Significance of Subchondral Bone Microfracture In The Human Osteoarthritic Tibial Plateau On Cartilage Tidemark Drift

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Abstract

Objective: To observe the significance of subchondral bone microfracture in the human osteoarthritic tibial plateau on cartilage tidemark drift.

Methods: Human knee tibial plateau cartilage samples with different OARSI grades were obtained, and the numbers of cartilage tidemarks and microfracture lines were determined after safranin O staining and compared among groups. Osteogenesis caused by microfracture in subchondral bone was observed and staged according to biological properties. Immunohistochemistry, western blotting, PCR and laser microdissection were performed to detect the biological properties of osteogenic areas caused by microfractures. The accompanying relationship between the ossification process and new tidemark formation in osteogenic areas caused by microfractures was then evaluated.

Results: With the increase in OARSI grade, the numbers of cartilage tidemarks and subchondral bone microfracture lines in human knee tibial plateau cartilage tissue samples showed increasing trends. The subchondral bone microfracture line was observed to extend as far as the basal area of hyaline cartilage and cause endochondral osteogenesis. The endochondral osteogenesis caused by subchondral microfracture was categorized into chondrocyte, calcareous sedimentary and ossification stages. A new tidemark appeared in the osteogenic region near the hyaline cartilage when the ossification process of the osteogenic region was completed.

Conclusions: Endochondral osteogenesis can occur at the base of hyaline cartilage induced by microfractures in the tibial plateau subchondral bone. A new cartilage tidemark can form on the side of the osteogenic tissue close to hyaline cartilage when the process of osteogenesis is completed. There is a positive correlation between the degree of cartilage tissue degeneration and the number of tidemarks in articular cartilage.

Introduction

Osteoarthritis (OA) is a common leading cause of persistent pain and disability in the ageing population [1–3]. As a slow progressive joint disease, OA is characterized by articular cartilage degeneration, corresponding changes in subchondral bone, osteophyte formation, muscle weakness, and inflammatory changes in the joint synovium and tendon [4, 5]. Academic circles have long thought that pathological changes in articular cartilage are the most typical characteristics of OA. Therefore, articular cartilage research has always been in the core position [6–8]. Articular cartilage can be divided into hyaline cartilage (noncalcified cartilage) and calcified cartilage, and the boundary between them is called a tidemark [9, 10]. The tidemark has been thought of as a metabolically active calcification front advancing in the direction of the noncalcified cartilage. Pathological sections of articular cartilage revealed that the tidemark was a basophilic line [11]. The scanning results of transmission electron microscopy showed that the tidemark is an area rich in calcium and phosphorus [12]. Under normal circumstances, the tidemark is very close to the calcified cartilage side, showing a wave shape, and

usually only one[9–12, 13]. However, when OA occurs, multiple tidemarks may appear and drift to the hyaline cartilage side.

In the research field of OA, the understanding and research of OA have mainly focused on articular cartilage, but as the research has expanded, the occurrence of OA and the physiopathology of subchondral bone have piqued the interest of scientists[14–16]. Although ‘subchondral bone’ has been defined in a number of ways, at present, subchondral bone is considered a bony component lying distal to calcified cartilage. The subchondral bone can be divided into two layers: the subchondral bone plate near the calcified cartilage layer and the subchondral trabecular bone far away from the calcified cartilage layer[17]. Subchondral bone deterioration is commonly associated with articular cartilage defects, and subchondral bone sclerosis, together with progressive cartilage degradation, is widely considered a hallmark of OA[18, 19]. OA is considered an organ disease that affects the whole joint. Subchondral bone plays a crucial role in the initiation and progression of OA. The subchondral bone plays an important role in damping and supporting normal joints and may also be important for cartilage nutrient supply and metabolism[20]. The subchondral bone and cartilage are dynamic load-bearing structures and play complementary roles in joint load-bearing. The subchondral bone support covers the articular cartilage and distributes the mechanical load on the articular surface, gradually transferring the pressure. The elastic modulus of normal subchondral bone is similar to that of cartilage tissue, functioning well as a stress buffer and to prevent excessive stress concentrations in the articular cartilage. When OA occurs, subchondral bone hardens, and its elastic modulus decreases, which will weaken its ability to buffer stress and thus increase the impact force transmitted to the articular cartilage, causing further damage to the articular cartilage. The elastic modulus of the subchondral bone plate in subchondral bone is obviously higher than that of subchondral trabecular bone, so when the proportion of cortical bone in subchondral bone increases, the total elastic modulus of subchondral bone will obviously increase. When OA occurs, whether articular cartilage degenerates first or subchondral bone assembles first is still controversial in academic circles[21–23]. However, in most studies, the articular cartilage is degenerated first, and then, the subchondral bone tissue is assembled under the damaged cartilage area. Hardened subchondral bone further reflects stress back to the articular cartilage tissue, and this will also substantially increase articular cartilage damage or loss. Studies have shown that when OA occurs, there will be more microfractures in subchondral bone tissue[24–26]. However, after the occurrence of microfractures, how they affect the assembly of subchondral bone has not been explained in detail.

At present, it is still unclear what causes the replication and drift of cartilage tidemarks. The aim of our study was to observe how microfractures in subchondral bone tissue affect the variation in cartilage tidemarks in OA.

Materials And Methods

Patients and samples

This study was approved by the Ethics Committee of Shanxi Provincial People's Hospital (approval number: 2019018), and informed consent was obtained from each donor. In the present analysis, we studied a group of 28 patients (13 males and 15 females) with an average age of 57.3 years (57.3 ± 7.8). Eight young patients with an average age of 33.5 years (33.5 ± 8.2) suffered from amputation due to severe trauma (6 males and 2 females), and another twenty patients (7 males and 13 females) with an average age of 67.3 years (67.3 ± 5.6) received total knee replacement. All 91 tibial plateau cartilage specimens were derived from 28 patients, and the tissue was cut for histological sectioning and was stained with safranin O to determine the OARSI grade of the cartilage tissue. All methods were performed in accordance with relevant guidelines and regulations. All experimental procedures followed the Declaration of Helsinki.

Histologic assessment

Cartilage tissues, including subchondral bone, were removed from the harvested tibial platform and immersed in 37% formalin for 48 h. Asurgical electric saw was used to make cartilage specimens into 10 mm×10 mm specimens, and the specimens were marked with serial numbers. Each cartilage specimen (5 mm×10 mm) was used to make histological sections, and the specific process was as follows. The specimens were decalcified in 20% ethylenediaminetetraacetic acid solution (pH 7.2), and decalcification was confirmed by acupuncture. Every cartilage specimen was then embedded in a single paraffin block. Histological sections with a thickness of 5 μ m were cut with a pathological microtome. All histological sections were stained with safranin O. Cartilage degradation was quantified using the Osteoarthritis Research Society International (OARSI) grading system [27]. Three independent observers scored each section in a blinded manner, and the OARSI grade of each specimen was determined. Ultimately, 91 cartilage specimens were identified as OARSI grade 0 in eight cases, OARSI grade 2 in nine cases, OARSI grade 3 in twelve cases, OARSI grade 4 in fourteen cases, OARSI grade 5 in twenty-two cases, and OARSI grade 6 in twenty-six cases.

All histological sections were stained with safranin O and observed by optical microscopy. Five regions were randomly selected from each cartilage specimen in the field of view magnified 100 times by microscope, and the numbers of cartilage tidemarks and subchondral bone microfracture lines were counted. Continuous tidemarks and microfracture lines were counted as one. The score of each cartilage specimen was calculated according to the OARSI scoring system.

Immunohistochemistry

Histological sections were following conventional procedures, and immunohistochemical (IHC) staining was used to detect Collagen II (1:200, ab34712, Abcam, USA) and Collagen I (1:100, ab34710, Abcam, USA) in the osteogenic region caused by subchondral bone microfracture. We quantitatively scored the IHC results according to the percentage of positive chondrocytes and the staining intensity, as described below. We rated the intensity of staining on a scale of 0 to 3: 0, negative; 1, weak; 2, moderate; and 3, strong. We assigned the following proportion scores: 0 if 0% of the chondrocytes showed positive staining, 1 if 0–1% of the chondrocytes were stained, 2 if 2–10% were stained, 3 if 11–30% were stained,

4 if 31–70% were stained, and 5 if 71–100% were stained. We then combined the proportions and intensity scores to obtain a total score (range: 0–8), as described previously[28]. The results were assessed by 2 experienced pathologists in a blinded manner.

Polymerase chain reaction (PCR)

The remaining 5 mm×10 mm cartilage tissue of each specimen was obtained, the articular cartilage tissue was cut off with a scalpel until the calcified cartilage layer was reached, and then a subchondral bone tissue sample approximately 3 mm thick above the bone tissue was obtained with a surgical chainsaw. The bone tissue was crushed after grinding with the aid of liquid nitrogen. Total RNA was extracted from the tissue using TRIzol reagent (Thermo Fisher Scientific). The quality and quantity of total RNA samples were tested using a NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific). A preparation of RNA with an A260/A280 ratio of 1.8 ~ 2.0 was used for analysis of mRNA expression. Individual RNA samples (1 µg per sample) were reverse transcribed into cDNA using the PrimeScript RT Master Mix kit (Takara, Shiga, Japan) according to the manufacturer's instructions. The relative expression levels of target gene mRNA to the control 18S rRNA transcripts were determined by RT-PCR using SYBR Premix Ex Taq™ (Takara) and the specific primers in the IQ5 Multicolor Real-Time PCR Detection system (Bio-Rad Laboratories, Hercules, CA, USA). The sequences of the primers were forward 5'-CCCCTGCTATTTTCATCGACCC-3' and reverse 5'-GACACACGGCTCCACTTGAT-3' for Collagen II and forward 5'-ACTGAGAGGCTCCGAGAAATG-3' and reverse 5'-GAACCCCGCATCTTGGCTT-3' for Collagen I.

Western blotting

The ground tissue samples were lysed in RIPA lysis buffer containing PMSF, protease and phosphatase inhibitors (Keygen). A certain amount of protein was mixed with loading buffer, boiled for 10 minutes and subjected to SDS-PAGE followed by transfer to PVDF membranes. The blots were probed with primary antibodies after being blocked with 5% fat-free dry milk in TBST. Western blotting was used for detection of Collagen II (1:3000, ab188570, Abcam, USA) and Collagen I (1:3000, ab34710, Abcam, USA). The relative levels of the target protein and the control β-actin expression were determined by western blot analysis. The bound antibodies were detected with horseradish peroxidase (HRP)-conjugated secondary antibodies and visualized using an enhanced chemiluminescence reagent. The data were analysed by densitometric analysis using ImageJ software.

Laser capture and real-time PCR

To quantify the mRNA levels of Collagen II and Collagen I, a formalin-fixed, paraffin-embedded Paradise PLUS Reagent System (MDS Analytical Technologies/Molecular Devices, CA, USA) was used to extract and amplify RNA from histological section tissue with different osteogenic stages in the osteogenic region [33]. Tissue sections (10 µm) were air-dried and dehydrated through graded alcohols and subjected to laser-capture microdissection (LCM) within 2 h of deparaffinization as previously described [29]. The osteogenic region was captured on LCM Macro CapSure Caps (Applied Biosystems) using the Arcturus AutoPix Automated Laser Capture Microdissection System (Applied Biosystems). The mRNA was quantified by real-time PCR using the QuantiTect SYBR Green PCR kit (QIAGEN, Valencia, CA, USA)

with the CFX384 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Each reaction was performed in triplicate.

Statistical analysis

The data are expressed as the means $\pm$ SD and were statistically analysed using SPSS software (version 20.0; IBM Corporation) and Graph Prism Software (version 8.0; Graph Prism Software, Inc.). One-way ANOVA was used for comparisons among the three groups. A p -value ≤ 0.05 was considered to indicate a statistically significant difference.

Results

Positive correlation between the degree of knee tibial plateau cartilage degeneration and the number of cartilage tidemarks

To observe the relationship between the degree of degeneration of human knee tibial plateau cartilage tissue and the number of cartilage tidemarks, we divided all specimens into the OARSI 0-1 group, OARSI 2 group, OARSI 3 group, OARSI 4 group, OARSI 5 group, and OARSI 6 group using the OARSI grading system. The results of histological sections stained with safranin O showed that with the increase in OARSI grade of cartilage tissue, the number of cartilage tidemarks of corresponding specimens showed an obvious increasing trend. At the same time, after scoring all cartilage tissue specimens, we found that the OARSI score of cartilage tissue was positively correlated with the number of cartilage tidemarks of the corresponding specimens. In addition, in cartilage tissue with severe degeneration, the newly generated tidemarks were discontinuous and more tortuous, obviously different from the normal tidemarks, which featured regular waves. (Fig. 1)

Positive correlation between the degree of knee tibial plateau cartilage degeneration and the number of microfracture lines in subchondral bone

To observe the relationship between the degree of degeneration of human knee tibial plateau cartilage tissue and the number of microfracture lines in subchondral bone, we also divided all cartilage tissue specimens into groups according to the OARSI grading system. The results of histological sections stained with safranin O showed that with the increase in OARSI grade of cartilage tissue, the number of microfracture lines in subchondral bone of corresponding specimens showed an obvious increasing trend. Moreover, it was found that the microfracture area initiated the process of entochondrolysis. In addition, we found that the microfracture line in the subchondral bone of severely degenerated cartilage tissue was generally long, and a "split" phenomenon even appeared between the calcified cartilage layer and the subchondral cancellous bone. (Fig. 1, Fig. 2)

Staging of endochondral osteogenesis caused by subchondral microfractures

To study the process of local osteogenesis caused by microfracture in subchondral bone, we observed histological sections stained with safranin O and divided the osteogenesis process into three stages: the chondrocyte stage, the calcareous sedimentary stage and the ossification stage. We detected the expression levels of Collagen II and Collagen I in histological sections using immunohistochemistry. The results showed that in the chondrocyte stage, local cells expressed a large amount of Collagen II and a small amount of Collagen I, while in the calcareous sedimentary stage, local cells expressed decreased levels of Collagen II and increased levels of Collagen I. At the ossification stage, the osteogenic region essentially did not express Collagen II but expressed a large amount of Collagen I.

Collagen II is a marker protein of chondrocytes, and Collagen I is a marker protein of osteocytes. Therefore, this result showed that the osteogenic process was characterized by the differentiation from chondrocytes to osteocytes. At the same time, we extracted total mRNA and protein from the calcified cartilage layer and subchondral bone tissue and then performed PCR and western blot experiments. The results showed that with the development of osteogenesis, Collagen II mRNA and protein expression levels showed a decreasing trend, while Collagen I mRNA and protein expression levels showed an increasing trend. Then, we used laser microdissection technology to obtain tissue from the osteogenic region of microfractures and used micro-mRNA amplification technology to detect the expression of Collagen II mRNA and Collagen I mRNA in the stages of microfracture osteogenesis. The results showed that with the development of ossification in the osteogenic region, the expression level of Collagen II mRNA in local tissue showed a decreasing trend, while the expression level of Collagen I mRNA showed an obvious increasing trend. (Fig. 2)

Relationship between the completion of endochondral osteogenesis and new tidemark formation

To observe the relationship between microfractures in subchondral bone and the formation of new tidemarks, we observed the formation of tidemarks in different stages of osteogenesis on histological sections stained with safranin O. The results showed that there were no new tidemarks near hyaline cartilage in the osteogenic region during the chondrocyte stage. During calcareous sedimentation, the embryonic form of the tidemark appeared on the side of hyaline cartilage in the osteogenic region. During the ossification stage, a new tidemark appeared on the side of hyaline cartilage in the osteogenic region. These results indicated that when the subchondral bone microfracture caused the completion of endochondrostosis, a new cartilage tidemark appeared on the side of the hyaline cartilage in the osteogenic area. (Fig. 3, figure 4)

Discussion

We found that when cartilage tissue of the tibial plateau of human knee degenerates, many microfracture lines develop in subchondral bone, and these lines can reach the base of the hyaline cartilage. Microfractures cause local endochondral osteogenesis. After the chondrocyte stage, the calcareous sedimentary stage and the ossification stage, the microfracture area becomes bone tissue, and a new tidemark appears on the side of the hyaline cartilage. Because subchondral bone microfractures do not occur at the same time, the newly formed tidemark often forms independently and is discontinuous.

Osteoarthritis (OA), a highly prevalent, slowly progressing, chronic degenerative disease of diarthrodial joints, is characterized by progressive wear and fibrosis of articular cartilage tissue associated with synovitis changes, subchondral bone remodelling, and muscle strength changes around joints [1–3, 29, 30]. During the progression of the disease, all compartments of the joints undergo structural, functional and metabolic changes that involve cellular elements as well as components of the extracellular matrix. Classically, degenerative deterioration of the cartilage is thought to be the central process of osteoarthritis. According to biological properties, articular cartilage can be divided into hyaline cartilage (noncalcified cartilage) and calcified cartilage, and the boundary between them is the tidemark. During the normal development and growth of diarthrodial joints, the tidemark clearly represents a calcification front. In the normal adult joint, the tidemark is still a single structure and ceases the advancement of minerals into the hyaline cartilage. Under these conditions, although the tidemark still contains some tightly bound calcium, it may have ceased to function as a calcification front. In the process of studying OA, we often used the ratio of hyaline cartilage thickness to calcified cartilage thickness to measure the degeneration degree of articular cartilage. When OA occurs, the ratio of the two is significantly reduced, which means that the tidemark of the cartilage moves to the hyaline cartilage side [31].

The tidemark, a distinct boundary between hyaline cartilage and calcified articular cartilage and not an artefact, has been described as a basophilic single line that is approximately $2\text{ }\mu\text{m}\times 5\text{ }\mu\text{m}$ thick [32]. Histological measures of articular cartilage pathology, generally considered to be the reference standard for the presence and progression of OA, showed significant tidemark replication and cartilage degradation in the OA group compared to the control group. Continuous collagen fibrils cross the tidemark, indicating a strong link between hyaline cartilage and calcified cartilage. The sharp borderline between calcified cartilage and subchondral bone is called the 'cement line'. In contrast to the tidemark, however, no continuous collagen fibrils cross the cement line [33].

Articular cartilage overlies subchondral bone and provides a vital function of maintaining homeostasis of the joint environment. It encompasses superficial hyaline cartilage and deeper calcified cartilage. Calcified cartilage is permeable to small molecule transport and plays an important role in the biochemical interaction between hyaline cartilage and subchondral bone. The subchondral bone plate is a thin cortical lamella lying immediately beneath the calcified cartilage. This cortical endplate is not an impenetrable structure but possesses a marked porosity. It is invaded by channels that provide a direct link between the articular cartilage and the subchondral trabecular bone. A surprisingly high number of arterial and venous vessels, as well as nerves, penetrate through the channels and send tiny branches into

the calcified cartilage. The distribution and intensity of the channels depend not only on ageing but also on the magnitude of the compressive forces transmitted through the cartilage and the subchondral bone within and between joints. Studies have shown that when knee OA occurs in the late stage and subchondral bone is hardened, the permeability of the subchondral bone plate decreases markedly[34]. Under the subchondral bone plate is subchondral trabecular bone. Arising from the subchondral bone plate is the supporting trabeculae, which comprise subchondral trabecular bone together with deeper bone structures. Subchondral trabecular bone exerts important shock-absorbing and supportive functions in normal joints and may also be important for cartilage nutrient supply and metabolism. Subchondral bone and cartilage are dynamic stress-bearing structures that play complementary roles in load-bearing joints. Relative to the subchondral bone plate, subchondral trabecular bone is more porous and metabolically active, containing blood vessels and sensory nerves. In the late stage of OA, the subchondral bone exhibits obvious hardening, and a certain thickness of subchondral cancellous bone is transformed into cortical bone by bone remodelling. There are many microfracture lines in subchondral bone. In this experiment, we found that there was a positive correlation between the microfracture lines of subchondral bone and the degeneration degree of cartilage tissue. The subchondral bone microfracture line could reach the basal part of the hyaline cartilage, leading to the occurrence of endochondral osteogenesis.

In the early stages of OA in humans, elevated bone remodelling and subchondral bone loss have been observed and are considered determinants of OA progression[35]. In a rabbit model with preceding osteoporosis, subchondral bone microstructural damage caused by increased remodelling aggravated experimental OA[36]. Temporal subchondral bone loss in early OA has also been documented in a number of different animal models[37]. Although the underlying mechanism for the increased bone turnover and structural deterioration in the early phase of OA is not fully understood, several factors have been implicated, including microdamage repair, increased vascularity stimulated by angiogenic factors and enhanced bone-cartilage crosstalk via increased subchondral plate pores[38]. Furthermore, it has been suggested that elevated bone remodelling and the associated stimulated vascularity are indispensable for the progression of OA but not subchondral sclerosis. In the late stage of OA, the microarchitectural characteristics of subchondral bone include elevated apparent density, increased bone volume, thickening of subchondral bone plate, increased trabecular thickness, decreased trabecular separation and bone marrow spacing, and transformation of trabeculae from rod-like into plate-like morphology. The overlying calcified cartilage is also thickened, with advancement and duplication of the tidemark, which contributes to articular cartilage thinning and deterioration.

It is normally induced by overloading and appears in two different forms: linear microcracks and diffuse microdamage[39, 40]. Linear microcracks, defined as short interstitial cracks, play a vital role in the initiation and progression of OA. In general, microcracks have two main functions: first, they act as a nidus for the initiation of bone remodelling; and second, they provide a channel for the physiological communication between cartilage and subchondral bone, with more catabolic agents crossing the osteochondral junction, which may be accelerated by accompanying vascular invasion and cutting cones into cartilage. The mechanism for the promotion of bone remodelling by microcracks may involve

damage to osteocyte canalicular processes and subsequent osteocyte apoptosis, which may induce osteoclastic resorption and microcrack repair. The high bone turnover induced by microcracks leads to the thickening of subchondral plates and calcified cartilage with tidemark advancement, which eventually causes thinning of the hyaline cartilage.

Conclusions

In summary, we found through experimental observation that when knee OA occurs, microfracture lines will appear in the subchondral bone, which will start the process of endochondral osteogenesis. When osteogenesis is complete, a new tidemark appears near the hyaline cartilage in the osteogenic area, and the tidemark drift phenomenon occurs.

Abbreviations

OA:Osteoarthritis; OARSI :Osteoarthritis Research Society International; IHC:Immunohistochemistry;

Declarations

Ethics approval and consent to participate This study was approved by the Ethics Committee of Shanxi Provincial People's Hospital (approval number: 2019018) Tai Yuan,China).

Consent for Publication Not applicable.

Availability of data and material Not applicable.

Competing interests None declared.

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Authors contribution XJ Wang: study design, data acquisition, data analysis, wrote the manuscript.YM Zhang,X Lu: data analysis, discussion of results. W Tian,J Zhang and WW Xu: data acquisition, histochemistry and western blotting analysis. LJ Li: statistical analyses. F Chang: study design, manuscript correction. All authors have approved the submitted manuscript.

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Figures

Figure 1

Correlation between the OARSI grades of tibial platform articular cartilage tissue and number of cartilage tidemark. (A) Schematic diagram of normal articular cartilage tissue and OA articular cartilage tissue structure. (Scale=100 μ m). (B) Comparisons of safranin O staining patterns of different OARSI grades articular cartilage from human tibial platform and the number of tidemark in cartilage tissue. (Scale=100 μ m). (C) Statistical results of different OARSI grades articular cartilage and the number of tidemark. (D) Correlation analysis between OARSI score of articular cartilage specimens and the number of tidemark in cartilage. Statistical significance is shown for One-way ANOVA analysis, and SNK-q test was used for comparison among groups. Bars represent the mean $\pm$ SEM, * $p < 0.05$ compared to the Grade 0~1 group, # $p < 0.05$ compared to the Grade 2 group, & $p < 0.05$ compared to the Grade 3 group, \$ $p < 0.05$ compared to the Grade 4 group, @ $p < 0.05$ compared to the Grade 5 group.

Figure 2

Correlation between the OARSI grades of tibial platform articular cartilage tissue and number of microfracture line in cartilage. (A) Comparisons of safranin O staining patterns of different OARSI grades articular cartilage from human tibial platform and the number of microfracture line in cartilage. (Scale=100 μ m). (B) Statistical results of different OARSI grades articular cartilage and the microfracture line in cartilage. (C) Entochondrostosis initiated by microfracture in subchondral bone. Statistical significance is shown for One-way ANOVA analysis, and SNK-q test was used for comparison among groups. Bars represent the mean $\pm$ SEM, * p <0.05 compared to the Grade 0~1 group, # p <0.05 compared to the Grade 2 group, & p <0.05 compared to the Grade 3 group, \$ p <0.05 compared to the Grade 4 group, @ p <0.05 compared to the Grade 5 group.

Figure 3

Stages and biological properties of endochondral osteogenesis caused by microfractures in subchondral bone. (A) Stages and biological properties of endochondral osteogenesis in subchondral bone. (Scale=100 μ m). (B) Calcified cartilage and subchondral bone were taken from different cartilage samples for related detection. (Scale=100 μ m). (C) PCR results of calcified cartilage and subchondral bone from different endochondral osteogenesis processes. (D) Western-blot results of calcified cartilage and subchondral bone from different endochondral osteogenesis processes. (E) Microdissection technique was used to detect the levels of related mRNA in specimens with different endochondral osteogenesis stages. (Scale=100 μ m). Statistical significance is shown for One-way ANOVA analysis, and SNK-q test was used for comparison among groups. Bars represent the mean $\pm$ SEM, * p <0.05 compared to the Chondrocyte stage, # p <0.05 compared to the Calcareous sedimentary stage.

Figure 4

Significance of subchondral bone microfracture on new tidemark formation.(A) Relationship between osteogenic area and new tidemark in different stages of endochondral osteogenesis.(Osteogenic area,black arrow. New tidemark,yellow arrow. Scale=100 μ m).(B) The new tidemark appeared in the osteogenic area near the hyaline cartilage side when osteogenesis was completed.(Osteogenic area,black arrow. The already formed tidemark in cartilage,yellow arrow. The coming tidemark in cartilage,red arrow.Scale=100 μ m).Scale=100 μ m).(C) Schematic diagram of the influence of entochondroostosis caused by microfractures in subchondral bone on new tidemark formation.