Research Title: Multidisciplinary *In Silico* Ageing Model to develop Rotator Cuff Surgeries and Therapies

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Long-term aim: Our vision is to develop multi-scale *in silico* models of the young and aged human rotator cuff (Fig. 1). Development of improved repair methods is hindered by the lack of relevant animal model which represents anatomical equivalence to the human rotator cuff; the *in silico* model will revolutionise research in this field. The attachment of tendon to bone fails when high stress concentrations arise. *In silico* analysis of the young, aged and repaired rotator cuff will reveal the ideal, aged and post-surgical stress distributions respectively throughout the tissue. These stress data will indicate why rotator cuff tear is so prevalent in the aged. Further to this the model will be used to develop new concepts for rotator cuff repair. A BBSRC Future Leader Fellowship will be sought to pursue this research, which is in line with the BBSRC's strategic research priority area of Bioscience for Health, with a view to improve 'healthspan'.

MICRA seedcorn funding aim: Develop methods for validating the micro-scale 3D finite element (FE) model of the bone-tendon attachment at the supraspinatus (SSP) tendon in the rotator cuff. The micro-scale model will form part of the full *in silico* model as illustrated in Fig. 1 & 2. The micro-scale FE model must be validated against experimental data to ensure that results are sound (Fig. 3.e). This validation will open the door to analyse stresses arising at the micro-scale in the aged and young rotator cuff, which will be presented for publication. This work-package will demonstrate proof of the *in silico* modelling concept, ahead of the BBSRC Future Leader Fellowship application.

Track Record: The lead investigator demonstrates past success in collaborative research across the engineering and clinical disciplines during PhD research into innovative hand tendon repairs, the outcome of which is currently being considered for patent application. This past research involved a similar workflow to that which is proposed herein, whereby existing repairs were examined using FE analysis and XμCT imaging, and novel repairs were prototyped via a validated FE model, demonstrating successful use of the proposed methods to address a clinical need.

Background: Musculoskeletal degeneration is one of the leading causes of age related disorders[2]. Partial or total rotator cuff tears are extremely common in aged individuals, presenting in 25% of over 65 year olds, rising to 50% of 80 year olds[3,4]. Injury presents as chronic pain, weakness and limited mobility in the joint, which significantly impacts upon quality of life and independence[5]. At present, surgical repairs are performed by re-approximating the tendon and bone using suture and bone anchors[6]. Unfortunately, current methods of repair are largely unsuccessful; recent clinical studies report re-tears in 9 % to 36 % of patients[7,8]. The development of new therapies is limited since *in vivo* animal models do not readily translate to human physiology due to anatomical differences. *In silico* modelling offers a method of observing stress in the human rotator cuff which leads to failure, and will permit development and prototyping of new repair methods.

High stresses typically arise at the interface between ductile and stiff materials. In the healthy tendon-bone attachment the tendon inserts into bone via uncalcified fbrocartliage (uFC) and calcified fibrocartilage (cFC), providing a gradual transition of mechanical properties. This functional graduation arises from a gradient in structural and compositional features on the nano, micro and macro-scale, which all contribute to the effective stress transfer and prevent tearing[9,10]. However, with ageing the functional graduation deteriorates, and this is further compounded by scar tissue formation following rotator cuff tear[11].

In light of the contributions from structural and compositional features on multiple length-scales towards stress transfer at the bone-tendon junction, a multi-scale modelling approach is essential to represent the rotator cuff *in silico*. However, whilst FE models have been reported in literature for prototyping rotator cuff repair[12], these have been limited to 2D, and only represented the tendon-bone attachment at the macro-scale. One rotator cuff FE model incorporated the graduation in mechanical properties from tendon to bone[13], however this model neglected features such as the change in direction of collagen fibrils and the

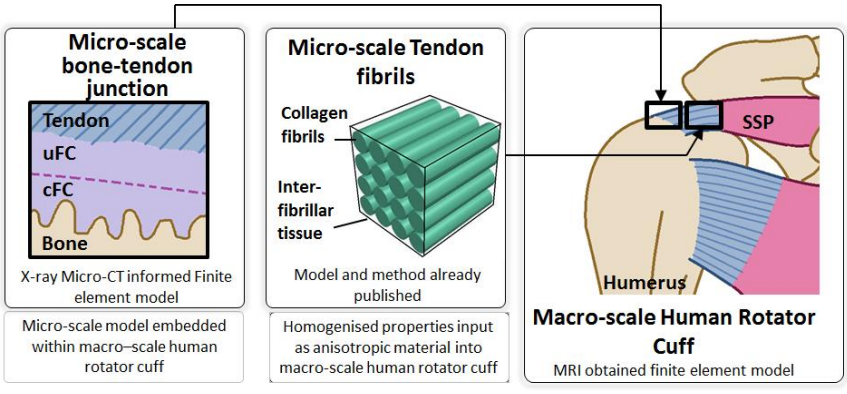

Fig. 1. The three sub-models which will make up the full multi-scale rotator cuff model, indicating how the sub-models integrate together.

Model	Sub-model	When will it be developed
Young	Micro-scale bone-tendon junction	Completed but requires validation (MICRA)
	Micro-scale tendon fibrils	Complete & published [1]
	Macro-scale human rotator cuff	Fellowship
Old	Micro-scale bone-tendon junction	Validation methods during MICRA. Model during fellowship.
	Micro-scale tendon fibrils	Fellowship
	Macro-scale human rotator cuff	Fellowship

Fig. 2. Summary of all models, highlighting where the MICRA seedcorn funding aims will contribute to the wider landscape of the project (green).

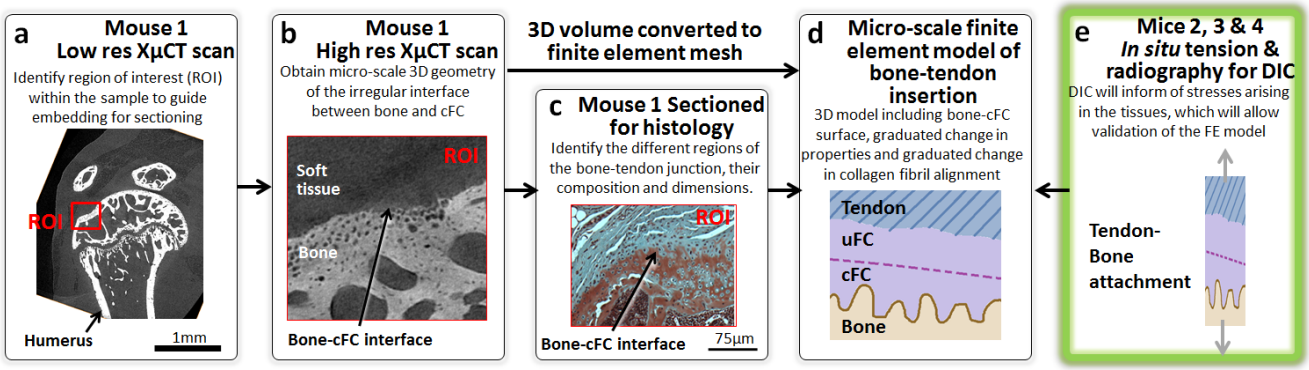

Fig. 3. Work-flow to develop and validate the micro-scale tendon-bone attachment sub-model. (MICRA seedcorn funded step in green)

micro-patterned bone surface, which are both crucial to effective stress transfer.

This study is timely due to recent advances in both X-ray Micro computed tomography (XμCT) imaging methods and FE analysis techniques which have made possible the development of this advanced rotator cuff model. It is now possible to reliably image soft tissue at sub-micron detail using XμCT due to the culmination of advances in staining methods[14] and lab based phase contrast imaging [15]. Within FE analysis, constitutive models have been developed to more realistically represent the non-linear, visco-hyperelastic behaviour of biological tissues *in silico*. Further to this, methods such as homogenisation have permitted models at different scales (from the micro- to macro-scale) to be linked[1].

Methodologies: Our FE models will be the first 3D *in silico* models of the rotator cuff to include the crucial multi-scale features of the tendon-bone interface in both the young and aged state. Two models, each built of three sub-models (Fig. 1&2) will be produced to represent different features within the tissue. The sub-models will be linked via homogenisation, or embedding micro-scale features within the macro-scale rotator cuff sub-model. Within the work package funded by MICRA seedcorn, methodologies will be developed to enable validation of both aged and young micro-scale tendon-bone insertion sub-models (Fig. 2).

Preliminary work already completed: The work-flow to develop and validate the micro-scale bone-tendon sub-model is illustrated in Fig. 3. The young micro-scale bone-tendon sub-model is currently being developed via this work-flow. XμCT imaging has enabled reliable reconstruction of the bone surface (Fig. 3.b), and histological analysis has confirmed that the surface is the bone-cFC interface (Fig. 3.c). The bone-cFC interface of the SSP tendon has been converted to a FE mesh and mechanical properties have been obtained from literature to inform the functional graduation between bone and tendon represented in the FE micro-scale sub-model. However, FE model validation is now necessary.

MICRA seedcorn funded work-package:

Validation of the FE sub-model will be provided via digital image correlation (DIC) to compare the stress distribution in a thin section of the bone-tendon (Fig 3.d), with that predicted within the micro-scale FE sub-model (Fig. 3.e). The bone-tendon SSP insertion will be identified within the murine shoulder (10 samples from 2 month old CD-1 mice already obtained) using XμCT imaging. This region of interest will then be dissected out, then cryo-sectioned at 20μm thickness. Samples will be loaded to failure in tension whilst obtaining radiograph images for DIC. Finally, XμCT will be performed to image the failure surfaces of the sample.

For success of this method, the MICRA seedcorn funding will enable several aspects to be optimised, which include: sample gripping, maintaining tissue hydration, and sample staining to improve contrast whilst minimising change in mechanical properties.

Once FE validation verifies the young FE micro-scale sub-model, the work-flow (fig. 3) will be repeated to build and validate an FE micro-scale model of the aged bone-tendon junction in future work.

This multidisciplinary project uses traditionally engineering techniques to address a clinical question. Crucial to maintaining clinical focus is the involvement of co-investigator Mr Usman Butt, who is a consultant shoulder, elbow and upper limb surgeon. Communication between the engineering and clinical fields will be supported via the use of a portable virtual reality workbench to visualise and examine data.

Budget: The MICRA seedcorn funding will pay for four days of XμCT imaging at the Henry Mosley X-ray Imaging Facility (HMXIF) at a total cost of £3,305.60, to identify the region of interest, ahead of dissection and sectioning for DIC. The school of materials fully supports this research, demonstrated by the provision of four extra days XμCT access at the HMXIF (funded by the EPSRC program grant), for development of the *in situ* radiography DIC methods (Fig. 3.e). The remaining £2694.40 will provide consumables for sample staining and preparation.

Output 1- Fellowship: Research arising from the MICRA seedcorn funding will contribute towards a BBSRC Future Leader Fellowship application to pursue the long term aim of developing the *in silico* young and aged, multi-scale human shoulder models, and using these models to develop new clinical techniques.

Output 2- Paper: The validation of the micro-scale FE models will lead to an original research article in a high impact journal. The article will detail age related changes to the micro-scale stress distribution at the bone-tendon junction, and how this contributes to the high incident rate of rotator cuff tear in the elderly.

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