(Two page abstract format: including figures and references. Please follow the model below.)

Temperature-responsive polymeric nanospheres containing methotrexate and gold nanoparticles: a multi-drug system for theranostics in rheumatoid arthritis

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Abstract (Arial 10)

Interest in developing multifunctional nanoparticles for a therapeutic application has recently gain particular focus in several diseases (cancer, infections, and inflammatory disorders) because of their unique theranostic properties. Theranostic combines therapeutics and diagnosis in a single multifunctional platform. These nano-carriers would preferentially target at the disease site, diagnose morphological changes of tissue of interest and provide effective therapy [1]. Multifunctional nanoparticles, containing stimuli-sensitive components, able to respond to internal and/or external triggers have appeared [2]. An example of such external trigger application is the photothermal driven drug delivery, through a nano-delivery system that, following intravenous administration, releases its payload at the site of interest upon application of a local near-infrared (NIR) light. NIR resonant nanomaterials, such as gold nanoshells [3] and gold nanoparticles strongly absorb NIR light producing local cytotoxic heat upon irradiation.

Chemo-photothermal therapy is a successful theranostic approach with the combination of chemotherapy and photothermal therapy that has recently emerged as a promising anticancer treatment [4]. Here, we intend to further explore the application of theranostic nanoparticles in the treatment of rheumatoid arthritis (RA). Permeability of rheumatoid synovium highly resembles solid tumours (e.g. leaky nature of their vasculature) [5] and the small synovial joints are within the penetration depth of NIR light, thus, multifunctional nanoparticles can be a promising tool for RA therapy.

In the present work, a novel stealth polymeric nanospheres platform able to carry anti-inflammatory drugs and an imaging agent was develop. The strategy to design a chemo-photothermal multifunctional platform was based on stealth polymeric nanospheres of pegylated-poly(DL-lactic-co-glycolic acid) (PEG-PLGA) containing methotrexate (MTX) and gold nanoparticles (Au NPs) for the treatment of RA as (i) MTX is effective in the management of RA when administered systematically or locally through intra-articular injections [6] and (ii) Au NPs have been used in RA therapy per se [7], but can also be used as contrast agent for photoacoustic imaging [8] and as an external trigger for thermo-responsive controlled drug delivery. Through emulsion-diffusion evaporation technique MTX was incorporated in the pegylated polymeric nanospheres in the presence or not of Au NPs. *In vitro* dug release assays revealed pH and temperature gold nanoparticles-dependence. Blank nanospheres exhibited negligible *in vitro* cytotoxicity, while MTX-loaded nanospheres hampered monocytes and macrophages viability at

a higher level than free MTX. Moreover, confocal fluorescent microscopy and flow cytometry revealed effective nanospheres internalization in human THP1 monocytes and macrophages. The cellular uptake was energy dependent and mediated by caveolae and clathrin-endocytosis mechanism. The MTX-loaded multifunctional nanospheres anti-inflammatory activity was evaluated using an *in vitro* model of RA involving monocytes and macrophages. Any change in the secretion of cytokines (IL-1β, IL-6 and TNF-α) in relation to the untreated stimulated cells indicate that the MTX and Au NPs incorporated on the multifunctional nanospheres retained their bioactivity and were able to reduce the inflammatory response *in vitro*. Results revealed that the MTX-loaded multifunctional nanospheres containing gold lead to a significant suppression of the pro-inflammatory cytokines produced by monocytes and macrophages, suggesting a favorable anti-inflammatory activity. These results confirm that the multifunctional nanospheres represent a promising theranostic platform for RA diagnosis and intracellular treatment, by combining methotrexate and gold nanoparticles for a highly effective targeted chemo-photothermal therapy.

References

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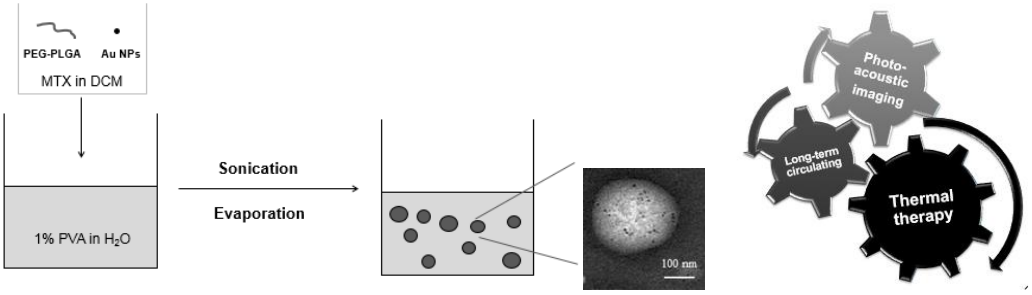
Figures



Schematic illustration of the synthesis of multifunctional nanospheres through a modified solvent evaporation single emulsion method for RA chemo-photothermal therapy.