Functionalized Solid Lipid Nanoparticles: a theranostic approach for the treatment of Rheumatoid Arthritis

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Abstract

Rheumatoid Arthritis (RA) is the most common autoimmune disease related to the joints and one of the most severe. Despite the intensive investigation, RA inflammatory process remains unknown and finding effective and long lasting therapies that specifically target RA is a challenging task. In RA the pro-inflammatory macrophages persist in the inflammation site and frequently overexpress cytokines and other biomolecule factors that amplify even more the inflammatory process. However, during RA, the macrophages also overexpress the CD64 surface marker that drives the search for new specific RA therapies.

This work proposed an innovative approach for RA therapy, taking advantage of the new emerging field of nanomedicine and the tools that it offers for targeted therapies. This study aimed to develop a targeted theranostic system for intravenous administration, using Solid Lipid Nanoparticles (SLN), a biocompatible and biodegradable colloidal delivery system, widely researched for medical applications, to function as a drug delivery system. The SLNs were encapsulated with methotrexate (MTX) and superparamagnetic iron oxide nanoparticles (SPIONs), to be used as therapeutic and imaging agents, respectively. The SLNs were also surface-functionalized with an anti-CD64 antibody that specifically targets RA-infected macrophages.

A total of eight different cetyl palmitate and stearic acid SLN formulations were produced using in an organic solvent-free emulsification-sonication method that combined high shear homogenization and ultra-sonication in order to compare the influence of each component present (MTX, SPIONs and anti-CD64) on NP characteristics. Particle size was assess by dynamic light scattering and analyzed by transmission electron microscopy and surface charge (zeta potential) mas measured by phase analysis light scattering. The placebo formulations showed sizes around 160 nm and zeta potentials of – 40 mV. Results also showed that MTX did not influence significantly NP properties, whereas SPIONs encapsulation caused an increase in both size and zeta values. The antibody conjugation caused an increased in zeta potential as expected but an unexpected decrease in NP size was observed. However, all the formulations presented sizes below 200 nm and zeta values lower than -12 mV, indicating suitable characteristics as nanosystems for intravenous administration. The stability of these formulations was also proven up to one month for the non-conjugated formulations. Nanoparticle morphology was analyzed by transmission electron microscopy (TEM).

TEM photographs indicated that the SPIONs were encapsulated inside the SLN matrix. Also, it was possible to observe small deformity and aggregation of NPs, while formulations without SPIONs presented a spherical shape with little aggregation. FT-IR was used to confirm the presence of MTX in the SLNs as well as the successful conjugation of the antibody to the SLN. MTX association efficiency

was determined by UV/Vis spectrophotometry, rendering values non-lower than 98% for both MTX-loaded SLNs and MTX- and SPIONs-loaded SLNs.

In vitro studies were performed with THP-1 cells and enabled to assess the cytotoxicity of the developed formulations. MTT and LDH assays demonstrated that the formulations were biocompatible and presented low cytotoxicity a concentrations lower than 500 µg/mL, but there were no significant changes when comparing the different formulations at the same concentrations unexpectedly.

This study could provide an effective and viable approach for future theranostic strategies. It was proven that the proposed NP were not cytotoxic, that both a therapeutic and imaging agent could be co-encapsulated and the SLN functionalized for a potential future application such as anti-body specific targeting. The proposed formulations are, therefore, promising candidates for future theranostic applications.

Figures

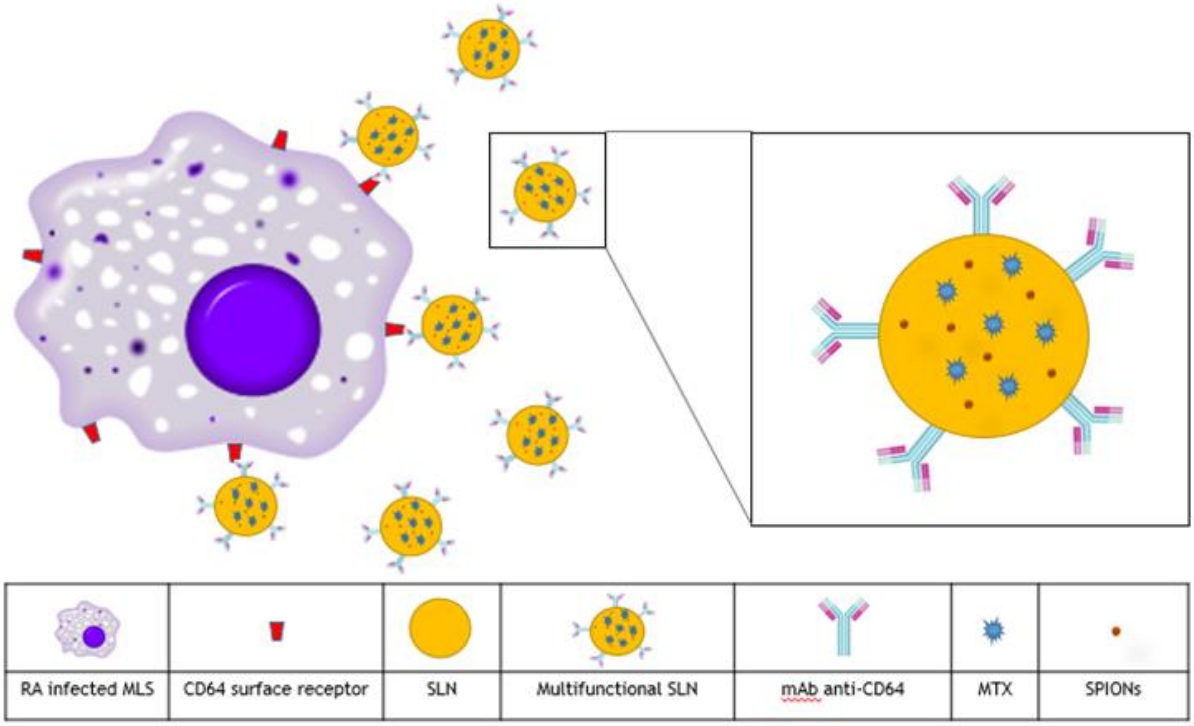

Figure 1 – Schematic representation of the proposed theranostic strategy for the treatment of RA.