Project Details	
Project Code	MRCPHS26Br Stevenson
Title	Disrupted Mechanical Signalling at the Golgi apparatus as a Driver of Osteoarthritis Progression
Research Theme	PHS
Project Type	Wet lab
Summary	Nearly 8% of the global population is living with osteoarthritis. The mechanisms of disease are poorly understood, however a major risk factor is altered mechanical loading of joints. This PhD project will investigate how the Golgi apparatus, a secretory pathway organelle, responds to mechanical signals and whether dysregulated extracellular matrix secretion contributes to osteoarthritis cartilage pathology. Analysis of GWAS studies will be used to identify osteoarthritis-linked Golgi proteins involved in disease progression, that will then be studied experimentally in 3D human cell culture and mouse tissue models of osteoarthritis. This is an exciting opportunity to uncover a novel cellular mechanism in joint disease.
Description	Osteoarthritis (OA) affects an estimated 10 million people in the UK and is characterised by the progressive breakdown of articular cartilage, leading to joint pain, stiffness, and reduced mobility. Mechanical signals are required for the maintenance of extracellular matrix (ECM) homeostasis in the joint, however abnormal mechanical loading is a risk factor for disease. A deeper understanding of how mechanical cues regulate chondrocyte behaviour is therefore critical for developing more effective treatments and interventions for OA. The composition and organisation of the ECM in articular cartilage is critical to its function. For example, proteoglycans like aggrecan embed within the collagen fibrillar network to provide biomechanical resilience, whilst other proteins transduce mechanical signals to chondrocytes to impact behaviour. These ECM proteins are transported through the Golgi during secretion, where they are modified to fine tune their chemistry in ways that affect how they assemble in the extracellular environment and consequently determine the mechanical properties of the ECM. Emerging evidence—including preliminary data from our lab—indicates that the Golgi itself is mechanosensitive. This suggests feedback loops exist between the Golgi and ECM that regulate ECM secretion and assembly to maintain tissue health. This project aims to test the hypothesis that disruption to these pathways contributes to the progression of OA and cartilage degeneration. Aim 1: Define structural changes in the secretory pathway following mechanical injury in OA models. Dr Blain will support the student in characterising alterations to secretory pathway architecture in OA by exploiting her traumatic injury-induced osteoarthritis in vivo mouse model. This will involve immunohistochemical analysis of archived murine cartilage samples collected at various timepoints following load-induced OA pathology. Dr Stevenson will also help the student to establish 3D culture models using human chondrocyte cell lines that can be treated with piezo-1 agonists to mimic loading. These in vitro models will permit the tracking of changes to Golgi morphology and transport in real time using fluorescent markers. The 3D cultures will also be subjected to cyclic

	compressive loading with the help of Dr Blain and instrumentation located in Cardiff. Aim 2: Identify OA-associated trafficking machinery from GWAS data. To find candidate mechano-sensors and effectors at the Golgi, the student will data-mine existing GWAS studies of hip and knee OA to look for trafficking genes containing SNPs associated with increased disease risk. We have a collaboration in place with Dr Ben Faber in population health at the University of Bristol to assist with this. This aim provides key opportunities for the student to take ownership of the project by making choices as to which candidates they wish to pursue through aim 3 and 4. Aim 3: Validate candidate trafficking components in 3D culture and OA models. A short list of candidate proteins, determined by the student, will then be validated with respect to their role in the mechanical regulation of Golgi function in vitro by 1) determining their cellular localisation with and without compressive loading and/or following piezo1 stimulation to look for Golgi recruitment, and 2) investigating whether loss of the protein abrogates Golgi responses to load. The most promising candidates will then be validated in the context of OA by performing immunohistochemistry on archived tissues samples as in aim 1. Aim 4: Elucidate the mechanistic role of a hit protein in disease pathology. One candidate protein, or a group of closely interacting proteins, will then be investigated in greater detail in the context of cartilage ECM homeostasis to provide insight into the mechanisms of disease. Mechano-sensitive protein function will be disrupted using either knockdown, over-expression, or Golgi mistargeting depending on the findings of aim 3, and the impact of this on ECM secretion and organisation determined using a combination of imaging and biochemical assays in 3D culture models. In particular, the trafficking and modification of proteoglycans will be investigated as they are both highly susceptible to Golgi dysfunction and significantly contribute to OA pathology. This PhD project is expected to provide novel insights into how Golgi mechano-dysregulation contributes to OA pathology and to identify key components of the underlying machinery. Given the novelty of this research area, a risk mitigation strategy is in place should suitable candidate proteins not be identified. Preliminary data from our lab has highlighted AKAP12 as a potential Golgi-associated mechano-transducer. As a scaffolding protein involved in actin cytoskeleton reorganisation and cell adhesion, AKAP12 is well positioned to mediate mechanical signalling. Crucially, our data show it is differentially recruited to the Golgi on soft versus stiff substrates, further supporting its role in mechano-transduction. The student will therefore be offered this protein to study if the need arises.

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