Camouflaging PLGA microspheres in a 3D biomimetic collagen scaffold to escape macrophage internalization

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Statement of Purpose: Several scaffolds functionalized with delivery systems have been developed for the controlled and sustained release of growth factors. Although this has been greatly attractive for tissue engineers, none of the current devices account for macrophage-mediated internalization of the delivery systems associated to the scaffold, resulting in their clearance from the implant and ultimate poor clinical outcomes [1]. Platforms able to overcome clearance mediated by macrophages are crucial to ensuring the proper release kinetics of bioactive molecules and guarantee the desired therapeutic effect. In this study our working hypothesis was that by fully embedding PLGA MicroSpheres (MS) in a highly structured and fibrous biomimetic collagen scaffold (camouflaging), it is possible to prevent their early detection and clearance by macrophages.

Methods: TRITC-labeled MS were produced through double emulsion method. Collagen scaffolds (CTRL), collagen scaffolds integrated with MS (MS INT) or with adsorbed MS (MS ADS) were fabricated as recently published [2]. MS internalization by J774 macrophages was studied by SEM and confocal laser microscopy. The extent of MS internalized per each experimental group was quantified by flow cytometry. Also, nitric oxide (NO) and TNF-α produced in response to the scaffolds were quantified by ELISA assays. The results were further assessed with bone marrow derived macrophages (BMDM). The release of the reporter protein FITC-Bovine Serum Albumine (BSA-FITC) from MS, MS ADS and from MS INT and MS INT seeded with J774 was assessed to evaluate whether the eventual internalization by macrophages would affect the release kinetics.

Results: SEM and confocal microscopy evaluation revealed the perfect camouflage of MS into the collagen matrix of MS INT, while MS ADS resulted simply exposed on the surface of the scaffold's pores. By flow cytometry, we demonstrated a 10-fold decrease in the number of particles internalized by macrophages, when they were integrated (MS INT) into the scaffold's matrix respect to MS ADS scaffold. This result suggested that the 3D scaffold was acting by camouflaging the MS (Fig. 1). These data were further confirmed with BMDM. The production of NO and TNF-α by macrophages (both J774 and BMDM) induced by MS INT was assessed in order to evaluate the inflammatory response to the scaffold. Macrophages' response to the MS INT resulted similar to that observed in the CTRL, while for MS ADS their production resulted significantly increased due to the higher MS phagocytosis. Ultimately, the release of BSA-FITC from MS ADS and MS INT was followed up to 7 days, as a proof of concept, to demonstrate the benefits of MS camouflaging over the controlled release of a reporter proteins. The cumulative release from MS INT resulted 10-fold less than from MS ADS. In addition, MS ADS showed that only a minor part of the protein was released. It resulted that after 7 days, the 70% of BSA-FITC was taken by macrophages when MS were not camouflaged, while only the 5% when MS were hided in the scaffold matrix. This strategy resulted in a 4-fold higher release of payload by the MS INT scaffold in comparison with MS ADS.

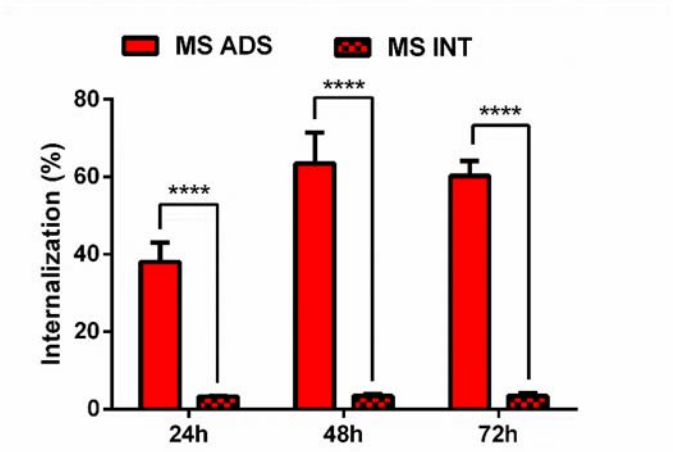

Figure 1. Quantification of the % of cells internalizing MS in MS ADS and MS INT scaffolds assessed by flow cytometry.

Conclusions: These data suggested that components of the extracellular matrix, like type I collagen, can be efficiently exploited to camouflage delivery systems.
Our study resulted significant as it represents an efficient strategy to preventing the early detection and internalization by macrophages of delivery systems from 3D scaffolds, contributing to preserve the release kinetics of their payload and reducing the inflammatory reaction, which is crucial to ensure the expected therapeutically outcome.

References:

[1] Franz, S. Biomaterials. (2011);32:6692-709;
[2] Minardi S. 10.19 (2014): 3813-3813.