Bismuth Ferrite Harmonic Nanoparticles and Biphotonic Microscopy: Innovative Approach to Track *In Vivo* Muscle Stem Cells, a Promising Candidate for Cell Therapy of Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a fatal X-linked recessive muscle disease that represents the most common form of muscular dystrophy, affecting one in 5,000 male births. It is caused by mutations or deletions in the gene encoding dystrophin leading to the lack of dystrophin protein. This results in muscle fiber degeneration followed by severe endomysial sclerosis, leading to progressive muscle weakness and premature death at the age of 20-30 years. Currently, there is no effective treatment for DMD. In 2011, we showed that a stem cell population (named MuStem cells) isolated from healthy dog skeletal muscle induces long-term muscle repair and striking clinical efficacy after its systemic delivery in the dystrophic dog representing the clinically relevant DMD animal model. During last years, our group isolated the human counterparts (hMuStem) and demonstrated that they share the same phenotypic and *in vitro* behavioral features of canine cells. Currently, little is known about the homing in the muscle tissue and the whole body distribution of the MuStem cells following a systemic delivery which are nevertheless essential to determine the safety and efficacy aspect of the therapeutic strategy. To address this need, we use bismuth ferrite harmonic nanoparticles (BFO-NP, 100-120 nm range) as probes for the tracking of MuStem cells to study *in vivo* the hMuStem cell engraftment.

The nanoparticles are very interesting tools because capable to generate both Second Harmonic Generation (SHG) and Third Harmonic Generation (THG) signals. The origin of the signals is related to the crystalline properties of the nanoparticles, in particular its peculiar non-centrosymmetric structure. Cell tracking is therefore possible from the NPs SHG and THG signals detected with a two-photon confocal microscope A1RMP Nikon and this work provides the opportunity for long-term, three-dimensional cell tracking.

Uncoated and poly-ethylene glycol(PEG)-coated BFO-NP were investigated to label hMuStem cells *in vitro*. The cells were exposed to BFO-NP and BFO-PEG-NP. Localisation and cytotoxicity of the NPs were investigated *in vitro* during two weeks.BFO-labeled cells were tracked in the mouse *Tibialis anterior* muscle after their intramuscular injection by using biphotonic microscopy.

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Images have been performed with an A1RMP biphotonic microscope from the APEX platform UMR703 INRA Oniris, Center of Excellence Nikon Nantes.