Investigation of Nanostar-Labeled Mesenchymal Stem Cells for In Vivo Cell Tracking in Osteoarthritis using Optoacoustic Imaging

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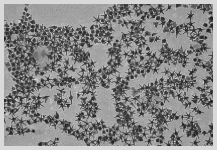
Background

This project will investigate the potential of gold nanostar (NS)-labeled mesenchymal stem cells (MSCs) in the treatment of osteoarthritis (OA) using optoacoustic imaging (OAI). OAI, specifically multi-spectral optoacoustic tomography (MSOT) has excellent resolution at increasing depths and capabilities in functional imaging.



A novel gold nanoparticle – the nanostar has been developed to enhance the signal response in OAI, as well as to permit tracking of MSCs.

Different nanostar surface coatings have been investigated for their uptake and effects on MSC characteristics: methoxy (NS-OMe) and carboxyl (NS-COOH). NS-SPIONs (superparamagnetic iron oxide nanoparticles) have also been produced to permit dual OAI and magnetic resonance imaging (MRI). Available carboxyl groups also allow for the conjugation of an antibody to NS, which will permit the tracking of MSC-derived extracellular vesicles (EVs). Initially, EVs isolated from MSCs will be labelled using antibody-conjugated NS to an EV marker e.g. CD63. If successful, CD63-conjugated NS will be used to track generation and release of EVs after therapeutic licensing of injected MSCs in the OA joint.

Aims & Objectives

1. Label MSCs and MSC-derived EVs in vitro with NS. Many factors affect the cellular uptake of NS:

- ★ NS Size
- ★ Agglomeration state
- ★ NS Shape
- ★ NS Concentration
- ★ NS Surface Coating
- ★ Incubation Time
- ★ Presence of Serum

2. Investigate the effect of NS on MSC characteristics (viability, trilineage differentiation capacity, surface immunophenotype, potency – immunosuppression/immunomodulation assays

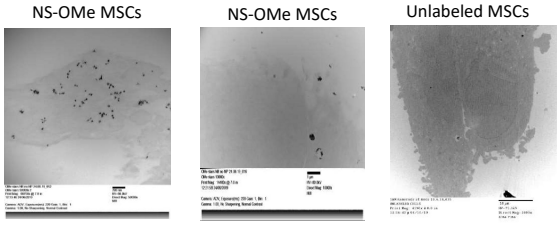
3. Intra-articularly administer NS-MSCs/EVs to healthy animals and mice induced to develop OA through destabilization of the medial meniscus (DMM) to determine the optimal concentration of NS required in vivo for MSOT detection, the optimal number of MSCs/EVs required for MSOT detection and to track biodistribution, engraftment and efficacy of NS-MSCs/EVs in vivo over-time.

Results

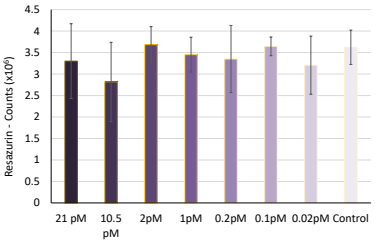
NS-OMe MSC Characterization

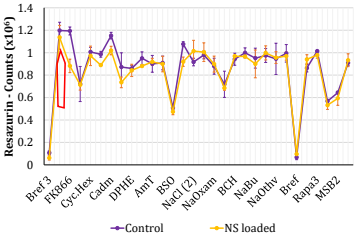
MSCs have been successfully labeled with NS-OMe as can be seen with transmission electron microscopy (TEM). Uptake of nanostars has not altered the cell phenotype in terms of viability, surface marker expression and tri-lineage differentiation capacity.

Transmission Electron Microscopy

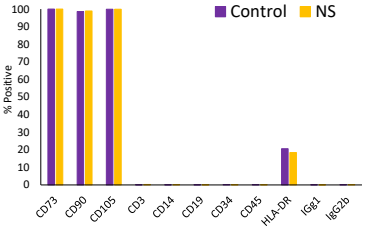


Viability of NS-labeled cells

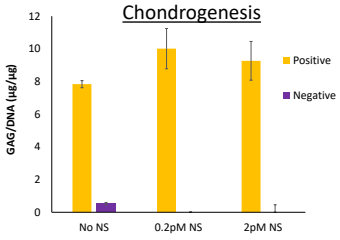


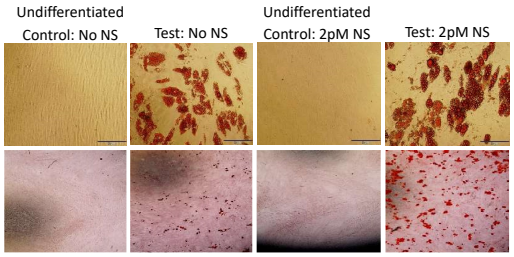


Surface Marker Expression



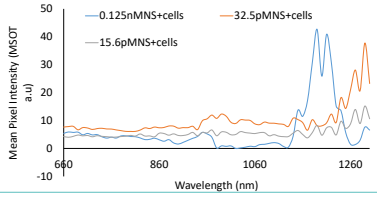
Chondrogenesis





Cellular Uptake of NS-COOH

The uptake of NS-COOH was not found to be optimal, as shown by a low mean pixel intensity on MSOT.



Dark field microscopy has also revealed inefficient uptake of NS-COOH. Dark field images suggested there is an accumulation of NS-COOH around the edges of cells.

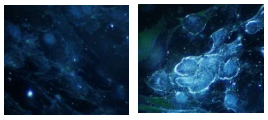
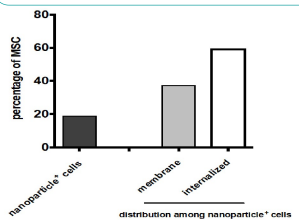
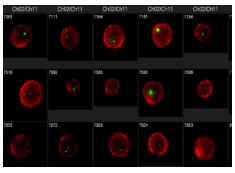
Unlabeled MSCs  1nM NS-COOH

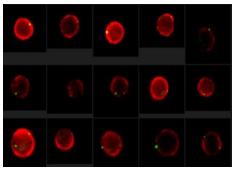
Image Stream analysis revealed the uptake of NS-COOH conjugated to an antibody is sub-optimal, with 20% of MSCs containing internalized or membrane-bound nanoparticles.



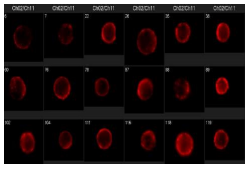
Internalized



Membrane-Bound

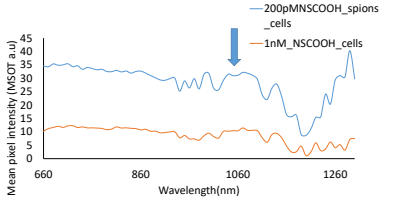


No Nanoparticles



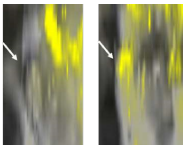
Cellular Uptake of NS-SPIONs

0.2nM NS-SPIONs demonstrated an increased mean pixel intensity on MSOT compared to 1nM NS-COOH



MSOT detection of NS in Joint

An enhanced signal in the knee joint (right) due to the presence of NS can be seen in comparison to baseline (left) as indicated by the white arrows.



Future Work

The optimal concentration of NS-SPIONs for MSOT detection will be determined. Methods to enhance NS uptake (electroporation/soluporation) will also be investigated.

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