The Supramolecular Structure and Assembly of Collagen Fibrils in Normal and Osteoarthritic Human Articular Cartilage by High-Resolution Scanning Electron Microscopy

INTRODUCTION: Articular cartilage (AC) is composed of a three-dimensional fibrillar meshwork of collagen types II, IX and XI that is embedded into a soft gel-like matrix of proteoglycans. The collagen fibrils provide the strength and compliance to transmit and dissipate mechanical forces in the AC during joint movement from the tissue level down to the nanometre scale. These bulk properties also depend on how collagen fibrils are physically interconnected with other extracellular matrix molecules. During the development of osteoarthritis (OA), the cartilage meshwork degenerates, changing the structure of individual collagen fibrils which results in functional impairments and, ultimately, to the breakdown of cartilage. Towards finding new binding sites to facilitate the development of drugs against OA it is important to understand the structure of collagen fibrils across hierarchical levels and to compare them to changes in fibrillar structures from diseased cartilage. By employing improved protocols for high-resolution scanning electron microscopy (SEM), we present distinct differences of the supramolecular structure of collagen type II containing cartilage fibrils in healthy knee- and hip articular cartilage. We show that collagen fibrils undergo drastic changes in OA.

METHODS: Proteoglycans were removed from articular cartilage specimens by 100 mM Soerensen’s phosphate buffer (pH 7.2) containing 1mg/ml hyaluronidase (type I-S Sigma, St Louis, MO, USA) and 1mg/ml trypsin (type I, Sigma, St Louis, MO, USA) and 0.5% sodium azide for 3 days at 37°C. The solution was renewed every 24 h. Specimens were then fixed with 2.5% glutaraldehyde (in PBS, 2.6 mM NaH₂PO₄, 3 mM NaH₂PO₄, 155 mM NaCl, 0.01% Na₂SO₄, w/v, pH 7.2) for 2.5 h at room temperature. Next, they were washed three times in ultrapure water and dehydrated in a series of graded ethanol concentrations (30%, 50%, 70%, 90%, 100%). After critical point drying, samples were sputter-coated with 3-5 nm of platinum and examined by SEM (Hitachi S-4800 FEG) operated at 1.5 - 5 kV accelerating voltage. Immunogold-labeling of extracted fibril using gold-conjugated antibodies was done based on the protocol published in [1].

RESULTS: Collagen fibrils in the human knee are composed of prototypic fibrils exhibiting a uniform diameter of 18 ± 5 nm, (n = 504, R² = 0.95), measured on larger fibrils clearly composed of at least two prototypic fibrils) in agreement with the findings based EM data [1] and X-ray diffraction [2] with their D-bands in register [1, 3] (Fig. 1a). Prototypic fibrils are wound in a right-handed helix along the fibril axis with a twist angle of ω = 13° ± 4.4° (n = 99, R² = 0.75) (see inset). Collagen fibrils exhibit a similar organization to multi-thread ropes which makes them more flexible than a single large fibril of the same diameter and, thus, better suited for a soft deformable material such as AC. In contrast to knee AC, femoral head AC fibrils (Fig. 1b) lack helicity (see inset, white arrows). This interesting result may be attributed to different joint loading conditions. During gait the local pressure on knee cartilage can vary in less than a second from no external load to several times bodyweight. The femoral head instead, as it is encapsulated in the hip joint, is always under a pre-stress load and local pressure during gait is distributed over a larger area than in the distal femur. A helical structure can then respond better to higher stresses before breaking, whereas a parallel structure is more suited for constant pre-load conditions, such as in suspension bridges. Degenerative processes in OA result e.g. in splitting of collagen fibrils as shown in Fig. 1c and progressively leading to smaller fibrils, and ultimately to a wool-like structure as depicted in Fig. 1d (grade 3, Outerbridge scale). This wool-like structure is made of prototypic fibrils with a mean diameter of 18 ± 4 nm (n = 255, R² = 0.69), in agreement with the diameter of prototypic fibrils found in normal cartilage. However, these prototypic fibrils exhibit a higher curvature per unit length, and they may have lost their potential to reorganize into thicker collagen fibrils. The inset in Fig. 1d shows immunolabeling using gold-conjugated antibodies to identify collagen type II as the quantitatively major component of the cartilage fibrils.

DISCUSSION: The thick and well-banded collagen fibrils are composed of prototypic fibrils that are aligned in register, thereby forming the overall D-band pattern of the mature collagen fibril. In the course of OA collagen fibrils split and reorganize to finally disassemble in their basic building blocks, the prototypic fibrils. This process ultimately results in the breakdown of cartilage. Our overall goal is to assess how changes in structure of collagen fibrils affect the mechanical properties of cartilage over different length scales to better understand cartilage function in health, ageing and disease. High-resolution SEM may thus be helpful in assessing the efficacy of drugs against OA as well as the structural quality of tissue engineered cartilage.