Multiphotonic Imaging and Bismuth Ferrite Harmonic Nanoparticles (BFO HNPs) to assess pre-clinically innovative therapeutic strategies through monitoring engraftment properties of injected cells

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In order to assess the therapeutic potential of cell-based strategies, it is of paramount importance to elaborate and validate tools for monitoring the behavior of injected cells in terms of tissue dissemination and engraftment properties. Here, we apply bismuth ferrite harmonic nanoparticles (BFO HNPs) to in vitro expanded human skeletal muscle-derived stem cells (hMuStem cells), an attractive therapeutic avenue for patients suffering from Duchenne Muscular Dystrophy (DMD) (1,2).

Recent works demonstrated the high biocompatibility of HNPs for human cell lines (3). Furthermore, the net advantages of HNPs with respect to other optically active NPs are (i) their efficacy as NIR-II probes, (ii) the possibility to use freely tune laser excitation for harmonic signals imaging and (iii) their properties to resist to bleaching (4,5). We firstly demonstrate the possibility of identifying approximately 100 nm HNPs in depth of muscle tissue at more than 1 mm from the surface, taking full advantage of the extended imaging penetration depth allowed by multiphoton microscopy in the second near-infrared window (NIR-II). Secondly, we show that the simultaneous detection of second- and third-harmonic generation (SHG and THG) from BFO HNPs helps separate their response from tissue background (endogenous SHG and THG from collagen and adipose tissue), with a net increase in imaging selectivity, which could be particularly important in pathologic context that is dened by a highly remodelling tissue.

Based on this successful assessment, we demonstrate the possibility of hMuStem cells labeling with HNPs. We monitor over 14 days any modication on morphology features and proliferation of hMuStem cells upon exposure to PEG-coated BFO HNPs at different concentrations, revealing their high biocompatibility. Successively, to assess the possibility of using HNP-based labeling to track cells of interest in tissue, BFO-PEG hMuStem cells were injected in mice muscle. We show the possibility to retrieve labeled hMuStem cells in mice muscle 24 h post-injection by detection of the intense SHG and THG signals generated from HNPs (Figure 1). These results show that the labeling of hMuStem cells with HNPs represents a promising approach for monitoring their engraftment properties.

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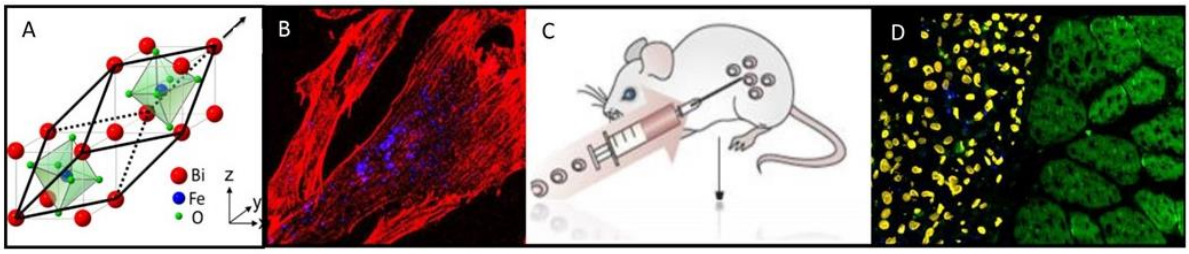

Figure 1 : A, Crystal structure of Bismuth Ferrite Harmonic Nanoparticles (BFO-HNPs); B, BFO-HNPs (SHG, blue) in an adult Muscle Stem cell visualized by its actin cytoskeleton (red); C, Injection of labeled cells into a mouse skeletal muscle; D, Visualization of cells labeled 24 hours after their administration by their nucleus (yellow) and nanoparticles HNPs (blue: harmonic signal).