The changing role of the superficial region in determining the dynamic compressive properties of articular cartilage during postnatal development

A.R. Gannon † ‡, T. Nagel §, A.P. Bell ‖, N.C. Avery ¶, D.J. Kelly † ‡ # *

| Department of Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland |
| Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Dublin, Ireland |
| CRANN Advanced Microscopy Laboratory, Trinity College Dublin, Ireland |
| Advanced Materials and Bioengineering Research Centre (AMBER), Royal College of Surgeons in Ireland, Trinity College Dublin, Dublin, Ireland |
| Collagen Research Group, Division of Molecular and Cellular Biology, University of Bristol, Langford, Bristol, UK |
| Collagen Research Group, Division of Molecular and Cellular Biology, University of Bristol, Langford, Bristol, UK |

A.R. Gannon † ‡, T. Nagel §, A.P. Bell ‖, N.C. Avery ¶, D.J. Kelly † ‡ # *

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Introduction

The composition and ultra-structure of articular cartilage varies through the depth of the tissue and is typically described as consisting of three distinct zones. When defined in terms of fibrillar orientation, these zones are commonly referred to as the tangential, transition and radial zones, or when defined in terms of depth they are commonly referred to as: the superficial tangential zone (STZ), the middle zone (MZ) and the deep zone (DZ). Each zone has a distinct extracellular matrix (ECM) composition, organisation and cell morphology. Interstitial water content decreases with depth from the articular surface, whilst proteoglycan content increases with distance from the articular surface. During development and maturation compositional and architectural changes occur in articular cartilage; it has been reported that collagen content (% dry weight) and cross-linking increase with age, whereas proteoglycan (PG) (% dry weight) content remains largely unchanged. Furthermore with age, the exclusively fine collagen fibrils of young cartilage develop in to the thicker and more varied fibril diameters of mature collagen fibrils. 

Conclusion: The findings demonstrate that the superficial region of articular cartilage undergoes dramatic structural adaptation with age, which in turn plays a key role in determining the dynamic compressive properties of the tissue.
articular cartilage (~1% collagen IX, ~3% collagen XI, ≥90% collagen II). In addition, the collagen architecture changes from a predominantly isotropic structure in immature articular cartilage to a mature arcade-like zonal structure first described by Benninghoff (1925)10–11. Such changes in biochemical composition and organization through the depth of articular cartilage with age likely lead to changes in the mechanical functionality of the tissue, yet to date this temporal and spatial relationship remains poorly understood. The primary mechanical functions of collagen fibrils in cartilage are twofold. First, they provide tensile stiffness and strength to the tissue. Second, the collagen network functions to restrain the swelling pressure of the embedded proteoglycans, which provides compressive stiffness to the tissue12,13. These trapped proteoglycans contain large amounts of glycosaminoglycans (GAG), particularly chondroitin sulphate and keratin sulphate that carry negative electrical charges in the physiological environment14,15. The density of these fixed charges is known as the fixed charge density (FCD)16. The swelling pressure exerted by this FCD, known as the Donnan osmotic fluid pressure, plays a key role in maintaining cartilage hydration and in determining the ability of the tissue to support compressive loads17–19. The compressive equivalent modulus of articular cartilage increases with depth from the articular surface, which correlates to increases in FCD in the deeper regions of the tissue, whereas dynamic properties have been shown to correlate with interstitial water and collagen content16. What remains to be fully elucidated is how these functional properties vary with age as a result of temporal changes to the biochemical composition and structural organization of articular cartilage.

The objective of this study was to explore how the equilibrium and dynamic compressive properties of articular cartilage change during postnatal development. In particular, we were interested in determining how changes to the superficial region (SR) of articular cartilage during skeletal development impact its functional properties, as biomechanical failure of this region of the tissue is postulated by many to be related to the development of osteoarthritis20,21. We have previously demonstrated that although this layer, in isolation, is less stiff in axial compression than the remainder of the tissue, it plays a key role in elevating the dynamic material properties of the entire tissue22. We speculated that this was due to the presence of the superficial region facilitating the generation of higher fluid load support during dynamic loading, which can be attributed to its high collagen fibril density and architectural organisation and hence low permeability. The question arises as to whether such functionality is present at birth, and if not, when these structural and compositional changes emerge in the SR of articular cartilage with skeletal maturity, and furthermore how they influence the biomechanical properties of the tissue. To address this fundamental question, we measured the equilibrium and dynamic compressive properties of articular cartilage from birth to skeletal maturity before and after removal of the SR of the tissue. Furthermore, we characterised the changing composition and organization of the tissue using biochemical, histological and microscopic techniques. We hypothesised that removal of the superficial region of articular cartilage would only negatively impact the dynamic modulus of mature tissue; since the superficial region is not yet developed for full function in skeletally immature articular cartilage.

Materials and methods

Sample preparation

Osteochondral cores were harvested from the medial and lateral trochlear ridges of the femoropatellar groove of porcine knee joints within 3 h of sacrifice as described previously23. Three age groups, representing different stages of maturation, were chosen for this study: 4 weeks (immature), 1 year, and 3 years (mature). Three pigs from each age group were used (n = 3), i.e., n = 3 independent replicates with three repeated measurements; therefore a total of nine pigs were used for this study. Three cores from each pig were prepared for mechanical testing and later used for biochemical analysis. One core from each animal per age group was chosen for helium ion microscopy (HIM) (n = 3 per age group); and finally a core from two animals within each age group was chosen for histological analysis. See SI section for more information.

Mechanical testing

Osteochondral cores were transferred to a confined compression chamber and attached to a standard materials testing machine with a 200 N XForce HP load cell (Zwick Roell Z005, Germany; resolution <0.2%). A preload of 0.05 N was applied to ensure contact between the articular surface and porous indenter (30 μm pore size, Aegis Advanced Materials Ltd., Worcestershire, UK). Cores were kept hydrated through immersion in a PBS bath at room temperature. Stress relaxation testing was performed, where a series of compressive strains were applied in increasing steps of 10% to a maximum of 30%. At each strain increment, peak strain was achieved within 500 s and the equilibrium stress was recorded after a relaxation period of 1800 s. Preliminary tests revealed that 1800 s was a sufficient relaxation period to allow the samples to fully equilibrate at all loading magnitudes previously outlined. A 1% amplitude sinusoidal strain was superimposed directly after relaxation at each static strain increment at a frequency of 1 Hz for five cycles. The aggregate modulus at each strain increment was calculated as the equivalent force divided by the specimen’s cross sectional area divided by the applied strain, whilst the dynamic modulus was calculated as the average force amplitude divided by the specimen’s cross sectional area divided by the average strain amplitude for all cycles. After dynamic testing the superficial region of the cartilage was removed using customised cutting tools for further testing in confined and unconfined compression. This region was taken as the top 15% of the total cartilage thickness based on zone thickness analysis of the mature tissue. The remaining osteochondral core was then placed back into the confined compression test chamber and retested using the same test sequence as outlined above.

For unconfined compression testing, osteochondral cores were placed between two impermeable steel platens, with and without their respective superficial regions. An identical testing regime to that described for confined compression testing was implemented, consisting of both stress relaxation and dynamic compression testing.

Biochemical, histological analysis and HIM

Sulphated GAG and collagen content were determined both biochemically and histologically; further details of which are available in SI. The collagen network was imaged using a helium ion microscope (HIM) (Orion® Plus, Carl Zeiss NTS) with an Everhart-Thornley detector producing an image from the secondary electrons generated by the incident helium ions, see SI section for more information. High resolution images were acquired in the superficial zone of the articular cartilage tissue classified as the top 100 μm of the tissue from the articular surface across age groups in order to visualise the changing morphology of individual network fibrils and fibril connections in this region and during maturation. The brightness and contrast of all images were optimised; no other post
processing procedures were performed. Fibril diameters were measured using ImageJ.

**Statistical analysis**

Statistical analysis was performed using a random effects model with Tukey’s post-hoc test for multiple comparisons, whilst a Durbin–Watson test was employed to test for the presence of autocorrelation in residuals. A two sample t-test was performed on the hydraulic permeability fit data. All tests were performed using the statistical software package MINITAB 15.1 (Minitab Ltd., Coventry, UK). Graphical and numerical results are displayed as mean with uncertainty expressed by 95% confidence intervals (CIs): mean (lower limit, upper limit). Significance was accepted at $P \leq 0.05$ or as indicated. Sample numbers varied according to respective comparison and are outlined in the results section of this manuscript and respective figure legends.

**Results**

**Removal of the SR negatively impacts the dynamic modulus of skeletally mature but not immature articular cartilage**

In general, the aggregate modulus (confined compression test) appeared to increase after removal of the superficial region (Fig. 1); although this was only significant for the 1 year old samples ($P = 0.0321$, $P = 0.0267$, $P = 0.0318$) at 10%, 20% and 30% applied strains respectively. The equilibrium Young’s modulus (unconfined compression test) did not significantly change after removal of the SR for any age group (Fig. 1), although the general trend was similar to the findings from confined compression testing. In contrast to the equilibrium results, the confined dynamic modulus (determined by dynamically loading tissue in confined compression) significantly decreased in the mature 1 year ($P = 0.0036$, $P = 0.0443$, $P = 0.05$ at 10%, 20% and 30% applied strains respectively) and 3 year ($P = 0.0132$, $P = 0.0242$, $P = 0.013$ at 10%, 20% and 30% applied strains respectively) old tissue after removal of the SR, whilst no significant change was observed after its removal in the 4 week old tissue (Fig. 2). A similar result was seen in the unconfined dynamic tests where a significant decrease in dynamic modulus was seen in the 1 year old tissue after removal of the SR ($P = 0.0201$ and $P = 0.015$ at 20% and 30% applied strains), with no significant change observed after its removal in the 4 week old tissue (Fig. 2).

The ratio of peak stress to equilibrium stress during stress relaxation testing (Fig. 3) also significantly decreased at 10% applied strain in the 1 year ($P = 0.0095$) and 3 year ($P = 0.0007$) old tissue after removal of the SR in confined compression. This was also observed for the 1 year old tissue respectively in unconfined compression ($P = 0.0097$, $P = 0.0178$, $P = 0.0347$ at 10%, 20% and 30% applied strains), whilst no significant difference was observed in the 4 week old tissue for either testing configuration.

The incremental aggregate modulus (Fig. 4), defined as the slope of a straight line fit to the equilibrium stress–strain curve in confined compression from 10% to 30% applied strain, significantly changed with age ($P < 0.0001$). The full thickness 3 year old mature tissue was significantly stiffer than the full thickness 1 year old tissue ($P = 0.0268$), whilst the 3 year old tissue, less its superficial region, was significantly stiffer than the 4 week and 1 year old tissues either with or without their SR ($P = 0.0078$, $P = 0.0021$, $P = 0.0002$ and $P = 0.0017$ respectively). Overall age also had a significant effect on the incremental Young’s modulus in unconfined compression ($P = 0.014$), however post-hoc tests revealed no significant differences between individual groups. Comparing the dynamic properties of intact samples (at 10% offset strain) across

![Fig. 1](image-url). Equilibrium aggregate moduli $H_a$ (MPa) and equilibrium Young's moduli (MPa) in confined compression and unconfined compression respectively of full thickness osteochondral cores and cores less their superficial region. Cores were obtained from the femoral trochlear ridges for different age groups: 4 week, 1 year and 3 year old. Bars show the mean ± 95% CI, n = 3 independent observations with three repeated measurements/group. All testing was carried out at increasing levels of applied strain: 10%, 20% and 30%. Connecting line does not imply linear relationship with strain; ‘a’ indicates a significant difference vs ‘Intact Osteochondral Core’ (10% strain); ‘b’ indicates a significant difference vs ‘Intact Osteochondral Core’ (20% strain); ‘c’ indicates a significant difference vs ‘Intact Osteochondral Core’ (30% strain); ‘d’ indicated a significant difference vs ‘Less Superficial Region’ (10% strain). $P$ value ranges for these differences are as follows: $\bullet$: $P = 0.0321$, $\gamma$: $P = 0.0267$, $\beta$: $P = 0.0318$, $\alpha$: $P = 0.0102$, $\pi$: $P = 0.0013$. 
Fig. 2. Dynamic moduli (MPa) in confined compression and unconfined compression respectively of full thickness osteochondral cores and cores less the superficial region. Cores were obtained from the femoral trochlear ridges for different age groups: 4 week, 1 year and 3 year old. Bars show the mean ± 95% CI, n = 3 independent observations with three repeated measurements/group. All dynamic testing was carried out at 1 Hz freq. and 1% amplitude at increasing levels of applied strain: 10%, 20% and 30%. Connecting line does not imply linear relationship with strain. 'a' indicates a significant difference vs ‘Intact Osteochondral Core’ (10% strain), 'b' indicates a significant difference vs ‘Intact Osteochondral Core’ (20% strain), 'c' indicates a significant difference vs ‘Intact Osteochondral Core’ (30% strain). P value ranges for these differences are as follows: a: P = 0.0364, b: P = 0.0036, c: P = 0.0443, d: P = 0.05, e: P = 0.0132, f: P = 0.0242, g: P = 0.013, h: P = 0.0201, i: P = 0.0038, j: P = 0.015.

Fig. 3. Peak stress:equilibrium stress ratio in confined compression and unconfined compression respectively of full thickness osteochondral cores and cores less their superficial region. Cores were obtained from the femoral trochlear ridges from different age groups: 4 week, 1 year and 3 year old. Bars show the mean ± 95% CI, n = 3 independent observations with three repeated measurements/group. All testing was carried out at increasing levels of applied strain: 10%, 20% and 30%. Connecting line does not imply linear relationship with strain. 'a' indicates a significant difference vs ‘Intact Osteochondral Core’ (10% strain). 'b' indicates a significant difference vs ‘Intact Osteochondral Core’ (20% strain). P value ranges for these differences are as follows: q: P = 0.0095, r: P = 0.0007, s: P = 0.0179, t: P = 0.0007, u: P = 0.0178, v: P = 0.0347.
different ages groups, it can be seen that the dynamic modulus in unconfined compression is significantly higher for the 3 year (\(P = 0.0044\)) old tissue compared to 4 week old tissue [Fig. 5].

Experimental data fit to a linear bi-phasic model of articular cartilage in confined compression revealed that the hydraulic permeability of the mature 3 year old cartilage was significantly higher (\(P = 0.039\)) after removal of the SR 1.54 \times 10^{-15} \text{ m}^3\text{N}^{-1}\text{s}^{-1} (1.06 \times 10^{-16}, 2.02 \times 10^{-16}) compared to full thickness cartilage 7.2 \times 10^{-16} \text{ m}^3\text{N}^{-1}\text{s}^{-1} (5.25 \times 10^{-16}, 9.16 \times 10^{-16}). The predicted permeability was over an order of magnitude higher in 4 week old tissues, and increased from 2.34 \times 10^{-14} \text{ m}^3\text{N}^{-1}\text{s}^{-1} (1.58 \times 10^{-16}, 3.21 \times 10^{-16}) to 5.06 \times 10^{-14} \text{ m}^3\text{N}^{-1}\text{s}^{-1} (2.96 \times 10^{-14}, 7.15 \times 10^{-14}) upon removal of the SR (\(P = 0.041\)).

Spatial changes in tissue composition with age cannot fully explain the functional role played by the superficial region of mature articular cartilage during dynamic compressive loading

Biochemical analysis revealed a significant effect of age (\(P < 0.0001\)) on sGAG content (normalised to % of tissue dry weight) [Fig. 6(b)]. The superficial region and the remaining cartilage tissue of the 1 and 3 year old skeletally mature joints contained significantly less sGAG than the 4 week old immature superficial region (\(P = 0.0275, P = 0.0092, P = 0.0051\) and \(P = 0.0022\)) and the remaining cartilage tissue (\(P = 0.00231, P = 0.0125\) and \(P = 0.0052\) respectively). Similarly when normalised to percentage wet weight; the superficial region and the remaining cartilage of the 3 year old mature tissue contained significantly less sGAG than the 4 week old superficial region (\(P = 0.0444\) and \(P = 0.05\)) respectively. Furthermore, the overall sGAG content (SR + remaining tissue) was significantly lower in the 1 (\(P = 0.0016\)) and 3 (\(P = 0.0001\)) year old tissue compared to the 4 week old tissue (data not shown). In contrast, collagen content (normalised to % of tissue dry weight) significantly increased with age (\(P = 0.0003\)). Both the superficial and remaining regions of the mature full thickness 3 year old tissue contained significantly higher amounts of collagen compared to the SR of the 4 week old tissue (\(P = 0.0304\) and \(P = 0.0397\) respectively) [Fig. 6(a)]. A similar and even more significant trend was seen when normalised to percentage wet weight. Furthermore, the overall collagen content (SR + remaining tissue) was significantly higher in the 3 (\(P = 0.0016\)) year old tissue compared to the 4 week old tissue (data not shown). In terms of changes in spatial tissue composition with age (% dry weight), no significant difference in collagen or sGAG content was observed between the SR and the remainder of the tissue within any given age group. Together these results suggest that relative changes in spatial tissue composition with age may not fully explain why removal of the SR leads to a reduction in the dynamic modulus in the mature articular cartilage.

These biochemical results are generally reflected in the histological analyses. Picrosirius red stained sections [Fig. 6(a)] point to an increase in staining between the 4 week old immature and 1 year and 3 year old mature tissue. Safranin O staining [Fig. 6(b)] demonstrated a decrease in staining between the 4 week old and the 1 and 3 year old samples.

The collagen network within the superficial region of articular cartilage dramatically reorganises with skeletal maturity

Given that the overall collagen content of SR of mature articular cartilage is not significantly different than the remaining tissue, we next sought to determine if changes to the organization of the SR during postnatal development might explain why removing this zone of the tissue negatively impacts its dynamic mechanical properties. Polarised light microscopy (PLM) revealed a thin, intensely birefringent and hence highly organised layer in the superficial region of the immature 4 week old tissue [Fig. 7], suggesting that even at this early age some level of organization exists.
in the superficial region. The majority of the remainder of the tissue displays large bands of non-birefringence, illustrating that the majority of the tissue is isotropic in structure. In the 3 year old, fully mature tissue, intense birefringent regions exist in the superficial and deep zone, clearly illustrating a fully developed Benninghoff architecture. The 1 year old tissue demonstrates the beginning of a skeletally mature architecture.

In order to further investigate the changing internal environment of the collagen architecture in the superficial region of the tissue, HIM was employed. The fibrils appear randomly organised in the 4 week old tissue, but appear more aligned parallel to the articular surface in the 1 and 3 year old tissue. A significant increase was observed in fibril diameter with age ($P < 0.0001$) (Fig. 8). Average fibril diameter increased from 14.37 nm (12.8, 15.93) in the 4 week old tissue significantly ($P < 0.0001$) to 22.91 nm (20.35, 25.48) in the 1 year old tissue, with a further significant increase ($P < 0.0001$) to 75.91 nm (61.45, 90.36) in the 3 year old tissue. In addition, a decrease in the number of collagen fibrils and fibril branching can be seen with age; this is most apparent comparing the 4 week old and 3 year old tissue (Fig. 8).

**Discussion**

The hypothesis of this study was that removal of the superficial region would only negatively impact the dynamic modulus of skeletally mature articular cartilage, as this region of the tissue is under-developed in skeletally immature joints. Previous studies have demonstrated that the compressive properties of articular cartilage increase with depth through the tissue\cite{2,21,23}, and similar to earlier findings\cite{26}, we observed an increase in the aggregate modulus upon removal of the SR (at all applied strains) for the 1 year old cartilage in confined compression (Fig. 1). In contrast to the equilibrium results, the dynamic modulus significantly decreased after removal of the SR in confined and unconfined compression for the 1 and 3 year old mature tissue (Fig. 2). Overall, sulphated GAG content (% dry weight) decreased significantly with
age whilst collagen content (% dry weight) significantly increased, however no significant difference was observed between the bulk biochemical composition of the SR and the remaining cartilage for any age-group. This suggests that temporal changes in the relative spatial biochemical composition of the tissue cannot fully explain why the superficial region of articular cartilage becomes so mechanically important with age. The results of this study would suggest that organizational changes to the collagen network of the SR of the tissue with age play a key role in determining the dynamic properties of the tissue. This was explored using HIM which demonstrated a change in fibril alignment in the superficial region of articular cartilage with age, as well as a significant increase in collagen fibril diameter and a decrease in fibril branching as the tissue matured.

In confined compression, characterised by one dimensional deformation, fluid flux occurs in the axial direction, forcing interstitial fluid through the SR of the tissue. An explanation for the reduction in the dynamic moduli in confined compression upon removal of the SR in mature articular cartilage may therefore be that the SR acts as a low permeability barrier to fluid flow, where its removal results in a reduced ability to maintain fluid load support. This argument is reinforced by the finding that the ratio of peak stress to equilibrium stress also significantly reduces after removal of the SR at 10% applied strain, and by the finding that the permeability of the tissue increases after removal of the SR. This is consistent with previous interpretations that the tangential zone acts to limit fluid flow out of and into articular cartilage. In contrast, removal of the SR did not influence the dynamic properties of the immature 4 week old tissue, which is likely due to the fact that the tissue is predominantly isotropic in structure and has yet to obtain a Benninghoff architecture (Fig. 7). Fibrils in the 4 week old tissue are approximately five times smaller than 3 year
old mature fibrils, which likely contributes to the finding of an overall higher permeability in immature cartilage. Furthermore, the densely packed and highly aligned collagen fibrils observed in the superficial region of mature articular cartilage are also hypothesized to contribute to the lower permeability observed in this tissue.

The finding that the equilibrium modulus increases upon removal of the SR may also be due to a number of factors. An increase in compressive stiffness with depth through articular cartilage is traditionally associated with an increase in sulphated GAG content, resulting in higher fixed charge densities which generate greater Donnan osmotic fluid pressures\(^{15}\). However, more recently, increases in compressive stiffness has also been correlated with increases in tissue collagen content\(^{2,29-32}\), in part due to its effect on extracellular water and the effective FCD within the tissue\(^{33}\). Whilst no significant difference was observed between the bulk biochemical composition of the SR and the remaining cartilage for any age-group, the sGAG content (% wet weight) and collagen content (% wet weight) reveal a trend toward a higher content in the DZ of each age group. Together this might lead to an increased compressive modulus in the deeper regions of the tissue. Clearly removing a softer (in equilibrium) SR from a plug of articular cartilage would lead to an increase in the equilibrium modulus of the remainder of the tissue.

Geometric differences between mature and immature tissue (specifically that mature tissue is significantly \((P < 0.001)\) thinner than immature tissue) were also considered as a possible alternative explanation for the fact that removal of the SR (defined as the top 15% of total tissue thickness) only negatively impacted the dynamic modulus of mature articular cartilage. In order to investigate this thickness effect further; cylindrical cores of articular cartilage were modelled\(^{34}\) as homogeneous, nonlinear elastic (Neo-Hookean) and linear biphasic in confined compression, with the same material properties (Young’s modulus \(E = 0.5\) MPa, Poisson’s ratio \(\nu = 0.2\), dynamic viscosity of the pore fluid \(\mu = 10^{-9}\) MPa*s, intrinsic permeability \(k = 7.5 \times 10^{-12}\) mm², porosity \(\phi = 0.8\) ) but different tissue thicknesses; see SI for further details. The simulated loading rates and magnitudes corresponded to the experimental protocol (see SI). Mature tissue was modelled with a thickness \(t = 1\) mm, whilst the immature tissue was modelled with \(t = 3\) mm. The results of these simulations indicate that as flow path length decreases with decreasing tissue thickness (i.e., as occurs with age), fluid flow induced viscous effects become less apparent relative to (thickness independent) elastic effects. Comparing dynamic moduli values in “thin” (mature) and “thick” (immature) tissue, removal of the superficial region induced a 5.46% drop in the dynamic modulus in the mature tissue compared to a 12.71% drop in the immature tissue, demonstrating that removal of the top 15% of a thick tissue (i.e., an immature tissue) has a more dramatic effect than removal of 15% of a thin tissue (i.e., the mature tissue). Experimentally, the opposite observation was made: the mature tissue was affected more by removal of the SR than the immature tissue. Therefore, the reduction of the dynamic modulus observed experimentally after removal of the SR in mature tissue is not due to geometric differences between the different age groups. This model prediction also at least partially explains why the dynamic modulus of the thicker immature articular cartilage in confined compression is higher than mature tissue (i.e., due to a greater flow path length in thicker

![Fig. 9. Finite element simulations of an osteochondral core with a fully developed mature Benninghoff architecture in unconfined compression. (A) Stress relaxation curve for the full thickness section (FT Mature) and after removal of the respective superficial region (Less SR) and subsequent dynamic loading of the sample (B) Radial displacement (mm) at the edge of the tissue in the superficial region and deep zone of the sample during stress relaxation and dynamic loading.](image)
tissue), and demonstrates that it is inappropriate to directly compare the absolute values of dynamic moduli in confined compression across different age groups.

Similar to the confined compression results, a significant drop in both the dynamic moduli (Fig. 2) and the ratio of peak stress to equilibrium stress (Fig. 3) was observed during unconfined compression testing after removal of the SR (for most strain levels) in the mature 1 year old articular cartilage. This was not observed in the immature tissue. This is speculated to be due to the mature superficial region acting to limit radial expansion (and hence reduced fluid load support) during unconfined compressive loading. A tissue plug with a fully developed Benninghoff architecture as described in16 was assumed for mature articular cartilage16. Details on the implementation can be found in the SI. These finite element simulations demonstrated a 34.34% decrease in the dynamic modulus after removal of the superficial region in the mature tissue [see inset of Fig. 9(a)].

The effect of the mature SR can be seen most clearly by examining the radial displacement (mm) at the edge of the tissue in the superficial region and deep zone of the sample [Fig. 9(b)]. Here much smaller sample displacements are predicted in the superficial region of the tissue during stress relaxation and dynamic testing. This further supports the theory that the SR can play a key role in unconfined compression by limiting radial expansion and thus maintaining fluid load support.15,17

The incremental aggregate moduli in confined compression, as well as the dynamic modulus in unconfined compression, were found to increase with age (Figs. 4 and 5). No significant changes in the bulk tissue mechanical properties between 4 week old and 1 year old tissue were observed, despite a significant drop in sGAG (% dry weight) content with age. These results are in agreement with previous studies that found an increase in compressive modulus between foetal bovine (0.11 ± 0.03 MPa) and adult bovine (0.31 ± 0.03 MPa) articular cartilage17, whilst other studies have observed an increase in equilibrium compressive modulus of New Zealand White rabbits in new-born (~0.5 MPa) to 18 months of age (~2.5 MPa) using creep indentation testing3. In addition, they highlight the importance of both increases in collagen (% dry weight) content17 and attainment of a Benninghoff architecture to the equilibrium and dynamic compressive properties of articular cartilage.

A fully developed superficial region has been shown to be of vital importance in maintaining the mechanical integrity of articular cartilage under a range of loading regimes17,33,35. The findings of this study demonstrate that the superficial region of articular cartilage undergoes structural adaptation with age, which in turn plays a key role in determining the dynamic compressive properties of the tissue. Further work is required to better understand the origin and impact of other sources of variability in articular cartilage, which were not directly considered in this study, such as heterogeneity in tissue composition, organization and biomechanics with location across a single joint. Understanding such mechanisms will play a key role in furthering our understanding of articular cartilage development and degeneration and has important implications for the field of functional cartilage tissue engineering, highlighting the significance of recapitulating the native cartilage organization in tissue engineered constructs for supporting in vivo loads.

Conflict of interest
The author declares that there is no conflict of interest.

Author contribution
Computer models were generated by TN. HIM was conducted by AB and AG. Biochemical analysis was carried out by NA. AG, TN and DK contributed equally to the conception and design of this study. All other acquisition and analysis of data and article drafting was carried out by AG. All authors approved the final draft of the manuscript.

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