of the cells injected in the heart and for the detection of their fate within the myocardium.

Immunohistochemical approaches have the strength of being widely available, allowing information to be gained about cell viability, location, and fate as well as quantitative results, but measurements are subjected to variability and sampling errors, because a limited number of optical fields per section is typically examined (Terrovitis *et al* 2010). Moreover, quantitative data are affected by artifacts due to labeling techniques (Laflamme and Murry 2005, Reinecke *et al* 2008).

Bioluminescence imaging presents other limitations mainly linked to the low spatial resolution and the chance to obtain only surface images (Terrovitis *et al* 2010).

Labeling of stem cells with radiotracers has been experimented for studies using single photon emission computed tomography (SPECT) imaging and positron emission tomography (PET) for tracking cells (Hou *et al* 2005, Freyman *et al* 2006). However, SPECT showed some limitations in delivering precise quantitative data, mainly because of the errors derived from photon scattering, while PET, despite being very sensitive, is expensive, not widely available, and usually requires an on-site or nearby cyclotron for production of the necessary tracers (Terrovitis *et al* 2010). In addition, due to the short half-life of clinical grade radiolabeled isotopes, time restrain represents a limiting factor for long-term observations.

The direct radiolabeling limitations were bypassed by the use of reporter genes (Acton and Zhou 2005, Beeres *et al* 2007, Serganova *et al* 2007), but quantification of the signal was found to be not as precise as with PET, because the uptake depends on several factors: number of labeled cells, injected dose, arterial concentration of the tracer, regional blood flow, and tracer uptake kinetics (Su *et al* 2004). Moreover, with reporter gene approaches the tracers have to be injected systemically and in large amount to reach an appreciable detection, with a consequent increase in circulating radioactivity that decreases, in turn, the signal-to-noise ratio.

Cell labeling with iron particles for visualization by magnetic resonance imaging (MRI) is another possibility and one of the most frequently applied methods for cell tracking. However, there are important pitfalls also associated to this technique, because iron nanoparticles released by dead cells are taken up by macrophages, persisting in the myocardium for up to 5 weeks and generating signals that could be misinterpreted as survived cells (Terrovitis *et al* 2010).

Recently, Giuliani *et al* (2011) showed that x-ray computed microtomography (microCT) offers the unique possibility of detecting in *ex vivo* conditions, with high definition and resolution, the 3D spatial distribution of rat cardiac progenitor cells (CPCs), previously labelled with iron oxide nanoparticles, inside infarcted rat heart one week after injection. No x-ray absorption contrast was found within a control rat heart, 1 week after injection of saline solution and rhodamine particles, suggesting that the microCT is able to specifically recognize the migrating labelled cells (Giuliani 2012).

However, there are still unresolved issues related to suitable assessments of myocardial regeneration by the available imaging methodologies. For instance, the inability to detect proliferation of the injected cells could result in an underestimation of cell engraftment due to hyper-dilution, possibly below the detection limit, or unequal distribution of the tracer on dividing cells. Furthermore, the use of nanoparticle tracers was proved to activate macrophages taking up tracers released by dying cells, as shown in by Gianella *et al* (2010) in case of Feridex-loaded endothelial progenitors into ischemic tissues.

Zehbe *et al* (2010) reported for the first time an unexpected evidence, revealing the microCT efficiency in tracking unlabeled stem cells once injected into a tissue or a substrate acting as scaffold. They proposed a rather simple methodology to control neuronal cell growth by applying an electrical potential. Synchrotron Radiation-based (SR) microCT was used to confirm in 3D the efficacy of the method. However, while the authors expected to observe just strongly absorbing deposited gold structures and weakly absorbing polymer substrates, interestingly, the unlabeled cells were well imaged and in good accordance with the fluorescence microscopic images acquired previously.

Furthermore, Albertini *et al* (2009) reported the first experience of SR-microCT study, exploiting the phase-contrast to visualize in 3D the extracellular matrix organization after *in vitro* seeding of bone marrow–derived human and murine mesenchymal stem cells induced to myogenic differentiation, labelled with iron oxide nanoparticles, and seeded onto polyglycolic acid–polylactic acid scaffolds (PGA-PLLA).

Indeed, conventional microCT relies on attenuation of the x-ray beam intensity when passing through the sample, while in PhC-microCT the beam phase shift caused by the sample is not measured directly, but is transformed into intensity variations (Snigirev and Snigireva 1995, Bravin *et al* 2013). This phase shift in non-mineralized biological tissues can be up to three orders of magnitude larger than attenuation (Momose *et al* 1996, Lewis *et al* 2003), explaining the highly increased contrast that has been observed with PhC-microCT, investigating different tissues and organs (Giuliani *et al* 2017).

Giuliani *et al* (2014) used the phase-contrast imaging to study *in vitro* cultures of different human progenitor cells, i.e. endothelial colony-forming cells from healthy controls and patients with Kaposi sarcoma and human CD133+ muscle-derived stem cells seeded onto PGA-PLLA. The method, despite the cells had not been labelled with any tracer, enabled to detect with high spatial resolution the 3D organization of cells on the bioscaffold and evaluation of the way and rate at which cells modified the construct at different time points from seeding.

Moreover, Fratini *et al* (2015) demonstrated that high-resolution x-ray phase contrast tomography is able to simultaneously visualize, in 3D and in *ex vivo* conditions, the micro-capillary network and the micrometric nerve fibers, the axon bundles and the neuron soma of the mouse's spinal cord. More recently, Bukreeva *et al* (2017) performed an innovative spatial statistical analysis on the motor neurons to obtain quantitative information on their 3D arrangement in healthy-mice spinal cord and in a mouse model of multiple sclerosis. It is interesting to note that all these results were obtained with scales ranging from millimeters to hundreds of nanometers, without contrast agents and in non-destructive ways.

Thus, in this demonstrative investigation, basing on the studies previously reported, we discuss the rightness of the x-ray phase-contrast microtomography (PhC-microCT) approach to track unlabeled cells within the myocardium.

2. Materials and methods

Ten Wistar male rats (12/14 weeks old, 350/400 g) were involved in the study and were kept, fed and sacrificed following a procedure approved by Veterinary Animal Care and Use Committee of the University of Parma, in compliance with National Ethical Guidelines (Italian Ministry of Health; DL.vo. 116, 1992) and the Guide for Care and Use of Laboratory Animals (NIH publication n. 85-23, revised 1996). In order to induce myocardial infarction in the left ventricle, a thoracotomy in correspondence of third intercostal space and a ligature around the left descending coronary vessel were performed, as reported by Giuliani *et al* (2011). Three weeks after surgical intervention, the rat chest was opened again to directly inject 5×10^5 stem cells in the ligature site.

Five animals received injection of rat cardiac progenitor cells (rCPCs), the remaining five ones the same quantity of human cardiac stromal cells (hCStCs). The rCPCs were obtained from atrial auricle of Wistar rats, as reported by Giuliani *et al* (2011), while the hCStCs were collected from the same site of donor patients undergoing cardiac surgery under the approval of Local Ethics Committee and after informed consent obtained in accordance with the Declaration of Helsinki (Rossini *et al* 2011).

Four cell manipulation protocols were adopted for both rCPCs and hCStCs: in the first one the cells were labelled with iron oxide nanoparticles (Feridex), in the second cells were cultured with Insulin-Transferrin-Sodium Selenite (ITS) growth factor, while the last two situations consisted in total absence or combined use of Feridex and ITS.

Times-to-sacrifice were chosen 24–48 h, 12–13 days, 21 days and 30 days after cell injection.

2.1. X-ray phase-contrast microtomography (PhC-microCT)

MicroCT experiments were carried out at the beamline ID19 of the European Synchrotron Radiation Facility (ESRF) of Grenoble, France. The photon energy was set to 15 keV with a multilayer monochromator (Weitkamp *et al* 2010). 2000 projections of 2048×2048 pixels each (pixel size of 5 μm) were acquired at equidistant angular positions over 360°. The exposure time for each projection was 0.3 s. The scans were performed on the intact rat hearts at a z distance between specimen and detector of 25 mm. The phase-contrast can be recovered from a single sample-detector distance under the assumption of a single material specimen and monochromatic illumination. Thus, phase retrieval was performed using the software ANKAphase (Weitkamp *et al* 2011) which is based upon an algorithm presented by Paganin *et al* (2002), assuming that

$$z \ll \frac{d^2}{\lambda}$$

where d is the size of the smallest objects identifiable in the specimen and λ is the x-ray wavelength. In this experiment $d \sim 10\ \mu$m and $\lambda = 0.826$ Å: therefore, the z distance was chosen according to the rationale that it should have been much smaller than 1200 mm.

3. Results

Figure 1(A) shows a schematic representation of the mechanisms through which CPCs are imaged by propagation-based PhC-microCT. The injected CPCs, at an initial stage, are not integrated with the hosting myocardium, as shown in figure 1 (panel A-I). Pronounced edge enhancement can be seen by imaging the injected CPC at a sample-detector distance included in the Fresnel region (Mayo *et al* 2002,

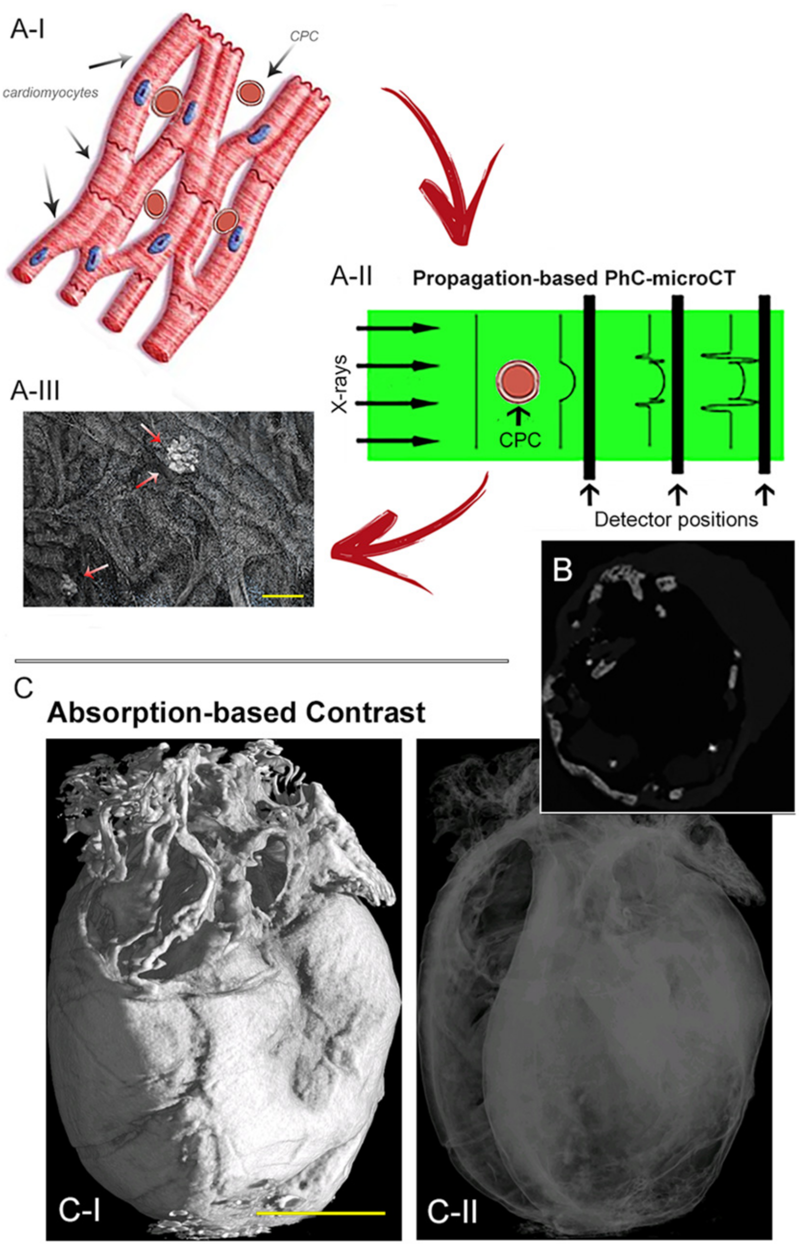

Figure 1. Evidences to confirm that the observed signal was actually due to phase contrast phenomena. In the case shown here, cardiac progenitor cells (CPCs) were rat-derived: analogue qualitative results were obtained for human-derived CStCs (data not shown). (A-I) Schematization of the investigated myocardium. The injected CPCs, at the stage here illustrated, are still undifferentiated and not integrated with the hosting tissue. (A-II) Propagation-based PhC-microCT: the detector is placed at a suitable distance from the sample in order to allow the detection of the typical edge-enhanced profile of the CPCs. (A-III) 3D Reconstruction of a rat heart subvolume (inner wall of the infarcted left-ventricle). The unlabeled CPCs are clearly visible inside the damaged ventricle, as indicated by the red arrows. Bar: 50 μm. (B) An axial slice, 5 μm of thickness, of the infarcted portion of a rat heart treated with unlabeled rCPCs. The axial section was reconstructed without the application of the Paganin filter to evaluate the edge-enhancement. (C) Infarcted rat heart injected with rCPCs, previously not labeled with tracers, observed by means of a conventional (absorption-based) microCT device (Skyscan 1174-Bruker, Kontich, Belgium). Solid (C-I) and semi-transparent (C-II) 3D reconstructions. The unlabeled CPCs are not visible anymore. Yellow bar: 300 μm.

Gureyev 2003) (figure 1, panel A-II). Thus, as shown in the 3D reconstruction of a rat heart subvolume—corresponding to the inner wall of the infarcted left-ventricle (figure 1, panel A-III)—the unlabeled CPCs (indicated by the red arrows) are clearly visible inside the damaged ventricle.

Indeed, the axial sections, reconstructed without the application of the Paganin filter, showed the presence of structures, *white spots*, mainly concentrated in the infarcted area of the ventricle (figure 1, panel B). This observation is not justified by any evident scientific motivation, since the cells had not been labeled with tracers before injection.

Moreover, this contrast cannot be explained arguing that the sample preparation procedure was affected by contaminants with high atomic number Z. Indeed, the non-infarcted heart of a control rat, injected with only vehicle saline solution containing rhodamine particles (but not cells) and prepared and sacrificed with the same procedure, did not show any contrasted signal in the whole heart, as shown and demonstrated by Giuliani (2012).

This unexpected signal was found to be due to phase contrast, using a comparative test performed on a laboratory-based microCT device. An infarcted rat heart injected with 5×10^5 rCPCs, previously cultivated in the absence of the Feridex tracer, was studied by means of a Skyscan 1174 (Bruker, Kontich, Belgium), with a voltage of 50 kV, no filter and pixel dimensions of 9.5 μm (figure 1, panel C-I). In fact, in conventional radiological imaging, image formation is based on differences in the absorption of sample details, i.e. dense structures absorb more than soft tissues. The conventional microCT is based on the mapping of the linear attenuation coefficient of x-rays that cross the studied sample, where the attenuation depends on the density of the object. Thus, if the white spot signal had been due to absorption, these spots would have been illuminated even using conventional microCT. Instead, as shown in figure 1, panel C-II, the strong contrast is no longer present in the 3D reconstruction, when the myocardium has been virtually rendered translucent to visualize the internal structures. This observation confirmed that the white spots were due to phase contrast.

We have also shown that the signal is related to the presence of the unlabeled injected cells with multiple evidences.

A photo of an infarcted rat heart and the PhC-microCT 3D reconstructions of three examined rat hearts were shown in figures 2(A) and (B)–(D), respectively.

First, it was found that the contrast signal was present (figure 2(B)) also in a non-infarcted rat heart, injected with the same CPCs amount of the infarcted rats. In this case, the signal was more homogeneously distributed within the heart, likely because the injected cells were not attracted by any injury.

Secondly, as investigated and described in Giuliani *et al* (2011), to further document cell homing, rCPCs were loaded, before their injection, with 585 Quantum Dots (QDot). In tissue preparations, rCPCs were found in all regions of the heart, although QDot-fluorescence signals were accumulated and uniformly distributed in the infarcted and peri-infarcted regions. Moreover, evidences of rCPC homing and engraftment have been also supported by Savi *et al* (2016) and a further example is reported in figure 3.

Third, the demonstrative study on the 10 infarcted rat hearts injected with CPCs seems to confirm, also on a quantitative level, that the observed phase-contrast signal derives from the injected cells. Indeed, we introduced three criteria of inclusion for the groups-of-study identification: (1) cell nature (rat CPCs or human CStCs), (2) treatment protocol, and (3) time from cell injection to rat sacrifice. The first assessment was performed fixing the former two variables, in order to study the effect of time from cell injection. Quantitative results are shown in figure 4.

In the early phase, between 24–48 h and 12–13 days, there was a clear increase of the aggregate cell volume in hearts injected with rCPCs, previously cultured with ITS and independently to the presence of the Feridex contrast agent, possibly indicating a proliferation of cardiac progenitor cells and/or an activation of the endogenous stem cells. Interestingly, the cell volume detected in the infarcted area decreased with time, when cells were previously labeled with Feridex.

In case of hearts injected with hCPCs, previously cultured with ITS and Feridex, the aggregate cell volume was confirmed to increase during the first 21 days, with an important reduction from 21 to 30 days from the cell injection. Coherently with the previous case, the cell volume detected in the infarcted area decreased with time, when cells were previously labeled with Feridex.

The aggregate cell volume was confirmed to decrease, from 21 to 30 days after the cell injection, also in the case of rat hearts injected with hCStCs, previously cultured with ITS and without Feridex. The 3D reconstructions are shown in figure 2 at 21 days (panel C) and 30 days (panel D) from cell injection. Notably, the overall cellular volume in this experimental condition reached, at 21 days from hCStCs injection, the amount of 5.3×10^9 μm^3, more than 3-fold greater than in hearts injected with hCStCs, cultured with ITS and Feridex, at the same time-point (figure 4).

4. Discussion

The propagation-based PhC-microCT (Snigirev and Snigireva 1995, Wilkins *et al* 1996, Cloetens *et al* 1999), by placing the detector and the sample at a moderate distance, produces contrast by Fresnel diffraction and is usually preferred to other phase-contrast methods when highly-resolved images are desired, because no optical components are necessary if a coherent x-ray source, like synchrotron light, is used.

In the present study, this method enabled us to track unlabeled CPCs injected in infarcted rat hearts at different time points, with fundamental information about their kinetics, confirmed in literature by histology and fluorescence analysis.

Our observations strongly support the following hypothesis:

1. The phase contrast is able to discriminate the myocardium from the injected cells due to changes in electronic density and/or misalignments in the form and lack of integration (engraftment) between the regular myocardial pattern and the injected cells;

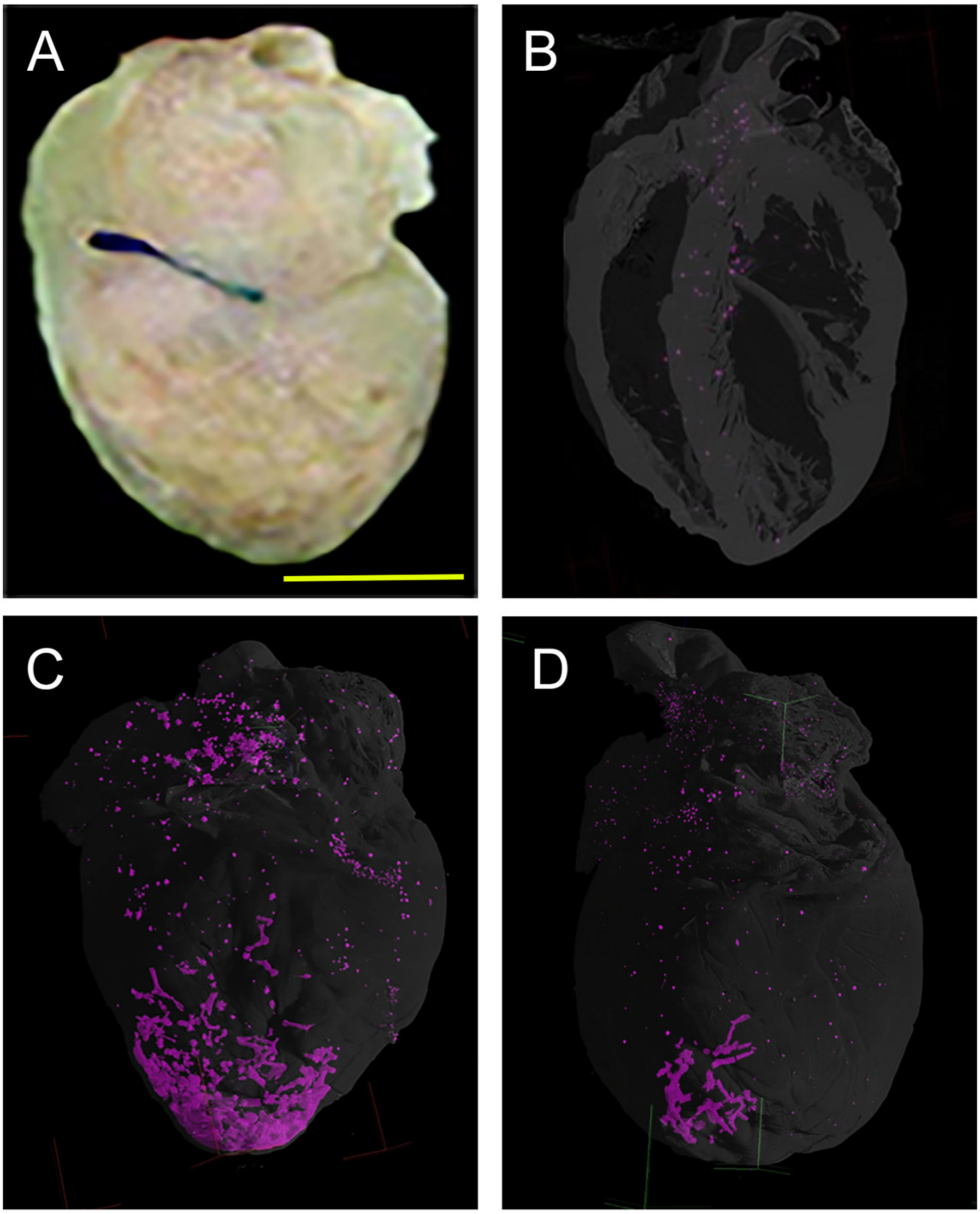

Figure 2. (A) Example of an investigated rat heart. The infarct was induced by binding of descending coronary vessel, as shown in the photo. Yellow bar: 300 μm. (B)–(D) Propagation-based PhC-microCT images: semi-transparent 3D reconstructions. (B) Longitudinal portion of a non-infarcted rat heart injected with CPCs, 1 week after the injection. The CPCs are clearly visible but with a more homogeneous distribution within the heart respect to the infarcted samples; (C), (D) infarcted rat heart injected with human cardiac stromal cells (hCStCs) not labeled with tracers but previously cultured with ITS (panel C at 21 days, panel D at 30 days from cell injection). Semi-transparent gray: myocardium; magenta: CPCs.

2. The aggregate cell volume increases for the first 21 days from cell injection, possibly indicating a proliferation of the injected cells and/or an activation of the endogenous stem cells;

3. The aggregate cell volume decreases from 21 to 30 days from cell injection, possibly indicating the completion of differentiation and cell integration in the myocardium. This observation is in line with a previous report (Rossini *et al* 2011), showing that after 21 days from injection in the same rat model of chronic MI, a fraction of the injected CStCs exhibited evident sarcomere striation and volume compatible with that of adult cardiac myocytes;

4. Feridex nanoparticles seem to interfere with the spontaneous regeneration process activated by CPCs, possibly because of an overlapping action of macrophages, as previously observed by Gianella *et al* (2010);

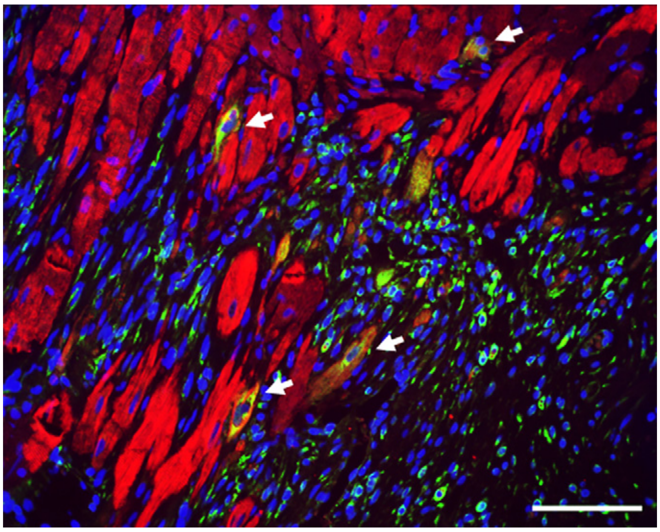

Figure 3. Myocardial regeneration in the peri-infarcted area of a rat heart injected with Green Fluorescence Protein (GFPpos) rat CPCs. Green fluorescence corresponds to GFP, red fluorescence to α-sarcomeric actin (α-SA). Arrows indicate cardiomyocytes generated by GFPpos CPCs, showing by yellowish fluorescence the co-expression of GFP (green) and α -SA (red). Resident spared cardiomyocytes are labelled by α-SA only. The blue fluorescence corresponds to DAPI staining of nuclei. Scale bar $= 50\ \mu$m.

5. ITS seems to enhance tissue regeneration, possibly favoring cell engraftment, as shown by Terrovitis *et al* (2010), and CPC proliferation.

Giuliani *et al* (2011) demonstrated by two alternative imaging techniques, based on different tracers, the ability of microCT to trace cells from the injection area to the infarcted area: the first was based on labeling the injected cells with QDots nanocrystals, the second was dependent on a genetic marker to visualize the progeny of the injected cells in the infarcted heart.

However, all these tracers influence a reliable quantitative analysis for the different reasons previously described and there are not, to date, alternative diagnostic techniques to PhC-microCT that do not require the use of tracers.

Thus, the full comprehension of the origin of the contrast in the injected cells is not trivial. In principle, two scenarios can originate edge-enhancement in PhC-microCT: the lacking of integration (engraftment) of the injected cells in the regular myocardial pattern and/or mismatches in electronic density between the injected cells and the surrounding tissues. While the first condition is certainly present, because we investigated the hearts at early stages after the CPCs injection, i.e. before the necessary times for a complete grafting, we strongly suspect that the contrast is mainly linked to mismatches in electronic density due to the presence of matrix metalloproteinases (MMPs) all around the injected stem cells. MMPs, also called matrixins, act in the extracellular environment of cells, degrading both matrix and non-matrix proteins. They were proved to have a fundamental role in morphogenesis, wound healing, tissue repair and remodeling after an injury, as in the case of a myocardial infarction (Nagase *et al* 2006).

Shen *et al* (2016) elucidated the effects of MMP inhibition on the therapeutic benefits of intramyocardial injection of platelet fibrin gel spiked with cardiac stem cells in a rat model of acute MI. In a syngeneic rat model of myocardial infarction, MMP inhibition blunted the recruitment of endogenous cardiovascular cells into the injected biomaterials, therefore hindering de novo angiogenesis and cardiomyogenesis.

However, these interactions are not fully understood and warrant further investigation, not only to better understand the origin of the edge-enhancement contrast but also and above all for their application as therapeutic tools to treat different diseases, including MI.

In this direction, our observations have two limitations: the small number of samples, which hinders any statistical speculation, and the absence of systematic assessments of the infarcted area, necessary to make a completely reliable quantitative comparison between the samples.

However, the calculated cell volumes were significantly and systematically different, depending on the different experimental parameters (time from the injection of the cells to the sacrifice, presence/absence of contrast medium and type of medium). Thus, this study deserves an accurate experimental verification using appropriate statistical approaches.

5. Conclusions

We have shown that PhC-microCT is an imaging method of fundamental interest in determining, in *ex-vivo* conditions, the fate of transplanted stem cells. The chance to avoid cell tracers, will substantially improve the reliability of quantitative analysis, being

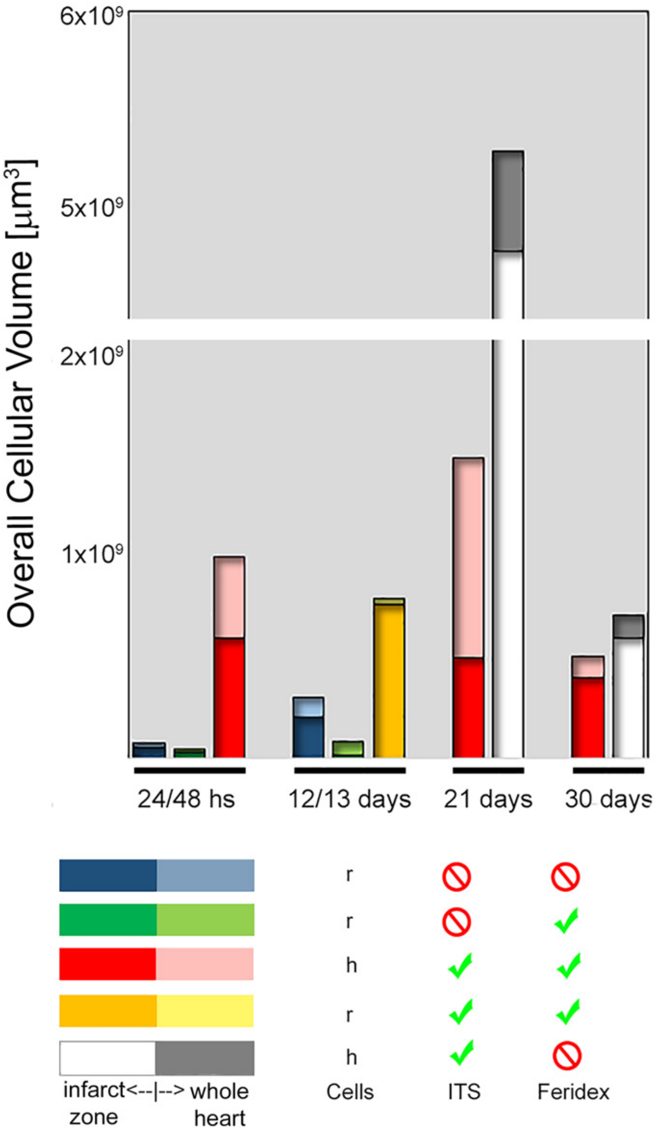

Figure 4. Overall cellular volume classified per group-of-study, basing on the conceptual legend in the bottom. h: human-derived CStCs; r: rat-derived CPCs.

the signal not anymore dependent on injected dose, concentration of the tracer, cell proliferation and tracer uptake kinetics. Hearts have been studied in *ex-vivo* conditions for several reasons: to increase the resolution for the injected cells detecting, to avoid movement problems due to the heartbeat and, last but not least, to avoid problems due to the x-ray dose.

However, the exploitation of phase contrast tomography promises to offer the interesting possibility of creating a fundamental change in medical radiological imaging. A considerable number of experiments have already demonstrated a significantly improved contrast compared to conventional methods, revealing soft tissue discrimination at micro and nanometric resolutions, at somewhat lower doses than those required by conventional radiological imaging.

Although the use of synchrotrons has revealed the possibilities offered by PhC-microCT, unfortunately the application of these ideas in a clinical context requires that the tomographic technology will be further improved in several areas, including x-ray sources, optics and detectors (Lewis 2004). Indeed, longitudinal studies at synchrotrons are currently hindered by the limited synchrotron accessibility (due to competitive application procedures for beamtime). In contrast, benchtop microCT devices usually present small-scale experimental configurations, i.e. comparable in size to the diagnostic CT scanners: therefore, they could be installed directly on the site where the research is performed, resulting in an improved accessibility.

Several different x-ray phase contrast settings have been developed over the last years to be implemented in laboratory microCT systems. Most of them (Propagation-based imaging, Grating interferometry, Edge illumination and Zernike phase contrast) were firstly realized for synchrotron radiation-based tomography and later modified for use with standard x-ray tubes.

They are mainly used to image soft (non-mineralized) tissues, often resolving the internal micro-morphology of stained and unstained tissues as well as the 3D distribution of cells within engineered constructs (Shearer *et al* 2016). Moreover, benchtop x-ray nanotomography was shown to efficiently resolve several native tissues and organs, allowing to also perform histological and immunohistochemical analysis after the microCT, showing that the exposure to x-rays and some x-ray contrast agents seem not affect the submicron morphology (Walton *et al* 2015).

However, the implementation of x-ray phase contrast tomography outside synchrotrons, to date, still presents several problems: x-rays offer limited spatial and temporal coherence, source drifts or environmental vibrations could affect the scanning and, main problem in the experimentation described in the present study, it is not possible to achieve sufficiently fast scan times because of the relatively low flux emitted by x-ray tubes. Indeed, laboratory-based phase contrast tomography still fails in achieving fast acquisitions, dramatically affecting the *in vivo* imaging of the heart due to cardiac motion.

The recent literature suggests that the previously mentioned restrictions of laboratory-based phase contrast tomography could be overcome in due course. Indeed, as discussed by Marenzana *et al* (2014), additional improvements may lead to a new generation of coded-aperture x-ray phase-contrast microCT scanners, suitable for *in vivo* longitudinal pre-clinical imaging of soft tissue and with resolutions significantly higher than the current magnetic resonance imaging.

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