Acadamh Ríoga na hÉireann
Royal Irish Academy

Charlemont grant report

Recipient name:	Dr Claudio Intini
Discipline and subject area:	Sciences
Amount and year awarded:	€2,500 in 2022
Title of project:	An innovative miR-activated scaffold for delivery of a therapeutic miRNA-221 inhibitor in human mesenchymal stem cells enhances cartilage repair in vivo.

Summary of findings:

The aim of this study was to develop and characterise an innovative microRNA (miR)-activated scaffold for enhanced cartilage repair through the delivery of a miR-221 inhibitor using a non-viral vector (cell-penetrating peptide "GET") incorporated into a collagen-based scaffold specifically designed for cartilage repair. To this end, the miR-activated scaffolds were cultured in chondrogenic conditions for 14 days using human mesenchymal stem cells (hMSCs). Then, the scaffolds were inserted in osteochondral defects sized (Ø3mm and 2mm depth) created in ex-vivo bovine osteochondral plugs and implanted subcutaneously in nude mice. Animals were sacrificed after four weeks and an evaluation of the tissue repair was done by histological assessment.

Full analysis in vitro confirmed the capability of the GET delivery system to act as an optimal carrier in a 3D scaffold platform. The incorporation of GET nanoparticles (NPs) into the optimised collagen-based scaffold platform for cartilage repair was demonstrated to successfully transfect hMSCs with the miRNA cargo in a sustained and controlled manner up to 14 days in vitro. These results are not unexpected and reflect our previous preliminary work platforms with a therapeutic miR-221 inhibitor cargo.

Complete analysis in vivo revealed that biologically, all scaffolds performed well, supporting effective hMSC viability and migration throughout the scaffolds. These results support the findings observed previously in our laboratory showing the capacity of highly porous interconnected collagen-based scaffolds to facilitate efficacious cellular viability and matrix formation. Moreover, hMSC viability and migration capacity throughout the scaffolds was not altered in scaffolds functionalised with GET with or without the miR-221 inhibitor. Taken together, it demonstrated the capacity of the non-viral peptide-based GET delivery system to act as an optimal carrier in vivo.

Furthermore, both scaffold variants revealed an ability to sustain a chondrocyte-like morphology for the majority of cells located in the scaffolds. In particular, the histological results showed cells characterised by a smooth spheroidal shape with few cytoplasmatic protrusions extending beyond the pericellular matrix. The length and number of chondrocyte's cytoplasmatic protrusions extending beyond the pericellular matrix have been associated with the "healthiness" of the cells and the overall tissue. In particular, a greater number of the chondrocyte cytoplasmatic extensions can be an indication of a chondrocyte phenotype associated with unbalanced homeostasis and catabolism. Therefore, although caution is recommended (since further investigation and deeper analysis are needed), the smooth spheroidal

morphology observed in the cells, which have few cytoplasmatic protrusions, may represent a chondrocyte-like morphology associated with healthy tissue regeneration and balanced homeostasis.

Results from the histological assessment also indicated good tissue repair with early cartilage-like matrix deposition in the area surrounding the scaffolds adjacent to the native cartilage for both miR-free and miR-activated scaffolds. However, although both scaffold variants performed well, with satisfying cartilage-like tissue deposition observed throughout the matrices, miR-221 activated scaffolds did not show an evident superior ability to enhance the deposition of early cartilage-like tissue after four weeks in vivo.

This result seems in conflict with a similar study showing a beneficial effect on cartilage repair when miR-221 was silenced in hMSC's seeded on an alginate-based gel by 12 weeks post-implantation in rats. We believe that this discrepancy could be attributed to a shorter time point of analysis in vivo for our study which was of only four weeks compared to the 12 weeks in the other study. In future investigations, it might be advisable to analyse longer time points to observe a significant effect of miR-221 silencing on cartilage repair.

Furthermore, although these biomimetic collage-based scaffolds have shown great potential to direct an effective cartilage repair, the need to improve the bone basal tissue integration with a better bonding of the scaffold to the adjacent native bone tissue remains. This is essential to promote an effective cartilage repair with infiltrating precursor cell population capable of differentiating to cartilage forming cells, resulting in repair of the surrounding tissue and remodelling of the implanted scaffold. To this end, the development of innovative press-fit fixation method of the scaffold within the chondral defect site might be necessary to permit an improved cell infiltration into the scaffold structure for enhanced cartilage repair. Moreover, the choice of an animal model with osteochondral defects better supplied by blood, and thus, by precursor cell population such as MSCs might be required to enhance cellular migration throughout the scaffold and consequent cartilage tissue remodelling and repair.

Taken together, this research proposal aimed to interestingly assess and validate the chondrogenic regenerative capacity of the miR-activated scaffold in vivo. However, although both miR-free (only GET) and miR-activated (GET+miR-221 inhibitor) scaffolds performed well in vivo, the delivery of miR-221 inhibitor using a collagen-based scaffold did not improve significantly the cartilage repair process in vivo. With this is mind, the approach presented in this research still offers a strong perspective on collagen-based scaffolds to be used as a simple "off the shelf" therapeutic approach to support improved cartilage repair in the clinic. However, in future investigations, it might be advisable to assess a beneficial effect of the miR-221 activated scaffolds: 1) with a longer time point in vivo than four weeks; 2) to select an animal model that is better supplied by blood and cells with regenerative potential; and 3) to develop an improved fixation method of the scaffold in the chondral defect to direct an effective bone basal integration and cellular migration to the scaffold.

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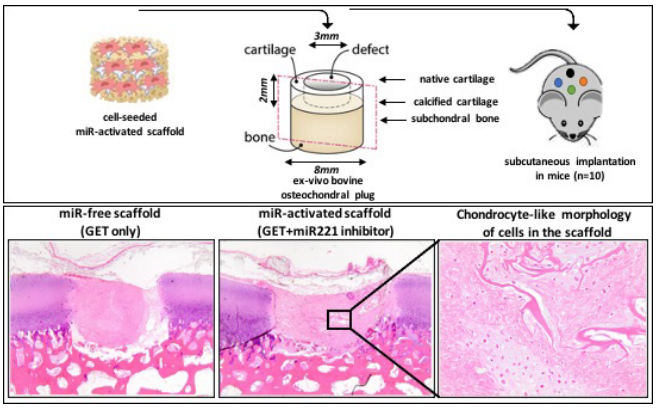

Plans for continuing collaboration:

There might be more collaborations between the laboratories given the positive experience gained during this one.

Published work and publication plans:

We plan to submit an article in a peer-reviewed journal based on the work involved in this project.

Dissemination and plans for future dissemination:

The work achieved in this project was presented with an oral talk at the Bioengineering in Ireland 2022 conference held in Galway, Ireland. Moreover, an abstract was submitted to the Orthopaedic Research Society (ORS) American Annual Meeting 2023. We will be notified in November if the abstract will be accepted for a talk or poster session.

Outreach and engagement activities:

Secondary school students from Dublin, Ireland were introduced to our proposed research activity in the grant.