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Title: Osteoarthritic bone marrow lesions almost exclusively co-locate with denuded cartilage: a 3D study using data from the Osteoarthritis Initiative

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ABSTRACT

Objectives: The aetiology of bone marrow lesions (BMLs) in knee osteoarthritis (OA) is poorly understood. We employed 3D active appearance modelling (AAM) to study the spatial distribution of BMLs in an OA cohort and compare this with the distribution of denuded cartilage.

Methods: Participants were selected from the Osteoarthritis Initiative progressor cohort with Kellgren-Lawrence scores ≥2, medial joint space narrowing and osteophytes. OA and ligamentous BMLs and articular cartilage were manually segmented. Bone surfaces were automatically segmented by AAM. Cartilage thickness of <0.5mm was defined as denuded and ≥0.5 to 1.5mm as severely damaged. Non-quantitative assessment and 3D population maps were used for analysing the comparative position of BMLs and damaged cartilage.

Results: 88 participants were included, 45 men, mean age (SD) was 61.3 (9.9) years, and mean BMI was 31.1 (4.6) kg/m^2. 227 OA and 107 ligamentous BMLs were identified, in 86.4 and 73.8% of participants; OA BMLs were larger. Denuded cartilage was predominantly confined to a central region on the medial femur and tibia, and the lateral facet of the trochlear femur. 67% of BMLs were co-located with denuded cartilage and a further 21% with severe cartilage damage. In the remaining 12%, 25/28 were associated with cartilage defects. 74% of all BMLs were directly opposing (kissing) another BML across the joint.

Conclusions: There was an almost exclusive relationship between the location of OA BML and cartilage denudation, which itself had a clear spatial pattern. We propose that OA, ligamentous and traumatic BMLs represent a bone response to abnormal loading.

Introduction

Osteoarthritis (OA) is the commonest arthritis and results in significant personal suffering and massive socio-economic impact globally. Current treatments are aimed at symptom modification and limited by efficacy, compliance and pharmacological toxicity. Greater understanding of OA pathological processes is needed to underpin the search for new therapies.

Magnetic resonance imaging (MRI) has improved understanding of OA pathology. It has demonstrated the prevalent nature of cartilage loss, which frequently leads to denuded areas of bone [1, 2]. MRI has also highlighted the whole-organ nature of the OA process and in particular has demonstrated highly prevalent bone marrow lesions (BMLs). BMLs, defined as ill-delineated regions of hyper-intensity in fat suppressed MRIs, are comprised of histological abnormalities such as fibrosis, necrosis, trabecular bone abnormalities and microfractures [3, 4]. They have been associated with both pain [5, 6] and progressive compartment-specific cartilage loss [7-9].

Little is known about the aetiology of OA BMLs, though fluctuation in size has been reported [10], as has response to pharmacological and mechanical intervention [11, 12]. BMLs have been classified into groups based on their location and presumed mechanism of formation [13, 14]. Trauma-associated BMLs are often associated with meniscal and ligamentous soft tissue injury. These BMLs reflect the mechanism of the injury sustained, for instance where two bones impact there will be "kissing" BMLs at the sites of impact, such as seen involving the lateral femoral condyle and posterolateral tibial plafond following a "pivot-shift" injury leading to an anterior cruciate ligament tear. Subchondral OA BMLs are associated with other MRI findings of OA and are not associated with a history of reported trauma or acute soft tissue injury.

Modern imaging analysis using active appearance models (AAMs), a form of statistical shape model, enables accurate quantification of tissue morphology and provides a consistent 3-dimensional

framework for reproducible measurement across a cohort of patients, allowing comparison of the spatial locations of different tissues.

Intact healthy hyaline cartilage protects the underlying bone from abnormal contact loading as a consequence of a matrix of collagen fibres and the material properties of cartilage [15, 16]. We hypothesised that OA BMLs represent a response of bone to abnormal loading, and that the bone beneath areas of cartilage loss should be more likely to contain OA BMLs. We explored this hypothesis in a subset of the NIH Osteoarthritis Initiative (OAI) selected for the presence of medial OA.

Methods

Participants were selected from the OAI progressor cohort (0.B.1 and 1.B.1) using radiographic criteria intended to enrich the sample for cartilage damage: presence of osteophytes, a KL score ≥2 and medial joint space narrowing greater than lateral joint space narrowing. All participants also had pain, aching or stiffness in the enrolment year. MR images were acquired in identical 3T systems (Siemens Magnetom Trio, Erlangen, Germany) using a standard knee coil across all clinical centres. The sagittal intermediate-weighted turbo spin echo fat suppression sequence (TSE) was used for manual segmentation of BMLs, and the sagittal water-enhanced dual echo steady-state (DESS) sequence for manual segmentation of the cartilage. Manual cartilage segmentation was supervised by a musculoskeletal radiologist and an expert segmenter as previously described [17]. Bone surfaces of the femur, patella and tibia were manually segmented in the DESS images, to assess the accuracy of the AAM bone searches. 2 segmenters were trained by Imorphics, and certified when able to repeatedly segment bone and cartilage with coefficient of variation of <3%. This equates to a 95% confidence limit of ± 0.1 mm on mean cartilage thickness of 2 mm.

Cartilage thickness maps for the femur, tibia and patella were produced as previously described [17, 18]. Briefly, all bones were automatically segmented from the DESS images using AAMs. This

provides a dense anatomically corresponded set of points on each bone surface. Cartilage thickness was measured at each correspondence point using a vector perpendicular to the surface, and finding its point of intersection, if any, with inner and outer cartilage surfaces. Denuded cartilage was defined as cartilage thickness of less than 0.5mm, the average length of a voxel edge. Severe cartilage damage was defined as between 0.5 and 1.5mm. Severe damage was defined as mean thickness in KL0 knees (2.25mm), less 1 SD (0.79mm) = 1.46mm, rounded to 1.5mm. Cartilage thickness maps were displayed on the mean bone shape, after subtracting the peripheral region around each cartilage plate, where cartilage thickness is normally below 0.5mm.

The procedure for manual BML segmentation was developed in association with a musculoskeletal radiologist and an experienced segmentation supervisor using EndPoint software (Imorphics, Manchester, UK). Subchondral BMLs were defined as ill-delineated regions of hyper-intensity located within the subchondral bone. We defined BMLs as lesions present on more than 1 slice and with a volume greater than 250 mm^3, to minimise inclusion of artefacts. BMLs were classified either as OA (based on their subchondral location) or ligamentous (if present away from the subchondral location, and overlapping ligament or meniscal attachments), and further divided into anatomical regions.

Eight anatomical regions were defined for OA BMLs (Figure 1A). MPF: medial patellofemoral region of the femur; MFT: medial femorotibial femur; LPF: lateral patellofemoral region femur; LFT: lateral femorotibial region femur; MT, LT: medial and lateral tibia; MP, LP: medial and lateral patella. BMLs were categorised as belonging to a compartment if the majority of the volume was present within that compartment. Nine anatomical regions were defined for ligamentous BMLs. ACLFemur, PCLFemur: attachments of the anterior and posterior cruciate ligaments on the femur; ACLTibia, PCLTibia, attachments of the anterior and posterior cruciate ligaments on the tibia; AntLatMen, AntMedMen: the anterior lateral and medial meniscal attachments; PostLatMen, PostMedMen, the

posterior lateral and medial meniscal attachments; and SITTM: the semitendinosus, iliotibial tract and medial collateral ligament attachments.

Accuracy of AAM bone searches were assessed by comparing them with manual segmentations of the same bone. Measurements of the distance from the automatically segmented AAM surface to the manually segmented surfaces were measured perpendicular to the surface at each correspondence point in the AAM surface (52,892 points in the femur; 34,382 in the tibia; and 17,582 in the patella). Results were expressed as mean (RMS) point-to-surface error, and 95^{th} percentile point-to-surface error.

3-dimensional images of the spatial distribution of BMLS within the mean bone shapes were then prepared by a step-wise process that involved: (1) creating 3D surfaces of all BMLs from manual segmentations of TSE images, using a marching cubes algorithm; (2) automatically searching TSE images using AAMs of the femur, tibia and patella bones to create AAM bone surfaces[19]; (3) rigidly warping individual BML surfaces to the mean bone shape with a transform calculated from the Euclidean alignment of each AAM bone surface to the mean bone shape (Euclidean alignment permits scaling of the object at the same time as a rigid alignment); (4) converting the warped BML surfaces for each TSE image into a binary image; and then (5) adding together the contents of the binary images into a combined reference image. Each voxel in the combined reference image then contains a count of how many times that voxel was included within a BML in the population.

Summary images were prepared showing the average position of OA and ligamentous BMLS by identifying all voxels in the reference image which had a value equal to or greater than that required to include approximately half of the total number of BML hits. The resultant voxels were displayed inside a semi-transparent mean bone surface. In a 'sensitivity' analysis, summary images representing 25% and 75% of all BML hits were also prepared, to demonstrate that the mean data were representative of the population and not driven by outliers.

For the non-quantitative assessments, 3-dimensional visualisations were created which showed the denuded and damaged cartilage masks in different colours on a semi-transparent bone, together with the BML surfaces (Figure 1B). This allowed 3D examination of the bone marrow lesions, and their spatial relationship to any cartilage denudation in each individual.

To further investigate the spatial relationships between individual OA BMLs and cartilage loss, 3 aspects of each BML were assessed. Firstly BMLs were categorised as either associated with denuded cartilage, associated with severe cartilage damage or not associated with either lesion, based on visual evaluation using the semi-transparent bone images as shown in Figure 1. BMLs were considered associated if approximately 25% or more of the denuded area was directly over the BML BMLs were categorised as large (>66% of the sub-region), medium (33-66%) or small (<33%) by a independent reader, blinded to the volumetric results). A BML was described as a kissing lesion if a BML existed in a directly opposed bone region (for example the MPF region of the femur is opposite the MP region of the patella). Ligamentous BMLs were not included in this analysis as they are not co-located with cartilage plates. In cases where no relationship was found between BMLs and cartilage lesions according to our denuded and severe damage definitions, the presence or absence of cartilage defects was recorded.

Results

88 participants were included in this study, 45 were male. The mean age was 61 (range 45-78) years and median BMI was 31 (range 22-49) kg/m^2. Mean anatomical alignment, assessed by goniometer, was -1° (range -12° to +7°, varus mal-alignment negative). The accuracy of the AAM bone search for femur (95th percentile) was 0.38(1.06) mm, tibia 0.31(0.84) mm, patella 0.27(0.71) mm.

Group level findings

Denuded cartilage showed a characteristic pattern in the femur and tibia, with denudation concentrated in specific anatomical regions (Figure 2). In this cohort selected for medial

radiographic OA, most cartilage loss was in the medial compartments of femur and tibia, with substantial loss also visualised in the patella and in the lateral patellofemoral compartment of the femur.

Osteoarthritis BMLs also showed a characteristic pattern of distribution (Figure 2). On visual inspection these areas strongly co-located with areas of cartilage denudation. The sensitivity analysis also demonstrated these patterns of co-location (data not shown). Ligamentous BMLs also demonstrated an expected pattern of distribution with the majority of these lesions at the cruciate ligament and meniscal attachment sites (Figure 3).

Individual participant findings

OA BMLs were almost twice as prevalent as ligamentous BMLs; they were seen in 76 (of 88) and 65 participants respectively. The majority of OA BMLs (66%) were large while most ligamentous lesions were smaller in size. 67% of individual OA BMLs were co-located with denuded cartilage and a further 21% co-located with severe cartilage damage (Table 1). In the remaining 12%, 25 of 28 were associated with cartilage defects (Figure 4). 74% of all OA BMLs were kissing lesions, directly opposing another OA BML. Only 2 of the 28 BMLs not associated with denuded or severely damaged cartilage were kissing lesions.

Discussion

Using accurate quantitative image analysis, confirmed by individual participant analysis, we have demonstrated for the first time the very strong 3D spatial relationship between OA BMLs and severe cartilage damage in the femur and tibia of knee OA, both at individual and group level. This relationship was not as obvious in the patella which frequently contained extensive BMLs. Previous studies have reported statistical associations of BMLs with compartmental cartilage alterations [8, 20-22]. However, these studies could not determine the 3D spatial relationship which is essential for proposing a causal relationship (beyond features which may be frequently correlated with increasing

OA structural progression). A unique feature of this study was the capability of AAMs to provide a standard frame of reference for both cartilage and BML measures derived from different MR sequences. Both TSE and DESS images were automatically segmented using the same set of AAMs for all bones, femur, tibia and patella, which allows direct comparison. These relationships are much less obvious in 2D slice-based methods, the current standard assessments for multiple OA tissue pathologies.

Previous studies have reported the distribution of cartilage loss within sub-regions of the knee[23]. This is also the first study to provide a detailed map of the location of cartilage denudation across all bone surfaces within the knee. The population map in Figure 2 demonstrates widespread areas with no cartilage loss and focal areas of denudation especially in the medial femorotibial joint. The presence of denudation in the lateral trochlea of the femur is notable, with up to 15% of the population having denuded cartilage in this area, compared with around 5% on the medial trochlea.

It is suggested that BMLs at attachments of ligaments are due to tractional trauma or repetitive microtrauma. Similarly other BMLs induced by trauma are a result of abnormal mechanical loads and represent a footprint of the injury mechanism [20, 21]. The very high co-location of OA BMLs and cartilage denudation demonstrated in the current study strongly suggests an underlying causative relationship. It seems probable that OA and ligamentous BMLs arise from the same process of abnormal mechanical loading. The high frequency of kissing lesions amongst the OA BMLs further supports this contention.

All the sites at which we observed BMLs have one thing in common: they represent sites where non-corticated bone is unprotected by cartilage. This is the normal situation at cruciate ligament and meniscal attachments, and also the 'acquired' OA state when cartilage is lost. Previous studies of trabecular bone morphometry support the concept of trabeculae aligning with force, and remodelling of trabeculae in areas of bone pathology [24, 25]. In line with our biomechanical loading theory, it is likely that areas of bone underlying denuded cartilage face repeated episodes of

abnormal loading, as a mechanical consequences of the loss of overlying cartilage [15, 16]. Although this is not a longitudinal study, differing and episodic loading of the knee across denuded areas could also explain the reported significant change in size of OA BMLs over time. This is further supported by the recent trial reducing load through bracing in patellofemoral OA, where reduction in in BML volume was seen [11]. It is also interesting to speculate how this relates to OA symptom changes, as in the same trial there was associated reduction in symptoms.

There are a number of limitations to this work. Firstly, this study addresses the cross-sectional and not longitudinal relationship of cartilage and BMLs. A detailed longitudinal cohort analysis would be the ideal study to confirm our results, though very large numbers of individuals would be required with very long-term follow-up to enable sufficient time for denudation to develop. Additionally, the numbers in this study were limited for feasibility reasons; the manual segmentation of both cartilage and BMLs is a time consuming process. Other relevant tissues such as meniscal pathologies were not analysed in this study, and may provide further insight into BML formation. This cohort had predominantly medial OA and the findings may not relate to severe lateral compartment knee OA. The use of visual assessment to score the association of BML and cartilage damage has limitations, and may introduce bias. It would have been preferable to count the number of denuded areas, however this was not possible as denuded and damaged cartilage regions often interconnect with each other and with the outer rim of thin cartilage.

This cross-sectional study cannot exclude the possibility that abnormal loading first causes damage to underlying bone, leading to future cartilage damage. However, we note that very few BMLs were not associated with cartilage damage, which would have been expected if this were a significant mechanism.

In summary, there was an almost exclusive relationship between the location of OA BML and cartilage denudation, which itself had a clear spatial pattern. We propose that OA and ligamentous

BMLs represent a similar bone response to the abnormal mechanical loading seen in traumatic BML formation.

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Table

Region	Denuded Cartilage	Severe cartilage damage	Not associated	Large	Medium	Small
			OA Bone Marrow Lesions			
Femur						
Medial Patellofemoral	8	2	6	7 (71%)	7 (0%)	2 (0%)
Medial Femorotibial	38	16	5	23 (78%)	28 (36%)	8 (13%)
Lateral Patellofemoral	24	9	3	22 (68%)	10 (40%)	4 (25%)
Lateral Femorotibial	3	2	4	0 (0%)	6 (0%)	3 (0%)
Tibia						
Medial Tibial	38	5	1	30 (80%)	11 (36%)	3 (67%)
Lateral Tibia			3	0 (0%)	0 (0%)	3 (0%)
Patella						
Medial Patella	6	4	1	4 (50%)	5 (20%)	2 (50%)
Lateral Patella	35	9	5	19 (74%)	18 (22%)	12 (17%)

Total OA Lesions	**152**	**47**	**28**	**105 (74%)**	**85 (27%)**	**37 (19%)**
Percentage of OA BML	**67%**	**21%**	**12%**			

Ligamentous Bone Marrow Lesions

Femur						
ACLFemur	-	-	-	0	3	1
PCLFemur	-	-	-	4	5	3
Tibia						
ACLTibia	-	-	-	13	30	8
PCLTibia	-	-	-	10	14	4
AntLatMen	-	-	-	0	1	0
AntMedMen	-	-	-	2	2	2
PostLatMen	-	-	-	0	0	0
PostMedMen	-	-	-	0	0	1
SITTM	-	-	-	0	4	0
Total ligamentous lesions	**-**	**-**	**-**	**29**	**59**	**19**

Table 1: Association of BMLs with denuded cartilage and kissing lesions, and size distribution of OA and ligamentous BMLs

Middle column shows the number of BMLs for each anatomical region. The figure in brackets shows percentage of BMLs that are associated with an opposing lesion ("kissing" lesion). Right-hand column shows breakdown of size in each anatomical region. ACL: anterior cruciate ligament, PCL: posterior cruciate ligament; ACLFemur, PCLFemur: attachments of the ACL and PCL on the femur; ACLTibia, PCLTibia, attachments of the ACL and PCL on the tibia; AntLatMen, AntMedMen: anterior lateral and medial meniscal attachments; PostLatMen, PostMedMen, posterior lateral and medial meniscal attachments; and SITTM: the semitendinosus, iliotibial tract and medial collateral ligament attachments.

References

1. Frobell RB, Wirth W, Nevitt M, Wyman BT, Benichou O, Dreher D, et al. Presence, location, type and size of denuded areas of subchondral bone in the knee as a function of radiographic stage of OA - data from the OA initiative. Osteoarthritis Cartilage. 2010 May; 18(5):668-676.
2. Moisio K, Eckstein F, Chmiel JS, Guermazi A, Prasad P, Almagor O, et al. Denuded subchondral bone and knee pain in persons with knee osteoarthritis. Arthritis and rheumatism. 2009 Dec; 60(12):3703-3710.
3. Zanetti M, Bruder E, Romero J, Hodler J. Bone marrow edema pattern in osteoarthritic knees: correlation between MR imaging and histologic findings. Radiology. 2000 Jun; 215(3):835-840.
4. Bergman AG, Willen HK, Lindstrand AL, Pettersson HT. Osteoarthritis of the knee: correlation of subchondral MR signal abnormalities with histopathologic and radiographic features. Skeletal radiology. 1994 Aug; 23(6):445-448.
5. Sowers MF, Hayes C, Jamadar D, Capul D, Lachance L, Jannausch M, et al. Magnetic resonance-detected subchondral bone marrow and cartilage defect characteristics associated with pain and X-ray-defined knee osteoarthritis. Osteoarthritis Cartilage. 2003 Jun; 11(6):387-393.
6. Felson DT, Chaisson CE, Hill CL, Totterman SM, Gale ME, Skinner KM, et al. The association of bone marrow lesions with pain in knee osteoarthritis. AnnInternMed. 2001 4/3/2001; 134(7):541-549.
7. Hunter DJ, Zhang Y, Niu J, Goggins J, Amin S, LaValley MP, et al. Increase in bone marrow lesions associated with cartilage loss: a longitudinal magnetic resonance imaging study of knee osteoarthritis. Arthritis Rheum. 2006 5/2006; 54(5):1529-1535.
8. Kijowski R, Stanton P, Fine J, De Smet A. Subchondral bone marrow edema in patients with degeneration of the articular cartilage of the knee joint. Radiology. 2006 Mar; 238(3):943-949.
9. Roemer FW, Guermazi A, Javaid MK, Lynch JA, Niu J, Zhang Y, et al. Change in MRI-detected subchondral bone marrow lesions is associated with cartilage loss: the MOST Study. A longitudinal multicentre study of knee osteoarthritis. Annals of the rheumatic diseases. 2009 Sep; 68(9):1461-1465.
10. Felson DT, Parkes MJ, Marjanovic EJ, Callaghan M, Gait A, Cootes T, et al. Bone marrow lesions in knee osteoarthritis change in 6-12 weeks. Osteoarthritis Cartilage. 2012 Dec; 20(12):1514-1518.
11. Callaghan MJ, Parkes MJ, Hutchinson CE, Gait AD, Forsythe LM, Marjanovic EJ, et al. A randomised trial of a brace for patellofemoral osteoarthritis targeting knee pain and bone marrow lesions. Annals of the rheumatic diseases. 2015 Jan 16.
12. Laslett LL, Dore DA, Quinn SJ, Boon P, Ryan E, Winzenberg TM, et al. Zoledronic acid reduces knee pain and bone marrow lesions over 1 year: a randomised controlled trial. Annals of the rheumatic diseases. 2012 Aug; 71(8):1322-1328.
13. Hernandez-Molina G, Guermazi A, Niu J, Gale D, Goggins J, Amin S, et al. Central bone marrow lesions in symptomatic knee osteoarthritis and their relationship to anterior cruciate ligament tears and cartilage loss. Arthritis and rheumatism. 2008 Jan; 58(1):130-136.
14. Roemer FW, Frobell R, Hunter DJ, Crema MD, Fischer W, Bohndorf K, et al. MRI-detected subchondral bone marrow signal alterations of the knee joint: terminology, imaging appearance, relevance and radiological differential diagnosis. Osteoarthritis Cartilage. 2009 Sep; 17(9):1115-1131.
15. Mononen ME, Mikkola MT, Julkunen P, Ojala R, Nieminen MT, Jurvelin JS, et al. Effect of superficial collagen patterns and fibrillation of femoral articular cartilage on knee joint mechanics-a 3D finite element analysis. J Biomech. 2012 Feb 2; 45(3):579-587.
16. Shirazi R, Shirazi-Adl A. Computational biomechanics of articular cartilage of human knee joint: effect of osteochondral defects. J Biomech. 2009 Nov 13; 42(15):2458-2465.

17. Hunter DJ, Bowes MA, Eaton CB, Holmes AP, Mann H, Kwoh CK, et al. Can cartilage loss be detected in knee osteoarthritis (OA) patients with 3-6 months' observation using advanced image analysis of 3T MRI? OsteoarthritisCartilage. 2010 5/2010; 18(5):677-683.
18. Williams TG, Holmes AP, Bowes M, Vincent G, Hutchinson CE, Waterton JC, et al. Measurement and visualisation of focal cartilage thickness change by MRI in a study of knee osteoarthritis using a novel image analysis tool. BrJRadiol. 2010 11/2010; 83(995):940-948.
19. Williams TG, Vincent G, Bowes MA, Cootes TF, Balamoody S, Hutchinson CE, et al. Automatic segmentation of bones and inter-image anatomical correspondence by volumetric statistical modelling of knee MRI. IEEE Press. p. 432-435.
20. Baranyay FJ, Wang Y, Wluka AE, English DR, Giles GG, Sullivan RO, et al. Association of bone marrow lesions with knee structures and risk factors for bone marrow lesions in the knees of clinically healthy, community-based adults. Seminars in arthritis and rheumatism. 2007 Oct; 37(2):112-118.
21. Guymer E, Baranyay F, Wluka AE, Hanna F, Bell RJ, Davis SR, et al. A study of the prevalence and associations of subchondral bone marrow lesions in the knees of healthy, middle-aged women. Osteoarthritis Cartilage. 2007 Dec; 15(12):1437-1442.
22. Hunter DJ, Zhang Y, Niu J, Goggins J, Amin S, LaValley MP, et al. Increase in bone marrow lesions associated with cartilage loss: a longitudinal magnetic resonance imaging study of knee osteoarthritis. Arthritis and rheumatism. 2006 May; 54(5):1529-1535.
23. Eckstein F, Burstein D, Link TM. Quantitative MRI of cartilage and bone: degenerative changes in osteoarthritis. NMR in biomedicine. 2006 Nov; 19(7):822-854.
24. Buckland-Wright JC, Lynch JA, Macfarlane DG. Fractal signature analysis measures cancellous bone organisation in macroradiographs of patients with knee osteoarthritis. Annals of the rheumatic diseases. 1996 Oct; 55(10):749-755.
25. Takechi H. Trabecular architecture of the knee joint. Acta Orthop Scand. 1977; 48(6):673-681.