Unravelling the Interaction of Enzymatic PLGA-Nanobots with the Innate Immune System

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Our body is composed of highly complex and sophisticated environments, which are one of the key factors hindering the efficient delivery of drugs.[1] Nanobots, which are nanoparticles able to actively move at the individual and collective level, have shown enormous advantages in the presence of high viscous media,[2] mucus barriers[3] and tumors (*in vitro* and *in vivo*)[4]. Moreover, nanobots active motion have also shown enhanced nucleic acid delivery capabilities, allowing more efficient gene therapies.[5] With all, nanobots have brought wide attention as the next generation of drug delivery systems. Despite the clear relevance of this new approach, the unknown interaction with the immune system present *in vivo,* could provoke a decrease in the efficiency and severe side effects. There is a huge need for fundamental studies in this direction. Moreover, if we can understand the interaction between both systems, we will be able to develop new strategies for treating immune disorders or for modulating specific immune responses.[6]

Here, we present the design and characterization of a new kind of enzymatic nanobot based on (poly(lactic-co-glycolic acid) (PLGA), an FDA-approved material already used in clinics.[7] By conforming a positive layer of polyethylenimine (PEI), glutaraldehyde chemistry was used for functionalizing their surface with urease, resulting in PLGA-PEI-Urease nanobots. Our nanobots have been well characterized regarding size, surface charge, polydispersity, enzyme surface distribution (by STORM), degradation, enzymatic activity and motion (swarming behavior).

The immune system is responsible for our protection, defending our body from foreign threats such as pathogens and particles. The first line of defense is composed by the innate immune system, and it is characterized by being non-specific and quick. Then, a specific systemic immune response, known as adaptive, will take place against the recognized threat. In the first case, there are two main components: the physical barriers (skin and mucus) and the phagocytic cells (monocytes, macrophages and dendritic cells).[8]

Skin and mucus are considered biological barriers that limit the entrance of external agents, including drugs and nanoparticles, into the body. As already mentioned, enzymatic nanobots can be used to efficiently overcome mucosal barriers.[3] Here, we wanted to explore the capability of urease-nanobots to go through the skin, the first line of defense of our body. For this purpose, *ex vivo* pig skin was used as the most similar to human model. We have preliminary seen, how only in the presence of the fuel, nanobots are able to not get stacked in the epidermis and reach deep regions in the dermis.

A human monocyte cell line (THP-1) has been used for obtaining macrophages by culturing in phorbol myristate acetate (PMA). The differentiation was characterized by phenotypical changes (adherence and shape) and following the change in expression of markers CD14, CD68, CD80 and CD163 by flow cytometry. Then, we characterized the viability of human monocytes and macrophages with different concentrations of fuel (urea), nanobots, and a combination of both at different time points. With the optimal conditions, we checked the up-take by imaging and flow cytometry. Finally, we analyzed if the presence of our nanobots could activate the innate immune system cells by checking marker expression and cytokines secretion.

With all, we have established the first steps to unravel the interaction between our enzymatic-nanobots and the innate immune system, aiming to understand how to avoid immunogenicity or to modulate immune reactions to our desire.

References

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