Recombined bone xenografts enhance tendon graft osteointegration of anterior cruciate ligament reconstruction

Weimin Pan · Yunyu Hu · Yiyong Wei · Long Bi ·
Dan Li · Jun Wang · Rong Lv · Jianwei Li · Zheng Cao

Received: 10 October 2008 / Revised: 24 November 2008 / Accepted: 24 November 2008 / Published online: 29 January 2009
© Springer-Verlag 2009

Abstract The objective of the study was to discover whether recombined bone xenograft (RBX), a porous solid material, could augment healing of the tendon-to-bone interface after anterior cruciate ligament (ACL) reconstruction. ACL reconstruction was performed bilaterally in 25 skeletally mature rabbits using long digital extensor tendon grafts. RBX was implanted into the treated knee, with the contralateral knee serving as control. Three rabbits were killed at postoperative weeks two, six and 12 for routine histology. The remaining 16 rabbits were killed at weeks six and 12, and their femur-graft-tibia complexes were harvested for mechanical testing. The treatment and control groups produced different histological findings at the interface between the tendon and bone. In the treatment group, large areas of chondrocyte-like cells were noted around the tendon-bone interface two weeks after the operation. At six weeks, more abundant bone formation was observed around the tendon. At 12 weeks, an immature neoenthesis structure was seen. In biomechanical evaluation six and 12 weeks after the operation, the ultimate strength of tendon in the bone tunnel was significantly higher in the treatment group than in the control group. RBX can augment the osteointegration of tendon to bone after ACL reconstruction.

Résumé L'objectif de cette étude est d'évaluer l'intérêt d'un matériel solide et poreux du type xénogreffe osseuse recombinaison. Cette greffe osseuse recombinaison (RBX), peut-

elle augmenter les chances de consolidation au niveau de l'interface tendon-os après reconstruction du ligament croisé antérieur (ACL). Matériel et méthode: une reconstruction du ligament croisé antérieur a été réalisée de façon bilatérale sur 25 lapins « squelettiques » en utilisant une greffe du tendon du long extenseur. RBX a été implanté dans les genoux traités, le genou controlatéral servant de contrôle. Trois lapins ont été sacrifiés à 2, 6 et 12 semaines avec un examen histologique de routine, les 16 lapins restant ont été sacrifiés à 6 et 12 semaines, le complexe osseux fémoro tibial ayant été testé mécaniquement. Résultat, le groupe contrôle non traité montre une différence histologique au niveau de l'interface entre le tendon et l'os. Dans le groupe traité par RBX, de larges plages cellulaires de chondrocytes-like ont été repérés autour de l'interface tendon-os 2 semaines après l'opération. 12 semaines après, des formations plus importantes ont été observées autour du tendon de même qu'une néoformation de type immature. L'évaluation bio mécanique a montré que 12 semaines après l'opération l'insertion tendineuse au niveau du tunnel osseux est beaucoup plus solide dans le groupe des lapins traités que dans le groupe contrôle. En conclusion le RBX peut augmenter l'ostéointégration osseuse de la jonction tendineuse dans les réparations du ligament croisé antérieur.

Abbreviations

ACL	Anterior cruciate ligament
RBX	Recombined bone xenograft
QSTG	Quadrupled semitendinosus and gracilis tendons
BPTB	Bone-patella tendon-bone
SEM	Scanning electron microscopy
BMPs	Bone morphogenetic proteins
H&E	Hematoxylin and eosin
SD	Standard deviation

W. Pan · Y. Hu (✉) · Y. Wei · L. Bi · D. Li · J. Wang · R. Lv ·
J. Li · Z. Cao
Institute of Orthopaedics and Traumatology, Xijing Hospital,
The Fourth Military Medical University,
Xi'an 710032, People's Republic of China
e-mail: orth12@hotmail.com

Introduction

Various graft sources and fixation materials have been tried in the pursuit of successful anterior cruciate ligament (ACL) reconstruction. Due to donor site morbidity issues, hamstring tendons and quadrupled semitendinosus and gracilis tendons (QSTG) have been used more often than bone-patella tendon-bone (BPTB) grafts [3]. However, slow healing rates while tendon grafts incorporate with bone delay patients' return to rehabilitation. The interface between grafted tendon and bone is thus a site of early failure for ACL reconstructions [15, 23].

Though the precise mechanism of interface between grafted tendon and the adjacent bone tissue is unclear, biological fixation of the grafts to bone is the goal for stability. Rodeo et al. found that healing occurs through the formation of fibrovascular tissue between the tendon and bone, followed by progressive bone ingrowth. The progressive increase in strength of the bone-tendon interface appeared to correlate with the degree of bone ingrowth [18]. Several other authors have also observed this phenomenon [8, 20]. It is advisable to enhance the rate and quality of osteointegration of grafted tendon at this site.

Experimental data have shown that some methods, such as mechanical stimulation [25], tendon wrapping with periosteum [6] and interface filling with growth factors [1, 27], bone marrow stromal cells [14] or biomaterials [17, 22], can enhance the underlying biological processes of tendon-bone tunnel osteointegration. Biomaterials have been developed to further promote the tendon-bone healing of grafted tendons. Current bone-tendon filling materials are mainly divided into two types: injectable and solid materials. Although injectable materials are reportedly more

applicable in clinical use, obtaining the appropriate quantity and seal is difficult for operators during clinical surgery. In order to avoid this issue, this paper focuses on the solid filling materials.

Our previous study demonstrated that the recombined bone xenograft (RBX), a new kind of biosynthetic solid material, promotes strong osteoinduction and is effective in augmenting bone ingrowth [5, 9–10]. We hypothesised that RBX would improve healing between tendon graft and bone tunnels in an intra-articular ligament reconstruction model. To test this hypothesis, we observed changes in the tendon-bone interface through histology, imaging and biomechanics.

Materials and methods

RBX preparation

Bovine cancellous bone granules 5–6 mm³ in size were defatted using chloroform and ethanol and deproteinised with hydrogen peroxide. Scanning electron microscopy (SEM) of the cancellous bone showed a regular porous structure, with pores 300–500 µm in diameter and walls 60–100 µm thick between the pores (Fig. 1a). Bone morphogenetic proteins (BMPs) were obtained according to the methods described by Urist et al. [24], resulting in a crude extract of BMPs. The cancellous bone carrier and BMPs were then recombined in the following manner: The BMP aggregate was redissolved in 4 M guanidine hydrochloride, and a certain amount of cancellous bone (with BMPs/carrier ratio 1:20 by weight) was added. Each granule contained 2 mg of crude BMPs. The air was then

Fig. 1 Scanning electron micrographs of the RBX. **a** The basic scaffold showed a regular porous structure, containing both big pores (300–500 µm in diameter) and smaller interconnected pores (50–80 µm in

diameter). **b** The thickness of the wall between the big pores in the RBX scaffolds was 60–100 µm. A cotton-like appearance of the crude bBMP fraction was found in the pores of the cancellous bone

dispelled from the pores of the cancellous bone framework in a vacuum. The resulting composite, designated as RBX, was then dialysed against distilled water, freeze-dried and sterilised with ethylene oxide. SEM of the composite demonstrated a network-like appearance of BMPs fraction precipitated in the pores of the cancellous graft (Fig. 1b). Cylindrical RBX was used in this study (radius: 2 mm; height: 5 mm)

Animal operation

The study was approved by the Research Ethics Committee of our institution. Twenty-five healthy, skeletally mature New Zealand white rabbits (22 weeks old; weight 2.7 ± 0.2 kg) were used. Bilateral ACL reconstructions were performed. One limb from each rabbit was treated with RBX, while the contralateral limb served as a control. Under general anaesthesia, the knee joint was reached via a medial parapatellar approach. After lateral patellar dislocation, the normal ACL was excised at its femoral and tibial origins. One 2.5-mm drill hole was made at the tibia and one at the lateral femoral condyle, through the footprints of the normal ACL. The long digital extensor tendon (3 cm in length and 2 mm in diameter) was identified and harvested. In the control group, both ends of the autograft were secured with Dexon 3-0 sutures, passed through the tibial and femoral drill holes and fixed at the tunnel exits with sutures tied over the neighbouring periosteum at maximum tension at 30° of knee flexion.

In the treatment group, two RBX bone cylinders were fixed to both ends of the graft using sutures. Each end of the graft was compressed to achieve a graft diameter of 2.4 or 2.5 mm, in order to obtain very strong graft ends [7]. The graft ends were then passed through the tibial and femoral drill holes from the intra-articular tunnel exits. Once the graft had been properly positioned in the tunnel, it was fixed using the same procedure used in the control group. Finally, the soft tissue was closed in layers. The rabbits were allowed to have free cage movement immediately after the operation. Three rabbits each were killed at postoperative weeks two, six and 12 for micro-computed tomography (CT) examination and subsequent routine histology. The remaining 16 rabbits were sacrificed at weeks six and 12, and their femur-graft-tibia complexes were harvested for mechanical testing.

Micro-CT examination

A desktop micro-CT system (eXplore Locus SP, GE Healthcare, Waukesha, WI, USA) was used to quantify and study the bone mineral density (BMD) and mineralised tissue ingrowth inside the tunnel. Specimens were scanned perpendicular to the long bone axis covering the entry and exit of the bone tunnel according to the protocol of “large

tube_14 μm _150 min_ss” for about 300 and 500 consecutive sections, respectively, for the femoral and tibial tunnels. The sections were three-dimensionally reconstructed using MicView software. To quantify the amount and quality of newly formed mineralised tissue over time, a 3-mm circular region of interest (ROI) inside the bone tunnel was chosen and three-dimensionally reconstructed using the software. After thresholding, the BMD (unit: mg/cm^3) of mineralised tissue inside the tendon-bone interface was calculated over the ROI.

Histological analysis

For histological analysis, samples were fixed in 10% neutral buffered formalin, decalcified and embedded in paraffin. Six-micron-thick sections were cut in the anterior-posterior direction, parallel to the longitudinal axis of the long bone. The sections were stained with hematoxylin and eosin (H&E), toluidine blue and modified trichrome stain for examination of healing at the tendon graft-bone tunnel interface. The sections were examined under light microscopy (Leica LA, Wetzlar, Germany).

Biomechanical testing

The graft-tunnel complexes were harvested and stored at -20°C until biomechanical testing. After the samples were thawed overnight at room temperature, the knee joints were carefully dissected to remove the surrounding soft tissue, until only the ACL graft remained as the physical connection between the two bones. The complexes were fixed in specially designed clamps, allowing tensile loading along the long axis of the graft in a materials testing machine (AGS, Shimadzu Corporation, Kyoto, Japan). A preload of 1 N and a load displacement rate of 50 mm/min tensile force were applied to the graft-tunnel complex until failure. The failure mode and the ultimate strength (N) were recorded.

Statistical analysis

Sample strength and stiffness values are expressed as the mean $\pm$ standard deviation (SD). The values were analysed using analysis of variance (ANOVA) tests. *P* values less than 0.05 were considered significant. The SPSS 11.0 statistics software was used in this study.

Results

Micro-CT examination

Micro-CT examination showed that RBX enhanced bone growth in the tunnel after ACL surgery. The average BMD

of newly formed mineralised tissue between tendon and graft was $101.7285 \pm 17.2138 \text{ mg/cm}^3$ at six weeks after the operation in the treatment group, $68.9320 \pm 11.7996 \text{ mg/cm}^3$ at six weeks in the control group, $152.5065 \pm 52.5724 \text{ mg/cm}^3$ at 12 weeks in the treatment group and $108.7622 \pm 14.4342 \text{ mg/cm}^3$ at 12 weeks in the control group. The BMD in the treated specimens was significantly greater than in the control specimens for the entire study population ($P < 0.05$) as well as at the individual six- and 12-week time points ($P = 0.011, 0.002$, respectively).

Histological findings in control limbs

At week two post-operation, a fibrovascular interface filled the tunnel between tendon and bone (Fig. 2a). This was composed of irregular vascular tissue and fibroblast, representing the vascular phase of healing. There were no obvious zones of organised collagen fibres or cartilage. At six weeks, the fibrovascular tissues were more organised (Fig. 3a). By 12 weeks, this interface was still distinct but definitely narrower; occasional perpendicular collagen fibres (like Sharpey's fibres) were observed crossing this

zone. There were few chondrocyte-like cells at week 12 (Fig. 4a).

Histological findings in treatment limbs

At two weeks, large areas of fairly disorganised chondrocyte-like cells were noted around the tendon-bone interface (Fig. 2b, c). The chondrocyte-like cells appeared immature, with pleomorphism of the cell size and shape. At six weeks, the chondrocyte-like cells were noted to be more orderly, having laid down a chondroosteoid matrix, and the cells appeared more regular in size and shape (Fig. 3b, c). The apposition between autograft and bone was not very close at this point. By 12 weeks, a transition from bone fibrocartilage to tendon was observed (Fig. 4d). The zones were not clearly delineated but instead showed a gradual blending of autograft into bone.

Biomechanical analysis

The ultimate load to failure was significantly greater in the RBX-treated group as compared with controls at six and

Fig. 2 Photomicrographs of a 2-week specimen. **a** The control specimen had a wider interface between tendon and bone. **b** In the limb treated with RBX (*T* tendon, *IF* interface, *B* bone, H&E stain, original magnification $\times 100$), large areas of chondrocyte-like cells that were fairly disorganised were noted between the tendon and bone. **c** The chondrocyte-like cells appeared immature (*black arrow*), with pleomorphism of the cell size and shape (toluidine blue stain, original magnification $\times 100$). **d** Slight amounts of proteoglycan (*black arrow*) being laid down between the tendon and bone (*T* tendon, *IF* interface, *B* bone, trichrome stain, original magnification $\times 100$)

Fig. 3 Photomicrographs of a 6-week specimen. There is a distinct tendon-bone interface in both the control (a) and treated (b) specimens. The interface tissue in the treated limb is more dense in the RBX-treated limb and appears cartilaginous, whereas there is a fibrous tissue interface in the control limb (*T* tendon, *IF* interface, *B* bone, H&E stain, original magnification $\times 100$). **c** Higher density of chondrocyte-like cells was noted around the tendon (toluidine blue stain, original magnification $\times 100$). **d** Newly formed bone was seen (black arrow) (*T* tendon, *IF* interface, *B* bone, trichrome stain, original magnification $\times 100$)

12 weeks (Table 1). The site of failure changed as healing time increased. Failure was more common in the tibial tunnel at six weeks. Six and seven of eight samples in the control and treatment groups, respectively, pulled out from the tibial tunnel. Midsubstance failure was more common at 12 weeks. Only one sample in the control group was pulled from the tibial tunnel and the remaining samples in the two groups were all broken in the central region of the tendon.

Discussion

A successful ACL reconstruction requires that ligament substitute tissue heals and firmly bonds to the walls of the bone tunnel. Forward and Cowan reported the tendon-bone incorporation in an extra-articular tendon-to-bone healing model, demonstrating that the biological response of the bone tunnel was “similar to the healing of a fracture”. Early tendon-bone anchorage occurred via a cartilage callus from which chondrogenic cells grew into. At three weeks, enchondral ossification began from the cartilage interface

into bony trabeculae. By three months, the osseous tissue that had annealed to the collagen fibrils showed signs of remodelling [2]. These descriptions are similar to the histological changes of tendon-bone incorporation observed in this study. However, at week two in the treatment group, although the material had partly been degraded, the interface between the tendon and the bone tunnel had been filled with many chondrocyte-like cells, whereas only fibrovascular tissue was present in the control group. As time elapsed, abundant new bone and collagen fibres firmly connected the interface. The increasing new bone volume suggested that the rate of bone formation was accelerated by RBX. All histological data show that the grafted tendon was in tight contact with the bone, with contact increasing rapidly through RBX augmentation. Biomechanical results support this from a functional point of view. Our results demonstrate that RBX may be used to biologically enhance the healing process between tendon grafts and their surrounding bony tunnels.

RBX is composed of antigen-extracted bovine cancellous bone and crude BMPs, with a certain amount of collagen

Fig. 4 Photomicrographs of 12-week specimens. In the treated limb (**b, c**), the tendon graft is surrounded by bone, whereas there is little bone formation around the tendon graft in the control limb (**a**) (**a, b** H&E stain, **c** trichrome stain, original magnification $\times 100$). **d** A transition was seen from bone fibrocartilage to tendon in some areas of the interface (*T* tendon, *IF* interface, *B* bone, H&E stain, original magnification $\times 100$)

matrix retained therein. Its uniqueness lies in the way that eliminating the antigenicity and compromising the osteoinductivity are separately taken into consideration in a common material base of a xenograft. As a unit, RBX not only has osteoconductivity but also osteoinductivity. On the other hand, RBX is derived from a biological source; thus, it fully degrades and is not retained. It would be a more effective material for bone tissue engineering.

Some studies have proposed that healing of the tendon-bone interface could be accelerated using tissue engineering techniques mainly focused on securing tendon grafts to bone via biological fixation [4, 21]. Since the results of our study also showed that bone ingrowth in the tunnel was

similar to “fracture healing”, we decided to apply tissue engineering techniques to improve bone ingrowth. Sufficient marrow in the bone tunnel provided a source of pluripotent cells with osteogenic potential, suggesting the potential use of growth factor combined with suitable scaffolding to augment bone growth around the grafted tendon. RBX combines two elements of tissue engineering, namely, the scaffolding matrix and the growth or differentiation factor; it can recruit and induce marrow cells on the scaffold to accelerate the repair of bone. Previous studies have applied type I collagen sponge and calcium phosphate matrix, fibrin glue separately combined with rhBMP-2, BMP-7 or bone protein (a bone-derived extract) to evaluate the augmentation of healing in translocated tendons within bone tunnels in ACL reconstruction [11, 13, 16, 19]. Though various extra-articular or intra-articular animal models and different transplants were employed, all observed that the limbs that received compounds showed more advanced healing and greater ultimate load to failure. However, in addition to maintaining local BMP concentration at sufficient levels over time, scaffolds should also provide a mechanically stable and biodegradable framework that enhances cell

Table 1 Ultimate pullout load

Time (weeks)	Ultimate pullout load (N)		Statistical significance
	Treatment group	Control group	
6	64.71 $\pm$ 21.36	42.69 $\pm$ 15.03	$P<0.05$
12	56.84 $\pm$ 16.81	38.29 $\pm$ 11.97	$P<0.05$

migration, attachment and differentiation. Moreover, the formation of new bone and degradation of the implanted material are both affected by the porous structure and organization of the optimised scaffold material, which should promote the ingrowth of bone tissue into the matrix and also encourage bone remodelling. As seen in Fig. 1, RBX has an innate macroporous structure with regular interconnected microspores and regular pore sizes, which facilitate ingrowth of the neotissues. BMPs are then incorporated into RBX. The matrix contained in the RBX scaffold not only facilitates cell adhesion to the scaffold but also acts as a slow-release depot, thus enhancing the role of crude BMPs as an osteoinductive agent. In our study, crude BMPs were used instead of rhBMP-2 or other kinds of BMP, as some studies have reported that the way forward in the therapeutic application of BMP is to use them in combination [12].

On the other hand, the mechanical load has a significant effect on the biochemical and biomechanical characteristics of soft and hard tissues. Yamakado et al. found that tensile stress enhances the healing process in tendon-bone junctions, compressive stress promotes chondroid formation and shear load has little or no effect on regeneration of the tendon-bone junction in an extra-articular animal model [26]. In this study, we put RBX, a porous solid biomaterial of three-dimensional structure, into the bone tunnel and speculated that it could cause compressive load on the grafted tendon from the beginning of healing. The compression should continue following the new bone ingrowth and remodelling on RBX. Our results showed more chondroid formation in the treatment group than in the control group.

There were several limitations in this study. One is that we used the long digital extensor tendon graft with suture fixation. We therefore could not completely mimic standard ACL reconstruction in humans, although the long digital extensor tendon graft is a very common graft used in animal studies for ACL reconstruction [1, 6, 19–20]. Second, we did not investigate the effect of single cancellous bone on bone formation because we consider RBX to be an entire functional unit. Our pilot study considered single cancellous bone as one part of RBX; it was found to act merely as a carrier, with no effect on new bone formation. Third, we hypothesised that compressive load affected healing due to RBX existence and remodelling, yet we could not prove the mechanism.

Conclusions

RBX enhanced bone formation around grafted tendon, achieving a more mature interface histologically and biomechanically. In the next phase of this work, we will

carry out longer-term analysis over at least 24–48 weeks, in order to evaluate some properties of tendon-bone interface healing promoted by RBX.

Conflict of interest We declare that we have no conflict of interest.

References

1. Anderson K, Seneviratne AM, Izawa K et al (2001) Augmentation of tendon healing in an intraarticular bone tunnel with use of a bone growth factor. *Am J Sports Med* 29:689–698
2. Forward AD, Cowan RJ (1963) Tendon suture to bone: an experimental investigation in rabbits. *J Bone Joint Surg Am* 45:807–823
3. Freedman KB, D'Amato MJ, Nedeff DD et al (2003) Arthroscopic anterior cruciate ligament reconstruction: a metaanalysis comparing patellar tendon and hamstring tendon grafts. *Am J Sports Med* 31:2–11
4. Hoffmann A, Gross G (2007) Tendon and ligament engineering in the adult organism: mesenchymal stem cells and gene-therapeutic approaches. *Int Orthop* 31:791–797
5. Hu YY (1993) Experimental studies on reconstituted bone xenograft and its clinical application (in Chinese). *Zhonghua Wai Ke Za Zhi* 31:709–713
6. Kyung HS, Kim SY, Oh CW et al (2003) Tendon-to-bone tunnel healing in a rabbit model: the effect of periosteum augmentation at the tendon-to-bone interface. *Knee Surg Sports Traumatol Arthrosc* 11:9–15
7. Liu SH, Kabo JM, Osti L (1995) Biomechanics of two types of bone-tendon-bone graft for ACL reconstruction. *J Bone Joint Surg Br* 77:232–235
8. Liu SH, Panossian V, al-Shaikh R et al (1997) Morphology and matrix composition during early tendon to bone healing. *Clin Orthop* 339:253–260
9. Wei L, Yunyu H, Yupu L (1991) Development and biological activity analysis of reconstituted bone xenograft. *Natl Med J China* 71:378–380
10. Bi L, Hu Y, Fan H (2007) Treatment of contaminated bone defects with clindamycin-reconstituted bone xenograft-composites. *J Biomed Mater Res B Appl Biomater* 82:418–427
11. Ma CB, Kawamura S, Deng XH et al (2007) Bone morphogenetic proteins-signaling plays a role in tendon-to-bone healing: a study of rhBMP-2 and noggin. *Am J Sports Med* 35:597–604
12. Matthews SJ (2005) Biological activity of bone morphogenetic proteins (BMP's). *Injury* 36(Suppl 3):S34–S37
13. Mihelic R, Pecina M, Jelic M et al (2004) Bone morphogenetic protein-7 (osteogenic protein-1) promotes tendon graft integration in anterior cruciate ligament reconstruction in sheep. *Am J Sports Med* 32:1619–1625
14. Ouyang HW, Goh JC, Lee EH (2004) Use of bone marrow stromal cells for tendon graft-to-bone healing: histological and immunohistochemical studies in a rabbit model. *Am J Sports Med* 32:321–327
15. Park MJ, Lee MC, Seong SC (2001) A comparative study of the healing of tendon autograft and tendon-bone autograft using patellar tendon in rabbits. *Int Orthop* 25:35–39
16. Pecina M, Giltaij LR, Vukicevic S (2001) Orthopaedic applications of osteogenic protein-1 (BMP-7). *Int Orthop* 25:203–208

17. Robertson WJ, Hatch JD, Rodeo SA (2007) Evaluation of tendon graft fixation using alpha-BSM calcium phosphate cement. *Arthroscopy* 23:1087–1092
18. Rodeo SA, Arnoczky SP, Torzilli PA et al (1993) Tendon-healing in a bone tunnel. A biomechanical and histological study in the dog. *J Bone Joint Surg Am* 75:1795–1803
19. Rodeo SA, Suzuki K, Deng XH et al (1999) Use of recombinant human bone morphogenetic protein-2 to enhance tendon healing in a bone tunnel. *Am J Sports Med* 27:476–488
20. Shaieb MD, Singer DI, Grimes J et al (2000) Evaluation of tendon-to-bone reattachment: a rabbit model. *Am J Orthop* 29:537–542
21. Spalazzi JP, Dagher E, Doty SB et al (2008) In vivo evaluation of a multiphased scaffold designed for orthopaedic interface tissue engineering and soft tissue-to-bone integration. *J Biomed Mater Res A* 86:1–12
22. Tien YC, Chih TT, Lin JH et al (2004) Augmentation of tendon-bone healing by the use of calcium-phosphate cement. *J Bone Joint Surg Br* 86:1072–1076
23. Tomita F, Yasuda K, Mikami S et al (2001) Comparisons of intraosseous graft healing between the doubled flexor tendon graft and the bone-patellar tendon-bone graft in anterior cruciate ligament reconstruction. *Arthroscopy* 17:461–476
24. Urist MR, Mikulski A, Lietze A (1979) Solubilized and insolubilized bone morphogenetic protein. *Proc Natl Acad Sci USA* 76:1828–1832
25. Wang CJ, Wang FS, Yang KD et al (2005) The effect of shock wave treatment at the tendon-bone interface-an histomorphological and biomechanical study in rabbits. *J Orthop Res* 23:274–280
26. Yamakado K, Kitaoka K, Yamada H et al (2002) The influence of mechanical stress on graft healing in a bone tunnel. *Arthroscopy* 18:82–90
27. Yamazaki S, Yasuda K, Tomita F et al (2005) The effect of transforming growth factor-beta1 on intraosseous healing of flexor tendon autograft replacement of anterior cruciate ligament in dogs. *Arthroscopy* 21:1034–1041