BIOPOLYMER NANOSTRUCTURES FOR PRECISION IMAGING: BASIC PRINCIPLES AND APPLICATIONS TO NANOMEDICINE

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Nowadays, CT and MR are the most widespread imaging modalities thanks to their ability to provide important anatomical information that are essential to achieve a reliable and accurate diagnosis. Despite the differences in their operating principles, each one with its own pros and cons, both techniques often require the injection of contrast agents (CAs) in order to make visible anatomical details otherwise not appreciable. Elements with high atomic number are used in CT to increase X-rays attenuation in the region of interest while, for MRI, paramagnetic atoms are chosen to act on the characteristic relaxation times of each tissue. Despite their extensive use, several drawbacks about the *in vivo* behavior of CAs have been recently highlighted. In fact, although different ligands are used to improve their performances and *in vivo* stability, CAs suffer from some limitations such as absence of specificity toward the target site, a rapid clearance from the bloodstream that allows short acquisition times and high toxicity due to their *in vivo* dissociation and deposition in organs such as brain, thyroid, and kidneys.

For this reason, a particular attention has been recently drawn to molecular imaging, interpreted as the characterization of biological and physiological processes at the cellular and/or molecular level. In addition to the possibility to drastically reduce the CAs dose, this methodology allows a dynamic and noninvasive monitoring of various diseases, before their clear macroscopic manifestation, leading to an early diagnosis. To achieve this goal, a contrast agent with high sensitivity and specificity to target a specific tissue or cell type is required for successful imaging. Moreover, this higher selectivity allows the combined use of more CAs heading toward the increasing use of multimodal imaging modalities such as PET/MRI and PET/CT that are able to provide anatomical details and functional information simultaneously.

Among all the possible probes, nanoparticles are emerging as a powerful tool for molecular and multimodal imaging thanks to their small size, their easily functionalizable surface and their ability to encapsulate more than one agent. In addition, through a suitable choice of the materials, it is possible to improve CAs biocompatibility but in particular influence their performances acting on the fundament parameters that leads to the generation of the imaging signal. For example, the presence of a hydrophilic polymer matrix around a gadolinium-based CAs allows to attract a large amount of water and increases the interactions between water molecules and the metal chelate promoting a relaxivity boosting. The effect, described by the *Hydrodenticity* concept, can be modulated through a proper control of the structural properties of polymer-based nanohydrogels affects the water molecules' dynamics resulting at specific condition in a relaxivity boost.

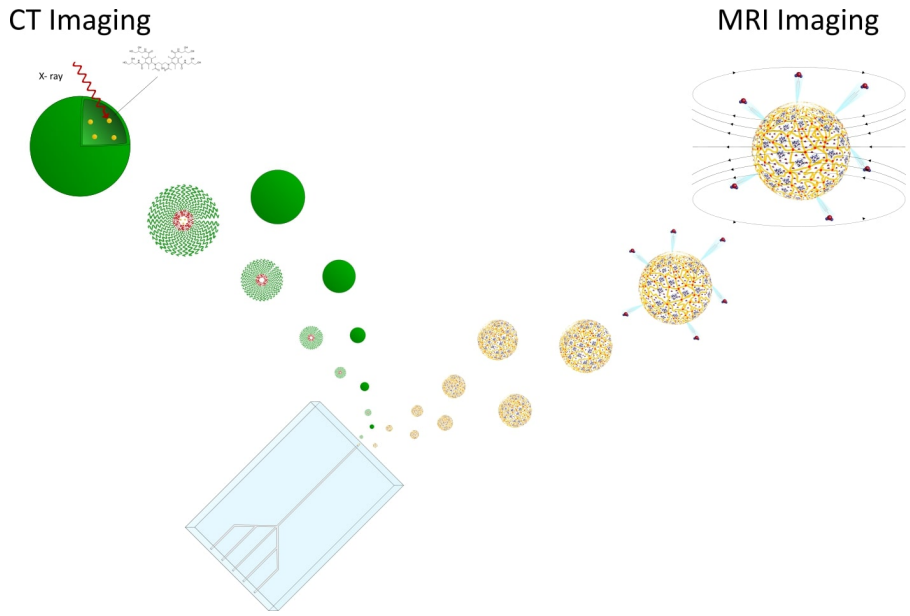
The present PhD work aims to build up innovative CAs, for both the single and multimodal imaging modalities, able to provide diagnostic information at molecular level through the use of biopolymeric nanocarriers. In particular starting by a detailed study of the interaction mechanisms between CAs and polymeric matrix and how this latter influences the CAs performances, the goal is to finely tune the nanostructures properties thanks to a microfluidic approach in order to improve the performances of clinically used CAs increasing their specificity and sensitivity towards the molecular target.

During the first year a bibliographic research has been carried out to understand the basic theoretical principles of CT and MR imaging, the limitations of the clinically relevant CAs and evaluate safer and more efficient alternatives. About MR, the unique paramagnetic properties of gadolinium have made it the main choice in the building up of CAs in the last 30 years. However, the highlighted toxicity of this compound, as result of its deposition in some body districts, has led to focus the attention on manganese which represents an optimal alternative to gadolinium thanks to the presence of an endogenous elimination pathway. Instead, despite their evident drawbacks, iodine based CAs still represent the more reasonable choice for CT imaging over more expensive or toxic elements such as gold and gadolinium too.

Starting by the acquired knowledge, the research has continued in the second year with the production of biopolymeric nanoparticles able to encapsulate CAs in order to deliver them more specifically to the target site or, in

the case of CT angiography, to lengthen their circulation in the bloodstream. The obtained nanoparticles have confirmed the ability of microfluidics to manipulate nanoparticles morphology and, in the specific case of MR imaging, the relaxivity boost, explained by the *Hydrodenticity* concept. In addition, preliminary multimodal imaging agents for CT/OI and MRI/OI have been developed thanks to the simultaneous encapsulation in a one step process.

Then, in the third year, the previously developed innovative imaging probes have been optimized and characterized in order to study their pharmacokinetics, the i*n vitro* and i*n vivo* toxicity and the imaging signal enhancement compared to clinically used CAs. In addition, predictive models, trained on experimental data, have been developed allowing to foretell the physicochemical and imaging properties of nanovectors for different combinations of input parameters and understand which of them have a greater impact on the desired outcome.



References:

Smeraldo, A., Netti, P.A., Torino E., "New Strategies in the Design of Paramagnetic CAs", *Contrast Media Mol Imaging*, p. 4327479, 2020, doi: https://doi.org/10.1155/2020/4327479.

Lee, N., Choi, S.H. and Hyeon, T. "Nano-Sized CT Contrast Agents", *Adv. Mater.*, vol. 25, no.19, pp.2641-2660,2013, doi: https://doi.org/10.1002/adma.201300081

McDonald RJ, McDonald JS, Kallmes DF, Jentoft ME, Murray DL, Thielen KR, Williamson EE, Eckel LJ. Intracranial Gadolinium Deposition after Contrast-enhanced MR Imaging", *Radiology*, vol.275, no.3, pp.772-82, 2015, doi: 10.1148/radiol.15150025

Andreucci M, Solomon R, Tasanarong A.,"Side effects of radiographic contrast media: pathogenesis, risk factors, and prevention", *Biomed Res Int.* ,2014, 741018, doi: 10.1155/2014/741018

James, M.L., Gambhir, S.S., "A molecular imaging primer: modalities, imaging agents, and applications", *Physiol Rev.*, vol. 92, no.2, pp. 897-965,2012, doi: 10.1152/physrev.00049.2010

Russo, M., Bevilacqua,P. , Netti, P.A. , Torino E., "A Microfluidic Platform to design crosslinked Hyaluronic Acid Nanoparticles (cHANPs) for enhanced MRI", *Sci Rep*, vol. 6, p. 37906, 2016, doi: https://doi.org/10.1038/srep37906

Vecchione,D. , Aiello, M. , Cavaliere,C. , Nicolai, E. , Netti, P.A., Torino E., "Hybrid core shell nanoparticles entrapping Gd-DTPA and 18F-FDG for simultaneous PET/MRI acquisitions" , *Nanomedicine (Lond)*, vol. 12, no. 18, pp. 2223-2231, 2017, doi: https://doi.org/10.2217/nnm-2017-0110

Tammaro, O., Costagliola di Polidoro, A., Romano, E., Netti, P.A., Torino E. "A Microfluidic Platform to design Multimodal PEG - crosslinked Hyaluronic Acid Nanoparticles (PEG-cHANPs) for diagnostic applications", *Sci Rep*, vol. 10, no. 1, p. 6028, 2020, doi: https://doi.org/10.1038/s41598-020-63234-x.

Aghajani, M., Shahverdi, A.R. & Amani, A.," The Use of Artificial Neural Networks for Optimizing Polydispersity Index (PDI) in Nanoprecipitation Process of Acetaminophen in Microfluidic Devices", *AAPS PharmSciTech,*vol. 13, pp. 1293–1301, 2012, doi:10.1208/s12249-012-9859-3

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