BIOACTIVE SUPERPARAMAGNETIC APATITIC PHASE AS A NOVEL NANOPLATFORM FOR mRNA DELIVERY

Hina Inam[1,2], Marta Tavoni[1], Federico Pupilli[1], Simone Sprio[1], Anna Tampieri[1*]
[1]Institute of Science, Technology and Sustainability for Ceramics), National Research Council of Italy (ISSMC-CNR), 48018 Faenza, Italy; [2] Department of Material Science and Technology, University of Parma, 43121 Parma, Italy

Exposure of cells to externally applied magnetic fields or to scaffolding materials with intrinsic magnetic properties (magnetic actuation) can regulate several biological responses. For instance, magnetic activation enhances bone and vascular remodelling in scaffold regeneration processes.
In the present work FeHA nanoparticles were synthesized under different conditions to optimize physicochemical and magnetization properties. FeHA was functionalized with siRNA molecules in aqueous environment, to assess the loading efficiency and capability to act as a magnetic carrier, suitable for controlled release of nucleic acids.
Magnetic hydroxyapatite nanoparticles were synthesized by co-precipitation method, which involve the controlled addition of H3PO4 solution into a aqueous solution of calcium hydroxide ($Ca(OH)_2$) under constant stirring at different temperatures <40°C. During the neutralization reaction, an aqueous solution of Fe(II) ($FeCl_2 \cdot 4H_2O$) and Fe(III) ($FeCl_3 \cdot 6H_2O$) were added simultaneously. The total amount of Fe ions with respect to Ca ions were adjusted so as to obtain Fe/Ca = 20 mol%. Fe(II) and Fe(III) were determined by ICP and UV-Vis spectroscopy. Citrate buffer (1wt% with respect to $Ca(OH)_2$) were added into the solution to improve the particles dispersibility and RNA binding capacity. The as-obtained solution was stirred for 3 h at the same synthesis temperature and rested overnight at room temperature before centrifugation at 10000 rpm. The resulting precipitate was dried in freeze drier and stored at 4°C. The resulting FeHA NPs were characterized by XRD, FTIR, SEM, DLS and ICP analysis. Afterwards, citrate-stabilized FeHA-NPs were functionalized with siRNA (SilencerTM GAPDH Positive control siRNA) by incubation at different NPs and siRNA ratio. Then, the prepared FeHA-citrate/siRNA complex was purified by dialysis process, and stored at 4°C. The supernatant was loaded into the wells of a dedicated siRNA Kit and the loading efficiency of siRNA was evaluated by a fluorescence-based RNA quantitative test. FeHA nanoparticles with superparamagnetic properties are interesting nanomaterials as drug carriers suitable to be guided by external magnetic fields. In this respect, citrate-modified FeHA showed enhanced dispersibility and ability to link siRNA molecules, with size suitable to be injected in the bloodstream and magnetically guided to carry nucleic acids to the desired target site. Also thanks to its excellent biocompatibility and bioresorbability, siRNA-functionalized FeHA is thus promising as a new tool for novel cell therapies, with the advantage to obtain a remotely controlled system permitting personalized and more effective therapies.

Key words: *Magnetic hydroxyapatite; Drug delivery; Regenerative medicine; Nano medicine*

REFERENCES

[1] S. Panseri, M. Montesi, M. Sandri, M. Iafisco, A. Adamiano, M. Ghetti, and A. Tampieri, "Magnetic labelling of mesenchymal stem cells with iron-doped hydroxyapatite nanoparticles as tool for cell therapy", Journal of Biomedical Nanotechnology, vol. 12, no. 5, pp. 909-921, 2016.
[2] H. Inam, S. Sprio, M. Tavoni, Z. Abbas, F. Pupilli, and A. Tampieri, "Magnetic Hydroxyapatite Nanoparticles in Regenerative Medicine and Nanomedicine", International Journal of Molecular Sciences, vol. 25, no. 5, p. 2809, 2024.
[3] A. Tampieri, T. D'Alessandro, M. Sandri, S. Sprio, E. Landi, L. Bertinetti, and J. Rivas, "Intrinsic magnetism and hyperthermia in bioactive Fe-doped hydroxyapatite", Acta Biomaterialia, vol. 8, no. 2, pp. 843-851, 2012.