A new strategy to enhance artificial ligament graft osseointegration in the bone tunnel using hydroxypropylcellulose

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Abstract

Purpose The aim of this study was to determine whether hydroxypropylcellulose (HPC) coating of polyethylene terephthalate (PET) artificial ligaments enhances graft osseointegration in the bone tunnel.

Methods Thirty New Zealand white rabbits underwent artificial ligament graft transplantation in the bilateral proximal tibia tunnels. One limb was implanted with an HPC-coated PET graft, and the contralateral limb was implanted with a non-HPC-coated PET graft as a control. The rabbits were then randomly sacrificed at weeks four and eight after surgery for biomechanical testing, histological examination, and histomorphometric and real-time polymerase chain reaction analysis.

Results At week four after surgery, there were no statistically significant differences in the load to failure or stiffness values between the experimental and control limbs ($P = 0.328$ and $P = 0.128$, respectively). At week eight after surgery, the mean load to failure and stiffness value of the experimental limbs was higher than that of the control limbs ($P < 0.001$ and $P = 0.018$, respectively). At week eight after surgery, some protruding new bone tissue from the host bone to the graft was found in the HPC-coated group, while a thick fibrous tissue band was observed at the interface between the graft and the

host bone in the control group. Histomorphometric analysis showed that the graft-bone interface width in the HPC-coated group was significantly narrower than that in the control group at week eight after surgery ($P < 0.001$). At weeks four and eight after surgery, the mRNA expression level of bone morphogenetic protein-2 in the HPC group was higher than that in the control group ($P = 0.001$ and $P = 0.010$, respectively). The mRNA expression level of osteopontin in the HPC group was higher than that in the control group only at week four after surgery ($P = 0.032$).

Conclusions Our data show that an HPC coating on the surface of PET artificial ligament grafts may induce artificial ligament graft osseointegration in the bone tunnel.

Introduction

The use of synthetic material to replace ruptured ligaments was proposed in the 1980s. However, poor results and high rates of failure were reported, and so the synthetic material became less popular [1, 2]. Improved surgical techniques and new designs providing more anatomical reconstruction may offer more satisfactory results. The Ligament Advanced Reinforcement System (LARS) artificial ligament (Surgical Implants and Devices, Arc-sur-Tille, France), which comprises fibers made of polyethylene terephthalate (PET), is reportedly a suitable material [3, 4]. At present, reconstruction of the cruciate ligament using an autogenous patellar tendon or semitendinosus muscle is most common, but has some drawbacks, including complications related to the harvesting of the graft, limitation of organization sources, breakage or loosening in the early stage of rehabilitation, and not allowing complete motion at an early stage [5, 6]. Allo-graft tendons are also associated with several risks, such as the potential for disease transmission, limited availability,

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inadequate ligamentization, and possible immunogenicity [7, 8]. The LARS artificial ligament does not have the above drawbacks. Thus, the LARS artificial ligament has been used in many countries worldwide [9]. However, as reported previously [10, 11], because the LARS ligament is hydrophobic and chemically inert, it integrates poorly into the surrounding bone. Therefore, how to promote osseointegration of the artificial ligament graft in the bone tunnel after implantation is an important issue that requires urgent resolution.

Hydroxypropylcellulose (HPC) is a nonionic, water-soluble cellulose ether polymer obtained by chemical reaction of the hydroxyl groups at positions 2, 3, and/or 6 of the glucose residues of cellulose [12, 13]. Because HPC has hydrophilic and bioadhesive properties [14–16], it is primarily used in oral and topical pharmaceutical formulations. However, it is not clear whether HPC-coated PET artificial ligament grafts have a greater osseointegration capability in the bone tunnel. Based on the above, we used HPC coating to modify the surface of PET artificial ligament grafts.

The purpose of this study was to investigate the effect of HPC coating on PET artificial ligament grafts in a rabbit extra-articular tendon-to-bone healing model. We hypothesized that the HPC coating would significantly enhance the osseointegration of the PET artificial ligament graft in the host bone.

Materials and methods

Preparation of the PET sheet with hydroxypropylcellulose coating

PET sheets ($\sim 20 \times 10$ mm) were taken from a LARS ligament (Surgical Implants and Devices, Arc-sur-Tille, France). They were cleaned in 75 % alcohol solution for four hours and then washed with a large amount of deionized water. Finally, they were air-dried for 24 hours. Klucel® brand HPC (Type LF, MW = 95,000, 3.8 “moles of substitution”) was obtained from Hercules (Wilmington, DE, USA). Powdered HPC was dissolved by extensive vortexing in room-temperature water to a concentration of 5 % m/m. The HPC solution was stirred for one hour to fully dissolve and then left overnight to allow bubbles to dissipate. The PET sheets were modified through plasma surface modification (HPD-100B Plasma Apparatus; Coronat Co. Ltd., Nanjing, China) at 70 V for 40 s and then immersed in the HPC solution for four hours. They were then air-dried for 24 hours. These PET sheets were rolled to 2 cm in length and ~ 3 mm in diameter. Before the animal surgery, both non-HPC-coated and HPC-coated PET sheets were subjected to scanning electron microscopy (SEM) (TS5136MM; Tescan, Brno, Czech Republic) at a 20-kV accelerating voltage to visualize surface morphology.

Animal study design and surgical procedure

All animal experiments were approved by the Institutional Animal Care and Use Committee at Fudan University and were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The animal surgical procedures were undertaken as described in our previous manuscript [17, 18]. Briefly, 30 mature male New Zealand rabbits (mean weight, 2.9 ± 0.3 kg) underwent a surgical extra-articular graft-to-bone surgical procedure. All rabbits were anaesthetised with 3 % pentobarbital (30 mg/kg). After skin disinfection and incision, a 3-mm-diameter bone tunnel was drilled in the proximal tibial metaphysis. The legs of the rabbits were randomly divided into experimental and control sides. The HPC-coated graft was pulled into one leg as the experimental group, while the non-HPC-coated graft was implanted into the contralateral leg as the control group. A nearly 0.5-cm-long graft was left outside the lateral tunnel entrance for the subsequent biomechanical test. The graft end was sutured with the adjacent periosteum and soft tissue at the tunnel entrance, and the wound was then closed layer by layer. Postoperatively, the animals were returned to their cages and allowed to move freely without immobilization. Buprenorphine (0.05 mg/kg) was used subcutaneously for pain control for three days. These rabbits were sacrificed at weeks four and eight after surgery for subsequent testing.

Mechanical testing

Rabbits from each group ($n = \text{ten}$) were sacrificed either four ($n = \text{five}$) or eight ($n = \text{five}$) weeks postoperatively, and the graft-tibia complex was immediately prepared for mechanical testing without being frozen. All scar tissue and sutures were carefully removed from the tibial tunnel exits. Mechanical testing was conducted using a materials testing system machine (8874; Instron Co., Norwood, MA, USA). The graft end outside of the lateral tunnel entrance was sutured by a No. 5 Ethibond suture for drawing, and the tibial portion was fixed firmly with a clamp. The bone tunnel was oriented parallel with the testing axis. Before the tensile test, the samples were preloaded with a static preload of 1 N for five minutes. After preconditioning, determination of the ultimate failure load was immediately carried out using an elongation rate of 2 mm/min, and the ultimate failure load (N) was recorded. For each sample, the test was considered completed when the graft ruptured or was pulled out of the bone tunnel.

Histological examination

The graft-tibia complexes of rabbits from each group ($n = \text{ten}$) were harvested either four ($n = \text{five}$) or eight ($n = \text{five}$) weeks postoperatively and subjected to histological examination.

After the rabbits were sacrificed, the graft-bone complex samples were fixed in 10 % formalin for 48 hours, embedded, and decalcified in a methyl methacrylate compound. The samples were sectioned perpendicularly to the longitudinal axis of the tibial tunnel with a thickness of 5 μ m using a microtome (SM2500; Leica, Nussloch, Germany). These sections were stained with hematoxylin and eosin (H&E) for routine light microscopic examination. The graft-bone interface was visualized by inverted light microscopy (IX71SBF-2; Olympus Optical Co., Tokyo, Japan), and digital images were taken using a DP Manager (Olympus Optical Co., Tokyo, Japan). The two investigators who performed the histological analysis were blinded to the animal surgery.

Histomorphometric analysis

Each section from the middle portion of the tunnel was divided into four quadrants to determine the width of the graft-bone interface. In each of the four quadrants, the width of the graft-bone interface was measured as the distance between the edge of the bone tunnel and the outer graft determined under $\times 200$ magnification. Four separate measurements were conducted in each of the quadrants, for a total of 16 measurements for each sample. The average width of the graft-bone interface of each sample was calculated. The two investigators who performed the histomorphometric analysis were blinded to the animal surgery.

Real-time polymerase chain reaction

The graft-tibia complex (n = five limbs in each group at four or eight weeks postoperatively) was harvested from each knee after sacrifice and subjected to real-time polymerase chain reaction (RT-PCR) analysis. Total RNA from interfacial samples between the host bone tunnel and graft were extracted using TRIzol reagent (10296010; Invitrogen, Carlsbad, CA, USA), based on the manufacturer's instructions. cDNA was generated using reverse transcriptase M-MLV (D2640A; Takara, Beijing, China), according to the manufacturer's protocol. Quantitative PCR was performed with SYBR Premix Ex Taq (DRR041A; Takara, Beijing, China) and then detected using an RT-PCR system (TP800; Takara, Kyoto, Japan). Bone morphogenetic protein-2 (BMP-2) and osteopontin expressions were normalized to that of β -actin.

The primers for RT-PCR were as follows: for β actin, forward 5'-CCA AGG CCA ACC GCG AGA AGA TGA-3' and reverse 5'-GCA GCG CGT AGC CCT CGT AGA TGG-3'; for BMP-2, forward 5'-GGA ATG ACT GGA TTG TGG CT-3' and reverse 5'-TGA GTT CTG TCG GGA CAC AG-3'; and for osteopontin, forward 5'-GTG GAC AGC GAG GAC TTG GAT G-3' and reverse 5'-GGC CTC GCG CTT ATATTG TCT GG-3'.

Statistical analysis

Statistical analysis was performed using the SPSS for Windows v. 13.0.0 statistical software (SPSS, Inc., USA). Numerical data were expressed as means $\pm$ standard deviations (SD). The data of the experimental and control groups were compared using a paired Student's t -test. Comparisons of groups at various time points were performed with an independent two-sample Student's t -test. All P values were two-sided, and a P value of <0.05 was considered to indicate statistical significance.

Results

SEM analysis

SEM showed that the surface of the non-HPC-coated PET grafts was smooth and clear (Fig. 1a). However, there was a thin coating on the fibre surface of the HPC-coated PET grafts (Fig. 1b).

Mechanical findings

Regardless of the implantation duration (four or eight weeks), all samples failed when they were pulled out from the bone tunnel. No PET grafts were ruptured. At week four after surgery, there was no statistically significant difference in the load to failure between the experimental and control limbs (45.2 ± 8.3 N versus 40.6 ± 5.3 N, respectively; $P = 0.328$). At week eight after surgery, the mean load to failure of the experimental limbs was higher than that of the control limbs (96.2 ± 7.3 N versus 58.0 ± 4.9 N, respectively; $P < 0.001$) (Fig. 2a). Likewise, at week four after surgery, there was no statistically significant difference in the stiffness values of the experimental and control limbs (5.2 ± 1.3 N/mm versus 3.8 ± 1.3 N/mm, respectively; $P = 0.128$). At week eight after surgery, the stiffness value of the experimental limbs was higher than that of the control limbs (8.0 ± 1.6 N/mm vs. 5.4 ± 1.1 N/mm, respectively; $P = 0.018$) (Fig. 2b).

Histological findings

At week four after surgery, inflammatory cells infiltrated the graft-bone interface in both the control and HPC-coated groups. Thick fibrous scar tissue had formed at the graft-to-bone interface at week four after surgery in both the control and HPC-coated groups. At week eight after surgery, some protruding new bone tissue from the host bone to the graft was found in the HPC-coated group, while a thick fibrous tissue band was observed at the interface between the graft and host bone in the control group. Furthermore, no obvious new bone had formed in the control group (Fig. 3).

Fig. 1 Scanning electron micrograph of the surfaces of the PET graft without and with HPC coating. **a** Surfaces of PET fibres without HPC coating. **b** Surfaces of PET fibers with HPC coating. Bar = 10 μ m. HPC hydroxypropylcellulose

Histomorphometric analysis

Histomorphometric analysis indicated no difference in the graft-bone interface width between the HPC-coated and

control groups at week four after surgery ($277.81 \pm 45.77 \mu\text{m}$ versus $302.31 \pm 30.30 \mu\text{m}$, respectively; $P = 0.084$). However, the graft-bone interface width in the HPC-coated group was significantly narrower than that in the control group at week eight after surgery ($112.62 \pm 30.94 \mu\text{m}$ versus $210.06 \pm 32.28 \mu\text{m}$, respectively; $P < 0.001$) (Fig. 4).

RT-PCR

At week four, the mRNA expression levels of BMP-2 and osteopontin in the HPC group were higher than those in the control group (0.65 ± 0.11 versus 0.34 ± 0.07 for BMP-2, $P = 0.001$; 0.23 ± 0.08 versus 0.12 ± 0.05 for osteopontin, $P = 0.032$). At week eight, the mRNA expression level of BMP-2 in the HPC group was higher than that in the control group (0.38 ± 0.03 versus 0.24 ± 0.09 , respectively; $P = 0.010$). However, there were no significant differences in the mRNA expression levels of osteopontin between the HPC and control groups (0.15 ± 0.03 versus 0.11 ± 0.02 , respectively; $P = 0.160$). Furthermore, both the mRNA expression levels of BMP-2 and osteopontin decreased with time (Fig. 5).

Discussion

Clinical outcomes after ACL reconstruction using tendon grafts are heavily reliant upon secure integration between the tendon and bone. During the early postoperative period, the weakest link is the tendon–bone interface, which does not regenerate into the fibrocartilaginous-type entheses seen in the native ACL insertion site [19–21]. Research on methods of enhancing and accelerating tendon-to-bone healing is ongoing; these methods include the application of periosteum augmentation, BMP, calcium-phosphate cement, granulocyte colony-stimulating factor, gene transfer, and so on [22–24], all of which may reduce the time required before the return to competitive sports, work, and other daily activities. Rodeo et al. [25] demonstrated a correlation between tendon–bone interface strength and the degree of bone ingrowth,

Fig. 2 Mechanical graft-bone healing examinations in a rabbit model at various time points after surgery. **a** Comparison of maximal failure load between the control and hydroxypropylcellulose (HPC) groups. **b** Comparison of stiffness at failure between the control and HPC groups

Fig. 3 Histological characterization of the control group (a and c) and the HPC group (b and d) at week four (a, b) and week eight (c, d) after surgery. Images are of the interface between the artificial ligament graft and the host bone (hematoxylin-eosin staining, magnification 200×). In the HPC group at eight weeks after surgery, new bone growth into the interface was observed (white arrow). HB host bone, G artificial ligament graft, IF interface, HPC hydroxypropylcellulose. Bar = 200 μ m

mineralization, and tissue maturation between the bone and tendon. At present, reports regarding methods of promoting coated PET graft bone healing in the bone tunnel are scarce.

HPC, a non-ionic and water-soluble polymer, is widely used in pharmaceutical and cosmetic formulations. It is also commonly used as a viscosity enhancer, binder, stabilizer, and emulsifying agent [26]. HPC is extensively used in oral formulations due to its non-toxic and non-irritant properties. In addition, it possesses mucoadhesive and hydrophilic characteristics [15, 16, 26]. In our study, we investigated the ability of an HPC coating to promote healing of PET artificial grafts in the tibial bone tunnel using a rabbit extra-articular tendon-bone healing model. Through plasma surface modification, the HPC solution was successfully coated on the fibre surface of PET sheets, and visualized by SEM.

Fig. 4 Width of the interface between the host bone and PET graft in the control and hydroxypropylcellulose (HPC)-coated groups

Fig. 5 mRNA levels of BMP-2 (a) and osteopontin (b) at weeks four and eight after surgery as determined by RT-PCR

Use of hydrophilic biomaterial can promote bone healing and osseointegration. Mardas et al. [27] evaluated new bone formation under etched titanium and modified-etched hydrophilic titanium (modSLA) domes placed on the calvarium of healthy, osteoporotic, and osteoporotic rabbits treated with bisphosphonates. They found that the use of a modSLA surface may promote bone healing and osseointegration in osteoporotic rabbits. Furthermore, platelet release has been shown to promote osteogenetic cell proliferation and differentiation. Kämmerer et al. [28] found that modifications of surface roughness appear to influence early platelet activation and cytokine release after 15 minutes, whereas surface chemistry modifications with increased hydrophilic properties and surface modifications lead to a further, more specific promotion of platelet activation and degranulation after 30 minutes. In the present study, we found that this HPC coating on the PET graft sheet improved the biomechanical properties of the graft in the bone tunnel. At week four after surgery, there was no statistically significant difference in the load to failure between the experimental and control limbs. However, at week eight after surgery, both the mean load to failure and the stiffness value of the experimental limbs were higher than those of the control limbs. This suggests that the hydrophilic HPC coating had a positive effect on the healing of the artificial ligament in the bone tunnel.

In our study, the histological results showed that at week eight after surgery, some protruding new bone tissue from the host bone to the graft was found in the HPC-coated group, while a thick fibrous tissue band was observed at the interface between the graft and host bone in the control group. In addition, no obvious new bone formed in the control group. This is possibly due to the fact that the HPC coating improved the hydrophilic nature of the surface of the PET sheet *in vivo*. The graft with the HPC coating had more osseointegration with the host bone at week eight after surgery compared with the control group.

Some studies have shown that the presence of several important proteins and growth factors surrounding the graft, such as BMP-2, BMP-7 and osteopontin, could favour osteogenesis [23, 29–32]. Hashimoto et al. [23] injected recombinant human BMP-2 (rhBMP-2) into the flexor digitorum communis tendon in the rabbit hind limb. One month following surgery, histomorphological examination confirmed direct insertion of tendon-bone structures in the proximal tibia of the rabbit. Ultimate failure loads of the BMP-2-generated tendon-bone junction were significantly higher than those in the control group. Wang et al. [30] also found that plasmid cytomegalovirus (pCMV)-BMP-2 gene therapy significantly improved tendon-to-bone healing and promoted angiogenesis and osteogenesis at the tendon-bone interface after ACL reconstruction in a rabbit model. In our study, we also found that at weeks four and eight after surgery, the mRNA level of BMP-2 in the HPC group was higher than that in the control group.

Osteopontin is a secreted adhesive glycoprophosphoprotein expressed by several cell types. It is normally produced in bone, teeth, kidneys, epithelial lining tissues, etc. It is involved in a number of physiologic and pathologic events, including angiogenesis and wound healing [31]. McKee et al. [32] found that during bone wound healing after surgical drilling and other forms of surgical cutting, the observed secretion of OPN by both macrophages and osteoblast-lineage cells, together with the localization of osteopontin to exposed mineral surfaces, suggests that osteopontin plays important roles in (i) the clearance of bone debris (powder) by macrophage phagocytosis after osteopontin opsonization and (ii) the formation of a cement line (plane) at the margins of the wound that integrates the new repaired bone with the existing drilled bone. In our study, at week four after surgery, the mRNA expression level of osteopontin in the HPC group was higher than that in the control group. At week eight after surgery, there was no significant difference in the mRNA level of osteopontin between the HPC and control groups. These results suggest that the enhancement of the hydrophilic nature of the PET graft could increase the expression of BMP-2 and osteopontin, which may advance the formation of new bone and finally promote graft-bone healing.

Several limitations to this study should be noted. First, a rabbit extra-articular tendon-to-bone healing model was used to investigate the effect of HPC-coated PET sheets in tendon-bone healing. This differs from the intra-articular tendon-bone healing required for ACL reconstruction. However, this model of graft implantation has been well-established in several tendon-bone healing studies [33, 34], and it is feasible for preliminary research of a new graft coating. Second, the thickness of the HPC coating was difficult to control, which may have influenced the effect of HPC coating on the graft-bone healing in the bone tunnel. Methods of controlling the thickness of the HPC coating should be elucidated in future studies.

In conclusion, our results demonstrate that an HPC coating can promote graft-bone healing of PET artificial grafts in the bone tunnel in a rabbit extra-articular graft implantation model. Our findings have great clinical significance in terms of enhancing tendon-bone healing in ACL reconstruction, and the methods we report here could be translated into a clinically appropriate treatment to enhance the repair rate of ACL reconstruction.

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Conflicts of interest The authors declare that they have no competing interests.

References

- Noyes FR, Barbe-Westin SD (1996) Surgical restoration to treat chronic deficiency of the posterolateral complex and cruciate ligaments of the knee joint. *Am J Sports Med* 24:415–426
- Mascarenhas R, Macdonald PB (2008) Anterior cruciate ligament reconstruction: a look at prosthetics-past, present and possible future. *Mcgill J Med* 11:29–37
- Cazenave A, Laboureaux JP (1990) Reconstruction of the anterior cruciate ligament: determination of the pre- and perioperative femoral isometric point. *Rev Chir Orthop Reparatrice Appar Mot* 76:288–292
- Dericks G Jr (1995) Ligament advanced reinforcement system anterior cruciate ligament reconstruction. *Op Tech Sports Med* 3:187–205
- Christen B, Jakob RP (1992) Fractures associated with patellar ligament grafts in cruciate ligament surgery. *J Bone Joint Surg Br* 74:617–619
- Bonamo JJ, Krinick RM, Sporn AA (1984) Rupture of the patellar ligament after use of its central third for anterior cruciate reconstruction. A report of two cases. *J Bone Joint Surg Am* 66:1294–1297
- Asselmeier MA, Caspari RB, Bottenfield S (1993) A review of allograft processing and sterilization techniques and their role in transmission of the human immunodeficiency virus. *Am J Sports Med* 21:170–175
- Jackson DW, Grood ES, Goldstein JD, Rosen MA, Kurzweil PR, Cummings JF et al (1993) A comparison of patellar tendon autograft and allograft used for anterior cruciate ligament reconstruction in the goat model. *Am J Sports Med* 21:176–185
- Legnani C, Ventura A, Terzaghi C, Borgo E, Albisetti W (2010) Anterior cruciate ligament reconstruction with synthetic grafts. A review of literature. *Int Orthop* 34:465–471
- Gao K, Chen S, Wang L, Zhang W, Kang Y, Dong Q et al (2010) Anterior cruciate ligament reconstruction with LARS artificial ligament: a multicenter study with 3- to 5-year follow-up. *Arthroscopy* 26:515–523
- Guidoin MF, Marois Y, Bejui J, Poddevin N, King MW, Guidoin R (2000) Analysis of retrieved polymer fiber based replacements for the ACL. *Biomaterials* 21:2461–2474
- Zhu L, Seburg RA, Tsai EW (2006) Determination of surface-bound hydroxypropylcellulose (HPC) on drug particles in colloidal dispersions using size exclusion chromatography: a comparison of ELS and RI detection. *J Pharm Biomed Anal* 40:1089–1096
- Ramachandran S, Chen S, Etzler F (1999) Rheological characterization of hydroxypropylcellulose gels. *Drug Dev Ind Pharm* 25:153–161
- Ma L, Kang H, Liu R, Huang Y (2010) Smart assembly behaviors of hydroxypropylcellulose-graft-poly(4-vinyl pyridine) copolymers in aqueous solution by thermo and pH stimuli. *Langmuir* 26:18519–18525
- Repka MA, ElSohly MA, Munjal M, Ross SA (2006) Temperature stability and bioadhesive properties of delta9-tetrahydrocannabinol incorporated hydroxypropylcellulose polymer matrix systems. *Drug Dev Ind Pharm* 32:21–32
- Yamada T, Saito N, Anraku M, Imai T, Otagiri M (2000) Physicochemical characterization of a new crystal form and improvements in the pharmaceutical properties of the poorly water-soluble antiosteoporosis drug 3,9-bis (N, N-dimethylcarbamoy-loxy)-5H-benzofuro [3,2-c] quinoline-6-one (KCA-098) by solid dispersion with hydroxypropylcellulose. *Pharm Dev Technol* 5:443–454
- Li H, Chen S, Wu Y, Jiang J, Ge Y, Gao K et al (2012) Enhancement of the osseointegration of a polyethylene terephthalate artificial ligament graft in a bone tunnel using 58S bioglass. *Int Orthop* 36:191–197
- Li H, Ge Y, Wu Y, Jiang J, Gao K, Zhang P et al (2011) Hydroxyapatite coating enhances polyethylene terephthalate artificial ligament graft osseointegration in the bone tunnel. *Int Orthop* 35:1561–1567
- Ballock RT, Woo SL, Lyon RM, Hollis JM, Akeson WH (1989) Use of patellar tendon autograft for anterior cruciate ligament reconstruction in the rabbit: a long-term histologic and biomechanical study. *J Orthop Res* 7:474–485
- Butler DL (1989) Kappa Delta Award paper. Anterior cruciate ligament: its normal response and replacement. *J Orthop Res* 7:910–921
- Grana WA, Egle DM, Mahnken R, Goodhart CW (1994) An analysis of autograft fixation after anterior cruciate ligament reconstruction in a rabbit model. *Am J Sports Med* 22:344–351
- Chen CH, Chen WJ, Shih CH, Yang CY, Liu SJ, Lin PY (2003) Enveloping the tendon graft with periosteum to enhance tendon-bone healing in a bone tunnel: a biomechanical and histologic study in rabbits. *Arthroscopy* 19:290–296
- Hashimoto Y, Yoshida G, Toyoda H, Takaoka K (2007) Generation of tendon-to-bone interface “entheses” with use of recombinant BMP-2 in a rabbit model. *J Orthop Res* 25:1415–1424
- Huangfu X, Zhao J (2007) Tendon-bone healing enhancement using injectable tricalcium phosphate in a dog anterior cruciate ligament reconstruction model. *Arthroscopy* 23:455–462
- Rodeo S, Arnoczky S, Torzilli P, Hidaka C, Warren R (1993) Tendon-healing in a bone tunnel: a biomechanical and histological study in the dog. *J Bone Joint Surg Am* 75:1795–1803
- Rokhade AP, Kulkarni PV, Mallikarjuna NN, Aminabhavi TM (2009) Preparation and characterization of novel semi-interpenetrating polymer network hydrogel microspheres of chitosan and hydroxypropyl cellulose for controlled release of chlorothiazide. *J Microencapsul* 26:27–36
- Mardas N, Schwarz F, Petrie A, Hakimi AR, Donos N (2011) The effect of SLActive surface in guided bone formation in osteoporotic-like conditions. *Clin Oral Implants Res* 22:406–415
- Kämmerer PW, Gabriel M, Al-Nawas B, Scholz T, Kirchmaier CM, Klein MO (2012) Early implant healing: promotion of platelet activation and cytokine release by topographical, chemical and biomimetic titanium surface modifications in vitro. *Clin Oral Implants Res* 23:504–510
- Mihelic R, Pecina M, Jelic M, Zoricic S, Kusec V, Simic P 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
- Wang CJ, Weng LH, Hsu SL, Sun YC, Yang YJ, Chan YS et al (2010) pCMV-BMP-2-transfected cell-mediated gene therapy in anterior cruciate ligament reconstruction in rabbits. *Arthroscopy* 26:968–976
- Standal T, Borset M, Sundan A (2004) Role of osteopontin in adhesion, migration, cell survival and bone remodeling. *Exp Oncol* 26:179–184
- McKee MD, Pedraza CE, Kaartinen MT (2011) Osteopontin and wound healing in bone. *Cells Tissues Organs* 194:313–319
- Chen CH, Liu HW, Tsai CL, Yu CM, Lin IH, Hsiue GH (2008) Photoencapsulation of bone morphogenetic protein-2 and periosteal progenitor cells improve tendon graft healing in a bone tunnel. *Am J Sports Med* 36:461–473
- Karaoglu S, Celik C, Korkusuz P (2009) The effects of bone marrow or periosteum on tendon-to-bone tunnel healing in a rabbit model. *Knee Surg Sports Traumatol Arthrosc* 17:170–178