

Early morphometric and anisotropic change in periarticular cancellous bone in a model of experimental knee osteoarthritis quantified using microcomputed tomography

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Abstract

Objective. To quantify early stage microstructural changes of periarticular cancellous bone in a canine anterior cruciate ligament transection model for experimental osteoarthritis.

Design. Unilateral transection of the anterior cruciate ligament was performed in 10 animals. Bone structure changes were quantified in five animals at 3-week post-transection and five animals at 12-week post-transection. An additional two non-operated animals were used as controls.

Background. Changes in trabecular architecture of the periarticular cancellous bone in early stage post-traumatic osteoarthritis is not well understood. Previous studies have found alterations in bone mineral density in experimental osteoarthritis suggesting adaptation of the trabecular structure. Early change of the periarticular bone following a ligament injury may contribute to the long-term development of osteoarthritis.

Methods. Bone cores from the medial condyles of the femoral and tibial pairs were scanned with a three-dimensional micro-tomographic system. Structural indices were quantified including bone volume ratio, bone surface ratio, trabecular thickness, trabecular separation, trabecular number, as well as structural anisotropy determined by the mean-intercept-length method.

Results. Significant structural changes were observed at 3-week post-transection, and were more prominent at 12-week post-transection. These changes were accompanied by decreasing anisotropy.

Conclusions. Periarticular cancellous bone microstructure is significantly altered in experimental osteoarthritis. These changes occurred as early as 3-week post-transection, and were large at 12-week post-transection.

Relevance

The pathogenesis of post-traumatic osteoarthritis is poorly understood, but it is clear that this disease involves the entire organ system of the joint, including the cartilages, synovium, ligaments, and bones. This study focuses on the changes that occur in the bones during the early stages following a joint injury, and contributes to a better overall understanding of the disease aetiology. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Post-traumatic osteoarthritis (OA) is characterized by changes throughout a joint, including the articular cartilage and periarticular bone. Considerable research has been aimed at the changes that occur in the joint

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Nomenclature			
Tb.Th	trabecular thickness	BS/BV	relative bone surface density
Tb.Sp	trabecular separation	DA ₁₃	degree of anisotropy: ratio of principle to tertiary eigenvalues of the fabric tensor
Tb.N	trabecular number	DA ₂₃	degree of anisotropy: ratio of secondary to tertiary eigenvalues of the fabric tensor
BV/TV	relative bone volume		

cartilages [1–3], however, the contribution of subchondral bone [4,5] in the pathogenesis of OA is less well understood. Subchondral bone, i.e., the thin subchondral plate and the underlying cancellous bone, is reportedly sensitive to changes in its physiological [6] and mechanical environment [7], with changes in the cancellous bone being particularly conspicuous.

The natural history of post-traumatic OA involves progressive changes in the joint beginning at the time of injury, and continuing until the late stages of the disease, which are characterized by joint space narrowing, full thickness cartilage erosion, osteophyte development, and subchondral sclerosis [8]. While late-stage subchondral bone changes have been described for ACL injured patients with developed symptoms of OA [9], little is known about changes that occur shortly after joint trauma. However, one study showed that areal bone mineral density (BMD) decreased up to 21% during one year after anterior cruciate ligament (ACL) rupture [10], though these patients were treated surgically. Nonetheless, the bone seems sensitive to injury.

In a longitudinal study with the ACL transected (ACLX) dog model for post-traumatic OA, Brandt and colleagues [4] reported degenerative changes in the knee joint 54-month post-ACLX that had similar characteristics to late-stage OA development in humans. Specifically investigating bone changes, Dedrick and colleagues [11] reported a significant increase in subchondral bone plate thickness at 54 months, but not at 3 and 18 months. This result is consistent with increased subchondral sclerosis found in late-stage human patients [8,10]. Notably, in this animal model, changes in the subchondral cancellous bone were evident at all three time points investigated (3, 18, 54 months). This suggests that the periarticular cancellous bone is more sensitive to the effects of joint injury than the subchondral plate, however, the temporal evolution of these changes, and the nature of the changes in the cancellous bone microarchitecture have not been described. Decreases in volumetric BMD have been reported as early as 3 weeks after injury in this model [12], suggesting that structural adaptations may be occurring.

The cancellous architecture is related to the mechanical properties of cancellous bone [13], and its measurement provides not only important information about the structural changes that occur in cancellous bone, but also provides clearer information about the significance of these changes in bone morphology and

bone strength. If the changes in bone morphology are irreversible, i.e., exceed the physiological capacity to adapt, then understanding when these changes occur may influence the decision to apply preventative measures early in the progression of the disease. Morphometric properties of the microarchitecture of cancellous bone can be calculated from three-dimensional (3D) imaging techniques such as serial sectioning [14] or microcomputed tomography (μ CT) [15,16]. Some established morphometric parameters [17] include bone volume ratio (BV/TV), bone surface ratio (BS/BV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp).

Anisotropy of the bone fabric, or the trabecular orientation, is closely related to the bone mechanical anisotropy [18]. The development and maintenance of the fabric anisotropy is influenced by the loading environment of the bone, and the change in anisotropy may indicate alterations in the loading conditions that occur in the post-traumatic knee joint. Although several methods for characterizing anisotropy are available [19], mean intercept length (MIL) [20] is a well-established method to quantify the degree of anisotropy (DA) and trabecular orientation.

The pathogenesis of post-traumatic OA is poorly understood, however, it is clear that this disease involves the entire organ system of the joint, including the cartilages, synovium, ligaments, and bones. Previously, significant changes in articular cartilage were reported, particularly in the medial aspect of the joint, as early as four weeks after surgery [21]. The present study focuses on the changes that occur in the subchondral bone architecture during the early stages following a cruciate ligament transection. It is hypothesized that a decrease in BMD after this post-traumatic knee injury is the result of morphological and anisotropic changes to the cancellous architecture. Thus, the objective of this study is to quantify these early morphometric and anisotropic changes to cancellous bone architecture in this canine model of experimental OA.

2. Methods

Skeletally mature, mixed-breed dogs ($n = 10$) were randomly assigned to one of two experimental groups: 3-week ($n = 5$) and 12-week ($n = 5$) post-unilateral ACLX. Two non-operated animals were used as a

baseline comparison with the contralateral limbs. Animal body masses ranged from 16 to 34 kg (mean 23.5 kg), and ages ranged from 1.5 to 3 yr (mean 2.5 yr). All procedures were approved by the University of Calgary Animal Care Committee. After unilateral ACLX by lateral arthrotomy as described previously [21–23] the dogs were housed in pens ($1.5 \times 2 \times 2 \text{ m}^3$) for the first two weeks and then allowed to ambulate freely in large pens ($9 \times 8.5 \times 2 \text{ m}^3$). Food and water were provided ad lib. Dogs rapidly returned to their pre-injury level of activity where eight animals showed no distinctive limp after two weeks, and the remaining two showed no limp after six weeks. At euthanasia, the femora and tibia were cleaned of all soft tissue, hermetically sealed, and stored at -80°C .

2.1. Specimen preparation

Cylindrical bone cores (dia. 6 mm, 12–14 mm length) were excised from the centre of the weight bearing regions of the medial femoral condyles and medial tibial plateaus for each knee using a hollow coring bit while the bones were secured in a custom designed jig, which consistently aligns the bones. Physiological saline (0.15 M NaCl) was applied through the bit to provide moisture and cooling. The cylindrical core included the cortical bone (1–2 mm) near the articular surface, and underlying cancellous bone (10–12 mm).

2.2. μCT scanning

The cores were scanned using a high resolution microtomographic (μCT) scanner [16] (μCT 20, Scanco Medical, Bassersdorf, Switzerland), providing a nominal isotropic resolution of $34 \mu\text{m}$ (voxel dimension). A constrained Gaussian filter ($\sigma = 1.2$, finite support of 2) was used to partly suppress the noise in the original volume data, and the data were globally thresholded (10.2% of maximal possible gray value) to extract the mineralized bone phase [24]. From the 3D image, a region of interest (ROI) was selected for structural analysis of the cancellous bone. The ROI was cylindrical (dia. 165 voxels), and included cancellous bone located from just below the cortical shell and extending up to 12 mm (353 voxels) into the cancellous region of the core. Some samples were shorter (between 10 and 12 mm) due to the physical limitations in smaller animals in which case the available cancellous bone was analysed.

2.3. 3D structural analysis

All structural analyses were based on the 3D image data sets. Bone volume ratio (BV/TV) was calculated by summing the number of voxels representing mineralized trabecular bone divided by the total volume, and expressed as a percentage. The bone surface ratio (BS/BV)

was calculated by summing the triangulated surface components of the mineralized bone phase [25] directly from the 3D data and dividing by the total bone phase volume. Trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and trabecular number (Tb.N) were also based directly on the 3D data using medial axis transformation and distance transformation [25,26], rather than with model-based assumptions of a rod or a parallel plate structure.

Mean intercept length (MIL) [20] was calculated on a 3D spherical subvolume of the bone image (dia. 5 mm), centered 4.5 mm below the cortical surface. The intersections between the bone–marrow interface were sampled along 500 parallel lines at 500 random orientations. The distribution of intercepts is ellipsoidal, from which the orientation and magnitude of the principal axes can be calculated. The principal axes of the ellipsoid are directly related to the anisotropy of the bone [18,20]. The primary degree of anisotropy (DA_{13}) was reported, which is the ratio of the primary and tertiary axis. The secondary degree of anisotropy (DA_{23}) was also reported, which is the ratio of the secondary and tertiary axes. A value of 1.0 for primary anisotropy indicates overall bone material isotropy, while a value of 1.0 for secondary anisotropy indicates transverse isotropy. The eigenvalues of the fabric tensor were normalized by the trace of the fabric tensor and are reported.

2.4. Statistical analysis

Statistical analysis of the results was done separately for the femoral and tibial data. For each set, the measured morphometric parameters was analysed using pair-wise comparisons (*t*-test) to assess changes in the 3- and 12-week post-ACLX groups between ACLX and contralateral limbs. Statistical significance was determined for a *P*-value of smaller than 0.05. The normal controls ($n=2$) provide a basis for comparison with the contralateral limb to ensure the contralateral joints are an appropriate control and these data were presented descriptively.

3. Results

The tibia and femur exhibited architectural changes in the subchondral bone at both 3- and 12-week post-ACLX. These changes can be observed visually, for example, in rendered 3D images of typical bone samples (based on having median morphological measures) from both the ACLX and contralateral medial femoral condyle (Fig. 1). Similar visual changes in trabecular bone architecture occur in the tibia.

At 3-week post-ACLX significant changes were found in the tibia, but not the femur, although the trends were similar. At 12-week post-ACLX, the

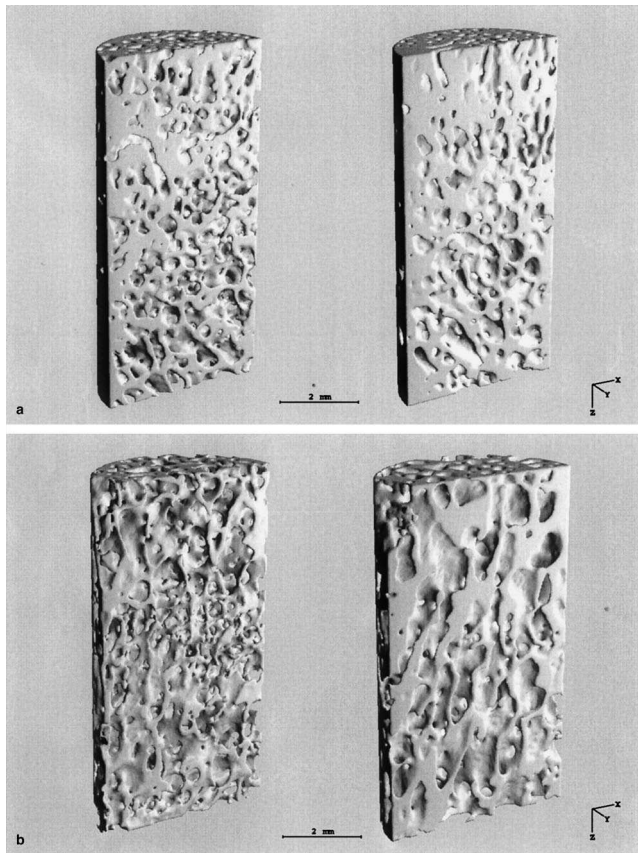


Fig. 1. Rendered 3D trabecular architecture of canine subchondral cancellous bone in the medial condyle of the distal femur using micromotomography (isotropic resolution 34 μm). The cylindrical examination volume is graphically dissected in half for presentation purposes. An image of the ACLX limb (left) and contralateral (right) is shown for a sample at: (a) 3-week post-ACLX and (b) 12-week post-ACLX (samples shown are representative of median morphometric parameters).

changes were larger than at 3-week, reaching statistical significance for both the tibia and femur. The morphometric changes can be characterized in general as decreasing BV/TV and Tb.Th (maximum changes were -41.5% and -32.7% , respectively), increasing BS/BV and Tb.Sp (maximum changes were 74.3% and 19.0% , respectively), and no detectable change in the Tb.N. The means and S.D. for each morphometric parameter are presented in Table 1, including the pair-wise differences. Significant differences between normal and contralateral control are indicated for $P < 0.05$. The results of the t -test to determine statistical significance with small pair-wise groups ($n = 5$) must be treated with some caution, however, careful examination of the data revealed no outliers, and a normal distribution (Kolmogorov-Smirnov test, $P = 0.92$), which indicated that the t -test was reasonable.

A trend toward decreased anisotropy (DA_{13}) was observed in both the tibia and femur following ACLX. There was a trend toward decreasing DA_{13} , and this

alteration was statistically significant in the tibia 12-week post-ACLX (-10.1% decrease). The secondary degree of anisotropy (DA_{23}) was decreased in the femur at 12-week post-ACLX (-5.8% decrease). The magnitudes and pair-wise differences of DA_{13} and DA_{23} are presented in Table 2, and the magnitudes of each of the principle axes (eigenvalues of the fabric tensor) are reported in Table 3.

The normal control knees are presented alongside the ACLX and contralateral control limbs of the 3-week and 12-week animals (Tables 1 and 2). With the possible exception of the BV/TV and BS/BV in the femur, the normal controls exhibited morphometric and anisotropic parameters that were similar to the contralateral control limbs.

4. Discussion

The trabecular structure of knee joint periarticular cancellous bone in this model of post-traumatic early OA was detectable as early as 3-week post-ACLX and was prominent at 12-week post-ACLX. Fig. 1 illustrates that architectural changes in the subchondral trabecular bone occur at both time points, and are particularly obvious at 12-week post-ACLX. These bone changes precede cartilage degeneration which typically are absent at 10–12 weeks, but are present by 32-week post-ACLX [21].

A limitation of the study was that the samples sizes were small, which can effect the results of the t -test. An examination of the data revealed no outliers that it was relatively normal, and had equal S.D.s This supports the validity of the pair-wise t -test, and statistically significant results were found for several of the bone changes.

The contralateral limb was used to assess all the bone parameters measured. Although the contralateral joint is an imperfect control, past studies utilizing the canine ACLX model for OA have found no evidence of significant changes in subchondral bone in the contralateral knee for up to 54-month post-ACLX [4,27,28]. In the present study, the contralateral limb was similar to the normal non-operated animals for all 14 morphologic and anisotropic parameters measured with the possible exception of two: BV/TV and BS/BV in the femur. The use of the contralateral limb is statistically advantageous, as bilateral variation within animals is smaller than among different animals [29]. The two non-operated control animals in this study were similar to the contralateral limbs, which ensures our data is consistent with previous reports suggesting the contralateral limb is an acceptable control.

Another possible limitation of this study was the influence of thresholding the 3D μCT data to obtain a binary representation of mineralized trabecular bone. This could influence estimation of morphological

Table 1

Summary of morphometric changes in canine model for early stage post-traumatic OA. Presented are the means and S.D. for each group, and the pair-wise difference between ACLX and contralateral (Δ). A positive (or negative) difference (Δ) is represented by an upward arrow (or downward arrow, respectively) if the difference is statistically significant based on a pair-wise t -test ($P < 0.05$). A horizontal arrow indicates that no statistically significant change was detected^a.

Index	Group	BV/TV [%]		BS/BV [1/mm]		Tb.Th [mm]		Tb.Sp [mm]		Tb.N [1/mm]	
		Mean (S.D.)	Δ (S.D.)	Mean (S.D.)	Δ (S.D.)	Mean (S.D.)	Δ (S.D.)	Mean (S.D.)	Δ (S.D.)	Mean (S.D.)	Δ (S.D.)
Femur	3-week ACLX	52.36 (3.93)	$\longleftrightarrow$ -4.91 (4.83)	10.22 (1.42)	$\longleftrightarrow$ 1.23 (1.03)	0.22 (0.02)	$\longleftrightarrow$ -0.018 (0.02)	0.31 (0.02)	$\longleftrightarrow$ 0.007 (0.03)	2.62 (0.14)	$\longleftrightarrow$ 0.048 (0.10)
	3-week ctrlat	57.27 (6.76)		8.99 (1.93)		0.24 (0.04)		0.30 (0.03)		2.58 (0.13)	
	12-week ACLX	38.47 (4.16)	$\downarrow$ -20.38 (4.99)	13.91 (0.99)	$\uparrow$ 5.91 (1.58)	0.18 (0.01)	$\downarrow$ -0.078 (0.02)	0.39 (0.06)	$\uparrow$ 0.061 (0.02)	2.34 (0.21)	$\longleftrightarrow$ -0.066 (0.07)
	12-week ctrlat	58.85 (1.84)		8.00 (0.66)		0.26 (0.02)		0.33 (0.04)		2.40 (0.19)	
	Normal unop.	50.67 (1.73)	n/a	10.06 (0.71)	n/a	0.22 (0.01)	n/a	0.35 (0.01)	n/a	2.37 (0.05)	n/a
	3-week ACLX	39.86 (7.54)	$\downarrow$ -14.11 (6.48)	12.39 (2.41)	$\uparrow$ 3.66 (1.30)	0.20 (0.03)	$\downarrow$ -0.046 (0.03)	0.40 (0.03)	$\uparrow$ 0.045 (0.04)	2.23 (0.10)	$\longleftrightarrow$ -0.079 (0.09)
	3-week ctrlat	53.97 (9.58)		8.72 (2.48)		0.25 (0.05)		0.35 (0.02)		2.31 (0.08)	
Tibia	12-week ACLX	28.33 (4.12)	$\downarrow$ -20.11 (5.44)	15.79 (1.98)	$\uparrow$ 6.73 (1.49)	0.16 (0.01)	$\downarrow$ -0.080 (0.03)	0.50 (0.05)	$\uparrow$ 0.080 (0.05)	1.89 (0.17)	$\longleftrightarrow$ -0.146 (0.15)
	12-week ctrlat	48.44 (5.25)		9.06 (1.49)		0.24 (0.03)		0.42 (0.03)		2.04 (0.10)	
	Normal unop.	49.77 (4.66)	n/a	8.67 (1.13)	n/a	0.25 (0.02)	n/a	0.40 (0.02)	n/a	2.06 (0.07)	n/a
	3-week ACLX	39.86 (7.54)	$\downarrow$ -14.11 (6.48)	12.39 (2.41)	$\uparrow$ 3.66 (1.30)	0.20 (0.03)	$\downarrow$ -0.046 (0.03)	0.40 (0.03)	$\uparrow$ 0.045 (0.04)	2.23 (0.10)	$\longleftrightarrow$ -0.079 (0.09)
	3-week ctrlat	53.97 (9.58)		8.72 (2.48)		0.25 (0.05)		0.35 (0.02)		2.31 (0.08)	

^a ACLX = anterior cruciate ligament transected; ctrlat = contralateral; normal unop. = normal unoperated; n/a = not applicable.

Table 2

Summary of anisotropic changes in canine model for early stage post-traumatic OA. Presented are the means and S.D. for each group, and the pair-wise difference between ACLX and contralateral (Δ). A positive (or negative) difference (Δ) is represented by an upward arrow (or downward arrow, respectively) if the difference is statistically significant by pair-wise *t*-test ($P < 0.05$). A horizontal arrow indicates no statistically significant change was detected^a

Index	Group	MIL: DA ₁₃ [1]		MIL: DA ₂₃ [1]	
		Mean (S.D.)	Δ (S.D.)	Mean (S.D.)	Δ (S.D.)
Femur	3-week ACLX	1.82 (0.13)	$\longleftrightarrow$	1.36 (0.08)	$\longleftrightarrow$
	3-week ctrlat	1.93 (0.14)	-0.11 (0.11)	1.39 (0.08)	-0.02 (0.04)
	12-week ACLX	1.87 (0.08)	$\longleftrightarrow$	1.31 (0.05)	$\downarrow$
	12-week ctrlat	2.02 (0.21)	-0.15 (0.13)	1.37 (0.08)	-0.08 (0.05)
	Normal unop.	1.99 (0.03)	n/a	1.39 (0.06)	n/a
Tibia	3-week ACLX	1.66 (0.13)	$\longleftrightarrow$	1.13 (0.06)	$\longleftrightarrow$
	3-week ctrlat	1.80 (0.10)	-0.14 (0.15)	1.14 (0.09)	-0.01 (0.04)
	12-week ACLX	1.63 (0.11)	$\downarrow$	1.15 (0.05)	$\longleftrightarrow$
	12-week ctrlat	1.81 (0.09)	-0.18 (0.12)	1.16 (0.09)	-0.01 (0.09)
	Normal unop.	1.84 (0.11)	n/a	1.17 (0.06)	n/a

^a ACLX = anterior cruciate ligament transected; ctrlat = contralateral; normal unop. = normal unoperated; n/a = not applicable.

Table 3

Summary of the mean and S.D. eigenvalues of the fabric tensor ($|H1|$, $|H2|$, $|H3|$) for each group. Eigenvalues have been normalized by the trace of the fabric tensor ($|H1| + |H2| + |H3|$)^a

Index	Group	$ H1 $	$ H2 $	$ H3 $
		Mean (S.D.)	Mean (S.D.)	Mean (S.D.)
Femur	3-week ACLX	0.44 (0.01)	0.33 (0.01)	0.24 (0.01)
	3-week ctrlat	0.45 (0.01)	0.32 (0.01)	0.23 (0.01)
	12-week ACLX	0.45 (0.01)	0.31 (0.01)	0.24 (0.01)
	12-week ctrlat	0.46 (0.02)	0.31 (0.01)	0.23 (0.02)
	Normal unop.	0.45 (0.01)	0.32 (0.01)	0.23 (0.01)
Tibia	3-week ACLX	0.44 (0.02)	0.30 (0.01)	0.27 (0.01)
	3-week ctrlat	0.46 (0.01)	0.29 (0.01)	0.25 (0.01)
	12-week ACLX	0.43 (0.02)	0.31 (0.01)	0.26 (0.01)
	12-week ctrlat	0.46 (0.02)	0.29 (0.02)	0.25 (0.01)
	Normal unop.	0.46 (0.01)	0.29 (0.01)	0.25 (0.01)

^a ACLX = anterior cruciate ligament transected; ctrlat = contralateral; normal unop. = normal unoperated; n/a = not applicable.

parameters [30], however, a consistently applied threshold ensures reliable comparison between specimens, although the absolute value of these measures may include a small amount of error. While the resolution of the μ CT measurements (34 μ m) could also influence the results, this source of error was unlikely to skew measures of trabecular thickness which were on the order of 160–260 μ m (Table 1) [31].

Direct 3D methods were used to calculate Tb.Th, Tb.Sp and Tb.N independently [26], instead of using the traditional morphometric methods [32] which require assumptions with about trabecular bone structure (i.e., plate- or rod-structure) and do not fully exploit the 3D data set. In longitudinal studies of diseased bone, the bone structure assumption may be violated thereby precluding the comparison of morphological parameters over time as, for example, in a rat model for osteopo-

rosis [33]. For this reason, we chose to use direct 3D methods which made full use of the 3D image data [26], and was independent of model based assumptions which may change during the development of a disease such as OA.

The trends in the morphological changes that we reported are similar to previous studies, however, the magnitude of these changes were different. Dedrick et al. [34] investigated changes in the subchondral bone in the canine model of OA between 3 and 54 months after ACLX. Generally, they found a progression of decreasing Tb.Th and increasing BS/BV in the tibial plateau, which was consistent with our findings. However, the magnitude of the Tb.Th and BS/BV changes reported by Dedrick et al. were greatest between 3 and 18 months ($\sim 7\%$), and only small changes occurred between 18 and 54 months ($< 2\%$). Combined with our

finding of decreases in Tb.Th (~32%) and increased BS/BV (~74%) between 3-week and 12-week post-ACLX, it appears that substantial morphological changes in the periarticular cancellous bone occurred early in this experimental model.

The decrease in BMD reported previously [12] is reflected in the decreased bone volume ratio (Table 1). While the thickness of the trabeculae decreased, there was a corresponding increase in the spacing between the trabeculae, and there was no detectable change in the trabecular number. These changes suggest that there was no loss of trabeculae, but rather a decrease in the thickness of the trabeculae. However, as the MIL measure indicates, the decrease in thickness was not uniform. Furthermore, the bone surface ratio dramatically increased as high as 74% (Table 1), thus it is a very sensitive index of trabecular structure. The functional significance of altered BS/BV may be related to the biological processes involved in bone remodelling, which predominantly occurs on the trabecular surface. The sensitivity of BS/BV using 3D microstructure data suggests that it is a potentially useful tool for early detection of trabecular bone resorption. This measure may become a useful tool as clinical measures of bone microstructure for disease detection become more widely available [35].

The changes in MIL, an index of anisotropy, indicate that trabecular morphology was not altered uniformly in all directions. This is obvious in Fig. 1 where the ACLX cancellous bone has a significantly less plate-like appearance than the corresponding contralateral limb. While the exact effect of altered anisotropy on the mechanical behaviour of the cancellous bone is unclear, it does provide insight into the possible mechanisms of bone adaptation that occur in the ACLX limb. Such a non-uniform change is likely governed by some mechanism that causes the trabeculae to preferentially thin along certain directions. A possible explanation is the altered loading configuration in the knee joint after ACLX where loading is reportedly reduced to about 35% of pre-operative levels two weeks after surgery, and slowly increases to 50–60% within 6–12 weeks [36,37]. With these loading conditions, coupled with altered contact patterns and stress distributions within the joint [38], a new mechanical environment exists for the bone that may lead to the measured non-uniform changes seen in trabecular thickness.

Overall, the changes in cancellous bone morphology and anisotropy measured suggest a decreased stiffness of the periarticular bone. These changes in the bone after joint trauma occur early and appear to persist both in dogs and, based on bone mineral density measures, in humans [4,8–10]. The implications of these presumed stiffness changes and their contribution to the development of OA remain unclear. A large stiffness gradient between cartilage and the underlying subchondral bone

may preclude any effect of the altered bone on the stress distribution either within, or at the interface with, the overlying cartilage. However, in a study investigating the effect of localized *increases* in subchondral bone stiffness due to implants [39], it was reported that increased subchondral bone stiffness within a localized region elevated the cartilage stresses near the cartilage–bone interface. This implies that there may be a relationship between subchondral bone stiffness and cartilage stress distributions, however, it is unclear whether a decrease in the global stiffness of the subchondral bone as suggested in the present study (e.g., –33% Tb.Th change) might have an effect. This is a topic of future study using finite element analysis.

In conclusion, significant periarticular bone changes occurred as early as 3-week post-ACLX, and these changes were more pronounced 12-week post-ACLX. In addition to morphological changes such as trabecular thickness and separation, the anisotropic properties were also altered. Whether these bone changes are irreversible is not known, however, it is clear that they occurred very early following the initial joint trauma. These data contribute to our understanding of the bone changes in this experimental model for post-traumatic OA.

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