ESRF	**Experiment title:** Intra-arterial transplantation of human AC133+ stem cells in muscular dystrophy: experimental study by MicroCT	**Experiment number**: MD-182
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Report:

The hope that many diseases can someday be treated with stem cell therapy is inspired by the historical success of marrow transplants in increasing the survival of patients.[1,2] Recently, it has been shown that hematopoietic $AC133^+$ stem cells could be used in curing the Duchenne muscular dystrophy.[3,4] Thus, one of the main reasons for studying these stem cells is their applicability in the therapy of neuromuscular disorders. Some $AC133^+$ cells became satellite cells, a type of cell present around muscle fibers that can move into the fiber and become muscle when repairs are needed. The circulating stem cells that can enter muscle via circulation may play role in maintenance or repair of muscle and it is clearly of interest to find ways of increasing their effectiveness.

One of the most promising approaches to understand the basic processes within the cell is magnetic nanoparticles labeling. The use of nanoparticles for biomedical applications, e.g. (a) as a tool for cell-biology research to separate and purify cell populations; (b) tissue repair; (c) drug delivery is a very promising tool.[5-8] In fact, traditional methods for evaluating biological cells are based on two dimensional (2D) techniques such

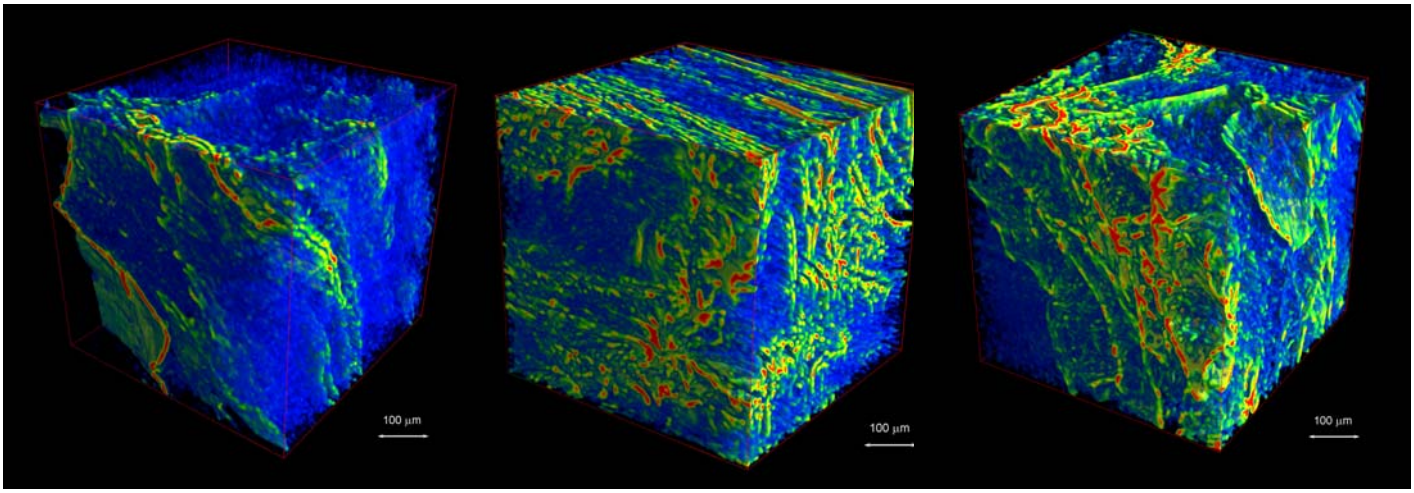
as histology, scanning electron and fluorescence microscopy imaging. Three-dimensional (3D) structure data and 3D quantitative analysis are difficult to obtain. Such techniques provide either integral information about the content of magnetic material along the beam direction or a relative local snapshot about the magnetic particle distribution limited by the number of histological cuts.[9] However, compared with traditional e.g. histology, magnetic resonance imaging (MR) microscopy enables a straightforward 3D characterization of samples. The data format is numeric in nature and more quantitative. It is free from sectioning-related artifacts and is much less labor-intensive. Furthermore, it is non-invasive and compatible with other techniques. The main disadvantage, what it cannot measure up to compared with histology is spatial resolution and the variety of contrasts. The resolution limitation of MR microscopy is believed to be about 10 μm which is coarser than histology and not high enough to examine morphology at least on cellular level.[10]

In the present work, we used X-ray computed microtomography (microCT) as possible experimental technique for 3D visualization of the presence of magnetic nanoparticles with resolution of about 1.6 μm. In the proposed experiment after the localization of the human steam cells within the mouse muscle, biopsies (each 4mm×4mm×4mm) were performed and the obtained specimens were analysed by microCT as described afterwards. Nine samples (obtained from nine equivalent mice's) were investigated, corresponding to three different times of observations after injection (namely 2, 12 and 24 h) and three different stem cell numbers (namely 50, 100, 500 X 10^3 cells). The presence of magnetic nanoparticles within specimens was also verified by conventional histology.

The MicroCT experiment was performed at ESRF with the following operating conditions: monochromatic beam; sample-to-detector distance 50 mm; detection system: Gadox scintillator associated to FReLoN CCD camera. The exposure time was 2 seconds per projection. The X-ray energy is an important parameter to obtain optimal conditions for the X-ray absorption contrast of the different phases contained in the investigated samples, and therefore for the quality of the images and the accuracy of the analysis. Several test experiments were performed by varying the X-ray energy values between 9 and 20 KeV. An optimal condition was found for at low energy of 15 KeV.

After scanning, the reconstruction result was produced as a set of horizontal cross-sections through the scanned object. An identification of magnetic particles within the samples can readily be performed by

observing these 2D slices. The particles are clear visible because of their high absorption and probably agglomeration. More details of 3D distribution of particles into the samples can be observed by 3D volume rendering. Injected cells were evident in red whereas muscle vessels appeared yellow (Figure).



50x10³ cells ***100x10³ cells*** ***500x10³ cells***

Figure: 3D display of subvolume of samples corresponding to three different stem cell numbers after injection 12 h.

Furthermore, it is possible to use image processing to make one or more phases translucent or even to "cancel" a phase in order to allow a more accurate observation of the spatial distribution of each phase. Another phase, hardly visible because of its low absorption, has been observed indistinctively in samples with or without injected human cells. This phase can be identified as fibrotic tissues and was visualized in blue. Using this method we recognized several injected cells between muscle fibers, around vessels 24h after intra-arterial injection. Moreover, a quantitative analysis of the whole reconstructed volume was also performed. The different timing investigated did not show differences in the location of stem cells: 24 hours after injection cells were distributed along the vessels as found in the samples isolated 2 and 12 hours after treatment. The variation of the parameter "stem cells number" (50, 100, 500 X 10^3 cells) let us to optimize the experimental conditions identifying in 50000 the inferior limit number of detectable cells into a murine muscle.

Thus, in this study MicroCT associated with X-ray synchrotron radiation was used to image, at a three-dimensional level, the human $AC133^+$ stem cells through magnetic particles labeling by intra-arterial

transplantation inside bioptic muscular tissue of dystrophies mice. It believes that it was possible because a high X-ray absorption of the nanoparticles. The result clearly indicates the possibility of further nondestructive analysis. For example, by means of modern image processing algorithms, appropriate techniques and resolution improvement, a quantitative analysis could be performed. The analysis could cover a wide range of different parameters like, e.g. the relative volume fraction of a sample containing a certain concentration of magnetic particles, the spatial distribution of particle clusters in the sample, and the size of regions enriched with magnetic particles.

References

1. Wagers, A. J., Sherwood, R. I., Christensen, J. L. and Weissman, I. L. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 297, 2256−2259 (2002)
2. Pittenger, M.F., Mackay, A.M., Beck, S.C., Jaiswal, R.K., Douglas, R., Mosca, J., Moorman, M., Simonetti, D., Craig, S., and Marshak, D.R. Multilineage potential of mesenchymal stem cells. *Science* 284, 143-147 (1999).
3. Torrente, Y., Belicchi, M., Sampaolesi, M., Pisati, F., Meregalli, M., D'Antona, G., Tonlorenzi, R., Porretti, L., Gavina, M., Mamchaoui, K., Pellegrino, M.A., Furling D., Mouly, V., Butler-Browne, G.S., Bottinelli, R., Cossu, G., Bresolin, N. Human circulating AC133+ stem cells replenish the satellite cell pool, restore dystrophin expression and ameliorate function upon transplantation in murine dystrophic skeletal muscle. *J. Clin. Invest.* 114, 182-195 (2004).
4. Sampaolesi, M., Torrente, Y., Innocenzi, A., Tonlorenzi, R., D'Antona, G., Pellegrino, M.A., Barresi, R., Bresolin, N., Gusella De Angelis, M.G., Campbell, K.P., Bottinelli, R., Cossu, G. Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesangioblasts. *Science* 301, 487-492 (2003).
5. Gupta, A.K., Gupta, M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials* 26, 3995–4021 (2005).

6. Neuberger, T., Schöpf, B., Hofmann, H., Hofmann, M., Rechenberg, B. Superparamagnetic nanoparticles for biomedical applications: Possibilities and limitations of a new drug delivery system. *J. Magnetism Mag. Mater.* 293, 483-496 (2005).
7. Pankhurst, Q.A., Connolly, J., Jones, S.K., Dobson, J. Applications of magnetic nanoparticles in biomedicine. *J. Phys. D: Appl. Phys.* 36,167–181 (2003).
8. Reimer, P., Weissleder, R. Development and experimental use of receptor specific MR contrast media. *Radiology* 36, 153–163 (1996).
9. Brunkea, O., Odenbacha, S., Fritsche, C., Hilger, I., Kaiser, W.A. Determination of magnetic particle distribution in biomedical applications by X-ray microtomography. *J. Magnetism Mag. Mater.* 289, 428–430 (2005).
10. Bulte, J.W.M., Van Zijl, P.C.M., Mori, S. Magnetic resonance microscopy and histology of the CNS. *Trends Biotech.* 20, 24 (2002).