Acadamh Ríoga na hÉireann
Royal Irish Academy

Charlemont grant report

Recipient name:	Dr Rebecca Rolfe
Discipline and subject area:	Sciences, Biology
Amount and year awarded:	€2,500
Title of project:	Generation of 3D tissue constructs for repair of tendon and ligament rupture

Summary of findings:

This funded research project was designed to investigate the development of tendons and establish an in vitro cell culture system to explore biological and mechanical aspects required to form functional load-bearing tissue in the adult. This approach is required to start to address the enormous clinical need to repair tendons by creating embryonic 3D models and then using genetic manipulation to control the necessary developmental processes. Within the project there were two main objectives outlined, (1) To establish scaffold free 3D mini-tendon constructs and (2) Optimise gene delivery into 3D mini-tendon constructs using tailor designed nanoparticles.

During this project we have successfully achieved multiple levels of optimisation to establish free 3D mini-tendon constructs, as outlined for objective 1, and now this will feed in to objective 2. We began by optimisation of embryonic tenocytes derived from chick metatarsal tendons at embryonic day 14. Primary tenocytes were dissociated from embryonic tissue and expanded to assess cell number and phenotype. We successful established an isolation protocol from these cells from embryonic tissue giving a large number of cells for plating in 3D conditions. Culture plates were set-up according to previously published protocols, using a sylgard substrate with two fixed pins positioned 10mm apart fixing a silk suture at either end (Figure 1A).

Subsequently cells were seeded, at a particular density in a fibrinogen/thrombin biopolymer within each culture plate. Optimisation of handling of the cells with the biopolymer was performed, in parallel with testing the biopolymer concentrations to create an attached 3D gel. Attachment of the fibrin embedded cell culture layer to the plates occurred successfully and then subsequent releasing of this layer was performed. Testing of the method to release was performed with multiple constructs, as to release adherence and allowing the construct to 'roll up' to create a 3D construct. Methods included scoring the circumference of the culture plate every 2 days with a pipette tip, and more rigorous lifting of the cell embedded gel to allow for 3D rolling. We next performed experiments to test the duration of rolling of the 3D construct, and profiled the phenotypic changes in the constructs over time (Figure 1A).

The next stages were to analyse the construct histologically and molecularly and initial experiments reveal that the construct show markers consisting with native tendons, indicating that we are truly capable of re-creating tendons in a dish (Figure 1B,C). Histological sections of the tissue engineering construction (TEC) revealed positive staining for collagen using trichrome Blue (Figure 1B) and fluorescently for a collagen binding protein marker, showing green fluorescence (Figure 1C2,C3) and cell nuclei (Figure 1C1, indicating that these cells are capable of differentiating according to the normal profile of tendons in vivo.

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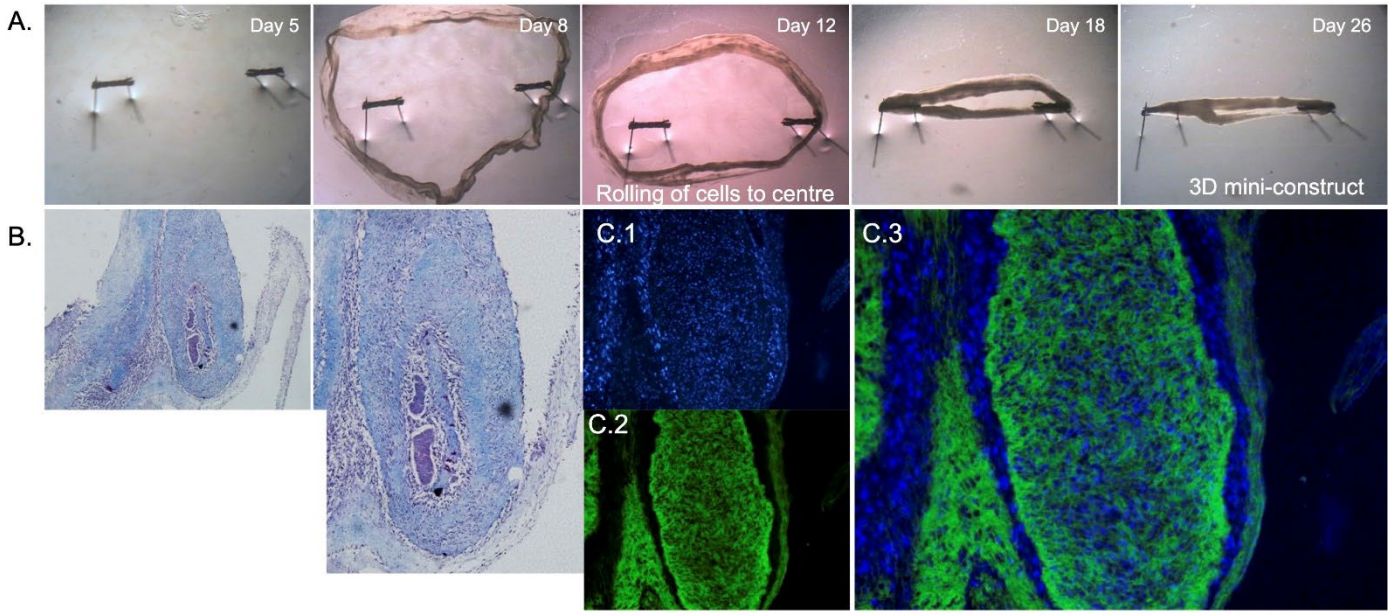

Generation of 3D Tissue Engineered mini-tendon constructs from embryonic cells. (A.) Phenotypic changes in formation of 3D constructs were profiled over time. (B) The construct in A was histologically processed and revealed positive staining for collagen staining with trichrome blue, and (C.) for a pan collagen binding protein showing green fluorescence (C2) and cell nuclei C1 Blue), with C3 showing a merge of cells and collagen staining.

The next steps in this project is for Objective 2: to optimise gene delivery into these 3D min-tendon constructs using tailor designed nanoparticles. In collaboration with the McCarthy group in Queens university Belfast the team will generate nanoparticles incorporating a readily detectable reporter gene encoding the Green Fluorescent Protein (GFP). The next phases of experimentation are to apply these fluorescently tagged nanoparticles to the successfully created mini-tendons. Nanoparticles can be delivered in culture medium or linked within a nanoparticle-hydrogel composite in contact with the differentiating cells, but this is the first time for their use in embryonic cells and will be tested extensively in the next phase of work. The dose and duration of gene delivery can be easily monitored via reporter gene expression which will enable a feedback system of determining the optimal parameters for maximal response. Once optimised, the system can be used for therapeutic gene delivery to prime appropriate signalling pathways and maturation to a robust load-bearing tissue which will be informed by our ongoing NSF-SFI-NI funded collaboration.

One of the most important intended outcomes of this project were to initiate a trans-disciplinary collaboration bringing together my expertise in developmental biology, tendon development and technical experience in primary and stem with the expertise of Prof. Helen McCarthy and her research team in nanoparticle gene delivery that is safe for development of clinical application. During this project this synergistic collaboration has been further supported successfully by awarding of a tripartite grant from the National Science foundation in the US, Science foundation Ireland in the Republic of Ireland and the Northern Ireland government. Partners in each jurisdiction cover multiple expertise, from bioengineering, developmental biology and nanomedicine. The ultimate goal of this synergistic collaboration is to incorporate the fundamental knowledge involved in generating normal tendon maturation to inform genetic engineering of robust load-bearing tissue constructs appropriate for clinical regeneration of tendon and ligament. The use of preliminary data generated from this Charlemont grant for this United States-Ireland-Northern Ireland Research & Development Partnership grant, together with current collaborators

at Penn State University led to this successful award. This work will continue to evaluate the capacity for primed mini-tendon constructs to integrate and repair in an animal model, feeding into larger clinically relevant translational models in the future.

Unfortunately due to COVID restrictions in the two labs during the time of this project, it was not possible to travel to the collaborators lab in QUB, nevertheless communication between partners occurred remotely with monthly meetings and individual aspects of the project continued. The project is now entering the second phase with the delivery of the fluorescently tagged nanoparticles imminently being transferred to the Trinity lab for incorporation into the mini-tendon constructs.

Plans for continuing collaboration:

As part of this project, I collaborated with Prof. Helen McCarthy's group in the School of Pharmacy in Queens University Belfast. During this project I had monthly meetings with researchers in this group learning the theoretical skills in nanoparticle synthesis and characterisation. In collaboration optimisation of the key formulation techniques to produce lyophilised nanoparticles encoding the GFP gene were being performed onsite in Belfast, and these are currently being produced to send to TCD and apply to the 3D mini-tendon models.

Unfortunately during the duration of this project it was not possible for me to attend QUB on site to visualise the production process (due to COVID lab restrictions and caring responsibilities), but communication and regular updates were provided between groups. Following the successful award of a United States-Ireland-Northern Ireland Research & Development Partnership grant this initiated collaboration is continuing and expanding positively between jurisdictions.

Our collaboration to date is developing a tailored nanoparticle gene delivery that is safe for clinical application, to prime appropriate molecular transitions in mini-tendon constructs, thereby stimulating the mechanostransduction and molecular pathways of normal tendon development.

Published work and publication plans:

As this is the beginning of this initiated collaboration and establishment of a complex embryonic culture system no publications have yet occurred to date, however, once nanoparticle delivery is established and other aspects of the newly funded project are developing, I anticipate multiple peer-reviewed publications to follow. All publications originating from Trinity that relate to this research project will be Open Access. Trinity is committed to the principles of open access and to this end, is a member of a transformative Open Access Publishing Agreement with Elseveir that allows affiliated authors to publish Open Access (Gold) in their journal titles.

Dissemination and plans for future dissemination:

This work is planned to be disseminated at International conferences of high calibre such as Gordon conferences, International Society of Developmental Biology and smaller limb development meetings as well as annual meetings of the Orthopaedic Research Society and the Tissue Engineering and Regenerative Medicine Society meetings in Europe and North America for more applied aspects of the work.

Collaborations and planned collaborations:

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During this project collaboration successful awarding of a tripartite grant from the National Science foundation in the US, Science foundation Ireland in the Republic of Ireland and the Northern Ireland government has occurred. The United States collaborators include Dr Spencer Szczesny, an Assistant Professor in the Departments of Biomedical Engineering and Orthopaedics & Rehabilitation at the Pennsylvania State University, who's research specialises in biomechanics and mechanobiology. The ROI lead is Professor Paula Murphy, a professor in developmental Biology in Trinity College Dublin, and the Northern Ireland partners include Professor Helen McCarthy and Dr Niamh Buckley. This original Charlemont grant has allowed for the initial collaboration that has led to the successful awarding of this tripartite grant (began first quarter 2022- end 2025) to continue this work. By integrating expertise in biomechanics, mechanobiology, developmental biology, and materials science, this new project hopes to address the critical barriers that have, to date, prevented the development of functional load-bearing tendon/ligament replacements.

Outreach and engagement activities:

As this is a new project, no direct communication events have occurred as of yet for presentations of this work, however there are activities planned; these include an open public lecture at a separate Academic Institution, for the general public and as part of undergraduate education stream. Interactive activities with secondary school students will be performed, highlighting this project's research and activities. All of our research work is communicated through social media outlets and publications through the host institutions communication team, so this project in the future will be actively promoted using local media and communication sources as required. Successful granting of subsequent funding that built on this research was recently highlighted in SFI media releases.

https://www.sfi.ie/research-news/news/us-ireland-research-2022/