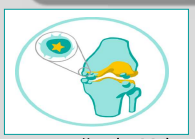
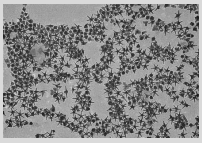
Investigation of Nanostar-Labeled Mesenchymal Stem Cells for In Vivo Cell Tracking in Osteoarthritis using Optoacoustic Imaging

Niamh Duffy[1], Georgina Shaw[1], Declan Byrnes[1], Soorya James[2], Martin Leahy[2], Mary Murphy[1]
[1]Regenerative Medicine Institute (REMEDI), NUIG, Galway, Ireland
[2]Tissue Optics & Microcirculation Imaging (TOMI), NUIG, Galway, Ireland

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 and assess the effect of various factors on 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).

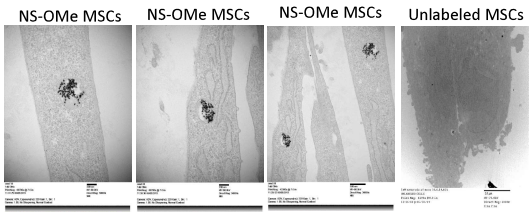
3. Intra-articularly administer NS-MSCs/EVs to healthy animals and mice induced to develop OA through destabilization of the medial meniscus (DMM) to determine the optimal concentration of NS required in vivo for MSOT detection, the optimal number of MSCs/EVs required for MSOT detection and to track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

Results

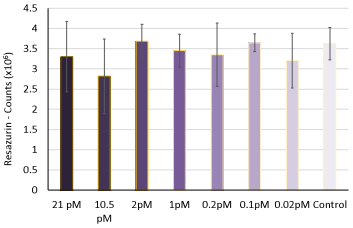
NS-OMe MSC Characterization

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

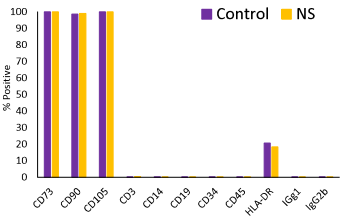
Transmission Electron Microscopy



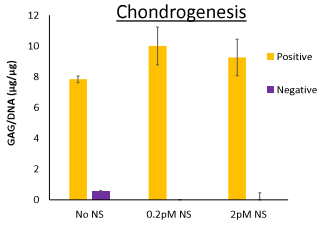
Viability of NS-labeled cells

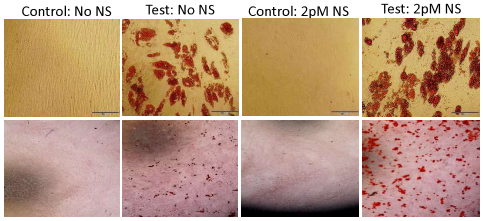


Surface Marker Expression

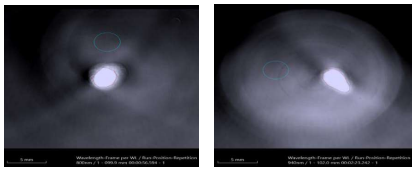


Chondrogenesis



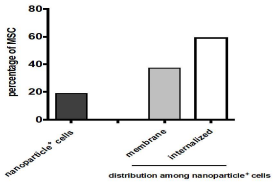


NS-OMe-labeled MSCs were visualised following encapsulation in sodium alginate microspheres using optoacoustic tomography in tissue-mimicking agar phantoms

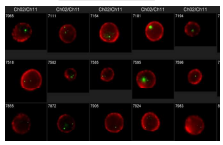


Cellular Uptake of NS-COOH

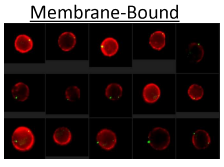
Image Stream analysis revealed uptake of NS-COOH conjugated to an antibody is sub-optimal, with 20% of MSCs containing internalized or membrane-bound nanoparticles.



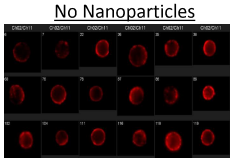
Internalized



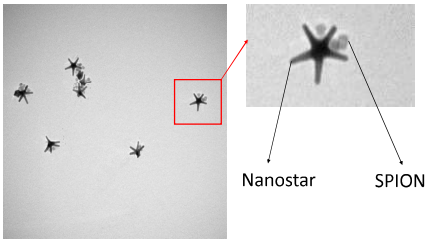
Membrane-Bound



No Nanoparticles



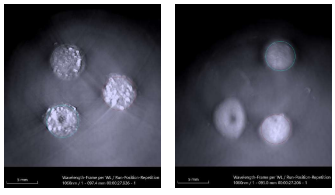
Conjugation of NS to SPIONs



Magnification: 150,000x

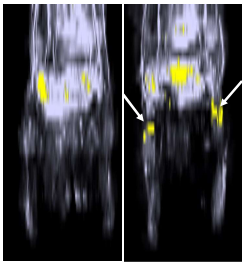
Cellular Uptake of NS-SPIONs

NS-SPION-labeled MSCs were visualised using optoacoustic tomography in tissue mimicking agar phantoms



MSOT Detection of NS in Joint

Enhanced signal in the knee joints (right image) due to the presence of NS was seen in comparison to baseline (left image) as indicated by the white arrows



Future Work

The optimal concentration of NS-SPIONs for MSOT detection will be determined in vitro prior to the onset of therapeutic in vivo studies.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 761214. The materials presented and the views expressed are the responsibility of the authors only. The EU Commission takes no responsibility for any use made of the information sent out.