International Orthopaedics (SICOT) (2005) 29: 67–72
DOI 10.1007/s00264-004-0622-6

ORIGINAL PAPER

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No effect of platelet-rich plasma with frozen or processed bone allograft around noncemented implants

Received: 2 November 2004 / Accepted: 4 November 2004 / Published online: 1 February 2005

Abstract We compared processed morselized bone allograft with fresh-frozen bone graft around noncemented titanium implants. Also, the influence of platelet-rich plasma (PRP) in combination with bone allograft was evaluated. Analysis was based on implant fixation and histomorphometry. PRP was prepared by isolating the buffy coat from autologous blood samples. Bone allograft was used fresh-frozen or processed by defatting, freeze drying, and irradiation. Cylindrical hydroxyapatite-coated titanium implants were inserted bilaterally in the femoral condyles of eight dogs. Each implant was surrounded by a 2.5-mm concentric gap, which was filled randomly according to the four treatment groups—group 1: fresh-frozen bone allograft; group 2: processed bone allograft; group 3: fresh-frozen bone allograft + PRP; group 4: processed bone allograft + PRP. Histological and mechanical evaluation demonstrated no influence of bone allograft processing. Even though the level of platelet in PRP was 7.7 times that found in whole blood, we found no improvement of bone formation or implant fixation by adding PRP.

Résumé Nous avons comparé l'allogreffe osseuse morcelée avec la greffe osseuse fraîche congelée autour d'implants en titane non-cimentés. L'influence du plasma riche en plaquettes (PRP) dans la combinaison avec l'allogreffe osseuse a aussi été évaluée. L'analyse était basée sur la fixation de l'implant et l'histomorphométrie. Le PRP a été préparé en isolant la couche leuco-plaquettaire du sang autologue après centrifugation. L'allogreffe osseuse a été utilisé fraiche et congelée ou a été traité par délipidation, lyophilisation et irradiation. Les implants de titane cylindriques enduit d'hydroxyapatite ont été insérés bilatéralement dans le condyle fémoral de huit chiens. Chaque implant a été entouré par un intervalle concentrique de 2.5 mm qui a été rempli de façon aléatoire d'après les quatre groupes de traitement—groupe-1: allogreffe congelée fraiche; groupe-2: allogreffe traitée; groupe-3: allogreffe congelée fraiche+PRP; groupe-4: allogreffe traitée+PRP. L'évaluation histologique et mécanique n'a démontré aucune influence du traitement de l'allogreffe osseuse. Bien que le niveau de plaquettes dans le PRP fût 7.7 fois celui du sang total, nous n'avons trouvé aucune amélioration de la formation osseuse ou de la fixation de l'implant en ajoutant du PRP.

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Introduction

Morselized, impacted bone allograft is widely used to restore the bone stock in the revision of failed joint replacements. Impacted bone allograft chips are often incorporated with fibrous tissue and not with bone [16]. Stimulation of bone formation into the impacted allograft is therefore of clinical interest.

Platelets are initiators of fracture healing. At the site of a fracture, platelets degranulate a number of growth factors, such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF) [22], all of which stimulate osteoblasts in vitro and bone formation in vivo [11]. Platelet-rich plasma (PRP) is a concentrate of platelets and white cells in a suspension of plasma. Local application of PRP at the site of a fracture or a grafted defect increases bone healing [15].

Bone allograft can be used fresh-frozen or processed. fresh-frozen bone allograft does carry a potential risk of

viral transmission and bacterial contamination [2, 14], but those risks can be almost entirely eliminated by various methods of preserving, processing, and sterilization. Bone graft processing, however, may alter the mechanical and biological properties [3, 17]. The aim of the present study was to evaluate the influence of bone graft processing and PRP on the biological activity of morselized bone allograft impacted around hydroxyapatite (HA)-coated titanium implants.

Materials and methods

Study design

Eight skeletally mature Labrador dogs with an average weight of 27.5 (23.7–30.5) kg were used for implantation. Two additional Labrador dogs served as bone graft donors. The protocol was accepted by the Danish Animal Research Committee. Animal handling was performed according to Danish laws and principles of animal care as described by NIH publication 85-23, revised, 1985. Two titanium implants were inserted extra-articularly into the femoral condyle: one medial and one lateral. Each implant was surrounded by a 2.5-mm gap. The four gaps surrounding the four implants in each dog were block-randomized to one of the following treatment groups—group 1: fresh-frozen bone allograft; group 2: processed bone allograft; group 3: fresh-frozen bone allograft + PRP; group 4: processed bone allograft + PRP.

Implants

Titanium alloy (Ti–6Al–4V) implants cylindrical in shape with a plasma-sprayed Ti porous coating were manufactured by Biomet Inc. (Warsaw, IN, USA) (Fig. 1). Mean pore diameter was 480 μm and porosity 44%. A 50-μm HA (Ca/P ratio 1.67) coating was plasma-sprayed on the porous Ti-alloy coating by BioInterfaces Inc. (San Diego, CA, USA). The final dimension of implants was a length of 10 mm and a diameter of 6 mm. The surface roughness (Ra) of HA-coated implants was 41 μm. Implants were sterilized by 35-kGy Co-60 gamma irradiation. A standardized 2.5-mm gap, from the implant surface to the border of the drill hole, was obtained by a footplate and washer.

Bone allograft

Proximal humerus, proximal femur, and proximal tibia were harvested from two dogs not included for implantation and stored at −80°C. After 3 weeks, the bone graft was thawed and soft tissue and cartilage was removed. Bone graft was milled twice using the finest grater using a standard bone mill. The bone-graft chips were divided into two portions. One portion was weighed into portions of 0.66 cm^3, packed into sterile containers, and stored at −80°C and later used as fresh-frozen bone allograft (Fig. 1). The other portion was further processed. First, it was washed down with pure water to remove marrow and blood cells. It was then soaked in a water bath and sonicated for 2 h (Branson 2210, Bransonic Ultrasonic Cleaner, Danbury, CT, USA) at a frequency of 47 kHz. Lipid was extracted in 70% ethanol over 4 h, and the graft was freeze-dried for 72 h at −55°C. It was then weighed into 0.66-cm^3 portions, irradiated with 18-kGy gamma irradiation, and stored at room temperature (Fig. 1). All procedures were done under strictly aseptic conditions, and bacterial cultures were taken at all stages of processing.

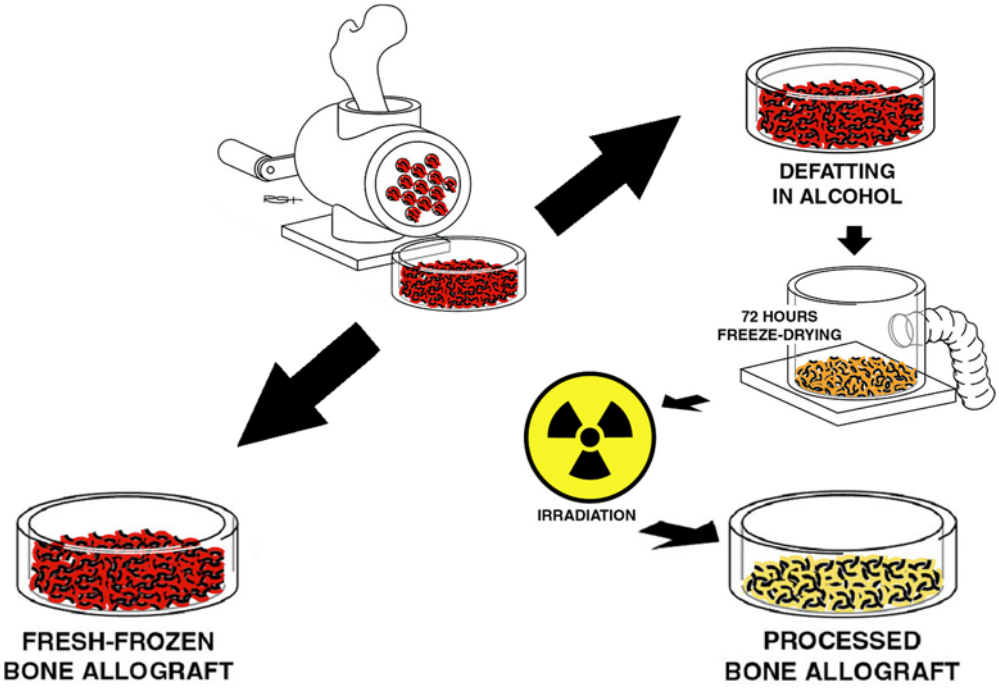

Fig. 1 Bone allograft was morselized in a grinder and divided into two portions. One portion was used fresh-frozen whereas the other portion was further processed by defatting, freeze drying, and irradiation.

Platelet-rich plasma

PRP was isolated from venous blood as previously described [9]. In the operation room, 0.5-ml PRP was mixed with 0.125-ml bovine thrombin/calcium chloride solution, 0.66 cm^3 of bone graft was added, and a gel was allowed to form for approximately 1 min before implantation.

Surgery

Anesthesia was induced by intravenous methohexitone (10 mg/kg) and maintained by halothane. Unloaded implants were inserted into the medial and lateral condyles in both knees as previously described [19]. A cylindrical hole of 10 mm was hand drilled and cleaned with saline. The implant was inserted, leaving a 2.5-mm gap (0.66 cm^3) between the implant surface and surrounding bone. The gap was filled according to the treatment groups. Before and after each operation, 1-g ampicillin (Anhypen; Gist-Brocades, Delft, Holland) was administered intravenously. The dogs were terminated by methohexitone (Brietal, Lilly; Denmark) sedation and an overdose of pentobarbital.

Preparation of tissue samples

Transverse sections of each implant were made on a diamond saw (Exact Apparatebau, Nordenstedt, Germany). First, the upper 1.5 mm was cut away. The next section, which was 5-mm thick, was put into 70% ethanol and prepared for histomorphometry. The lower 3.5 mm was stored at −20°C and later used for mechanical testing. All specimens were blinded until analyses of data.

Histomorphometry

The specimens were dehydrated in 70–100% ethanol containing 0.4% basic fuscin and subsequently embedded in methyl methacrylate (Technovit 7200 VLC, Exact, Apparatebau, Nordenstedt, Germany). Four sections of 25 μm, parallel to the long axis of the implant, were made on a microtome (Leiden, Holland) and surface-stained with 2% light green for 2 min [7]. This staining method stains mineralized tissue green and other tissue red (Fig. 3). Histomorphometry was done using the unbiased stereological vertical-section method. The microscope field was transmitted to a monitor, and a user-specified grid was superimposed using the software program CAST-Grid) (Olympus, Denmark). Bone ingrowth, defined as the fraction of the implant surface in contact with newly formed bone, was evaluated by the linear intercept technique [10]. Approximately 250 interceptions on the surface of each section were counted, and bone ingrowth was calculated as bone coverage of the implant surface as a percentage of total surface area. Volume fractions of woven bone, grafting material, and nonmineralized tissue in the gap were estimated at 0–1,000 μm (zone 1) and 1,500–2,500 μm (zone 2), respectively, from the implant surface (Fig. 2). Two hundred and fifty points per section were counted at a 100× magnification.

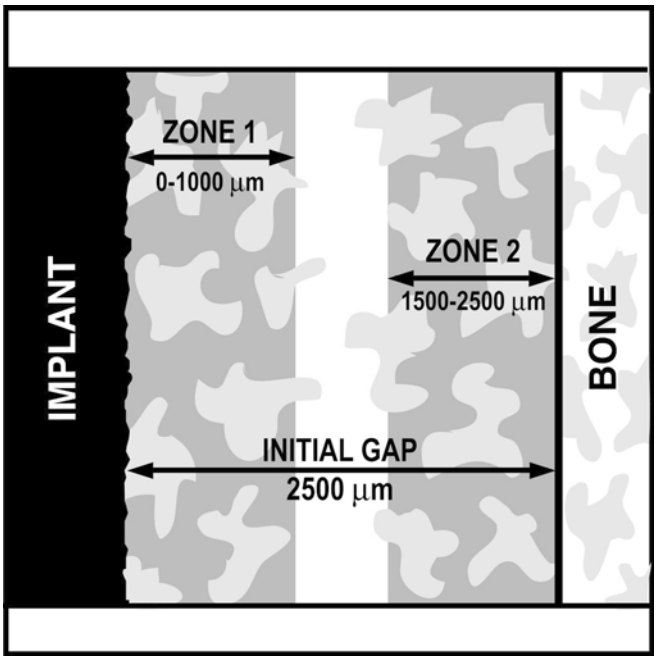

Fig. 2 Volume fractions of bone, bone allograft, and nonmineralized tissue were quantified in two zones—zone 1: 0–1,000 μm from implant surface; zone 2: 1,500–2,500 μm from implant surface.

Mechanical testing

A push-out test was performed using an Instron universal test machine (Instron Ltd., High Wycombe, UK). The implants were centralized over a metal platform with a circular hole supporting the bone specimen to within 500 μm of the interface. The implant was displaced at a velocity of 5 mm/min, and load-deformation curves were obtained. Ultimate shear strength (apparent stiffness) and energy absorption was determined as previously described [9].

Statistical analysis

Data are presented as median values and range in brackets. One-way analysis of variance (ANOVA) on ranks was applied, and groups were compared pairwise using Student–Newman–Keule. *P* values less than 0.05 were considered significant.

Results

All dogs were mobilized after 2 days. At autopsy, there were no signs of infections around any of the implants.

Table 1 Analyses of platelet-rich plasma (PRP) and whole blood [median (range)]. *n* final number of implants in each treatment group

Group	Baseline count	PRP	PRP/whole blood
Platelets ($\times10^9$/l), *n*=8	246 (132–321)	1,884 (1,156–2,742)	7.7 (6.0–8.9)
Leukocytes ($\times10^9$/l), *n*=8	8.1 (5.8–13.3)	71.7 (45.1–95.5)	9.1 (6.8–11.8)
Erythrocytes ($\times10^{12}$/l), *n*=8	6.0 (5.0–7.3)	8.6 (7.6–11.6)	1.5 (1.2–2.0)

PRP/whole blood was calculated for every single dog.

Table 2 Push-out data [median (range)]. *n* Final number of implants in each treatment group

Group	Ultimate shear strength (MPa)	Energy absorption (J/m^2)	Apparent stiffness (MPa/mm)
Fresh-frozen allograft, *n*=6	1.28 (0.65–2.59)	222 (91–598)	5.63 (1.9–10.7)
Processed allograft, *n*=7	1.20 (0.04–3.19)	213 (11–643)	5.03 (0.2–15.7)
Fresh-frozen allograft + PRP, *n*=7	1.13 (0.17–2.63)	223 (39–382)	4.35 (0.6–7.8)
Processed allograft + PRP, *n*=8	1.05 (0.09–1.99)	185 (21–368)	4.29 (0.2–9.7)

None of the differences in any of the parameters were statistically significant

Four implants were inserted too close to the joint space and were hence not surrounded by trabecular bone. These four implants were excluded from the study; the final number of implants in each treatment group is shown in Tables 2 and 3. Platelet and leucocyte counts were increased approximately eight- and nine-fold compared to venous blood (Table 1).

Processing of bone allograft markedly decreased all mechanical parameters, although not significantly (Table 2). PRP had little effect on fixation (Table 2).

Bone allograft and newly formed bone were stained green and were easily recognized in both zones in all four treatment groups. Bone allograft and newly formed bone could be distinguished by several histological characteristics. Newly formed bone is not remodeled after 3 weeks and is therefore woven bone in contrast to the lamellar structure and random orientation of bone allograft chips [9, 10]. Furthermore, bone allograft was recognizable by empty lacunae [9, 10] (Fig. 3). New bone was formed on the surface of the impacted graft chips. The thickness of the sections did not allow quantification of osteoblasts or osteoclasts. We found no signs of infection.

The implants grafted with fresh-frozen bone, with or without PRP, had up to 59% more bone ingrowth compared to implants treated with processed bone allograft (Table 3). Also, more newly formed bone was found in zone 1 in the groups grafted with fresh-frozen bone allograft in comparison with processed bone allograft. These findings were not statistically significant. Addition of PRP to the graft had only a minor influence on bone formation on the surface of the implant or new bone formation in the gap.

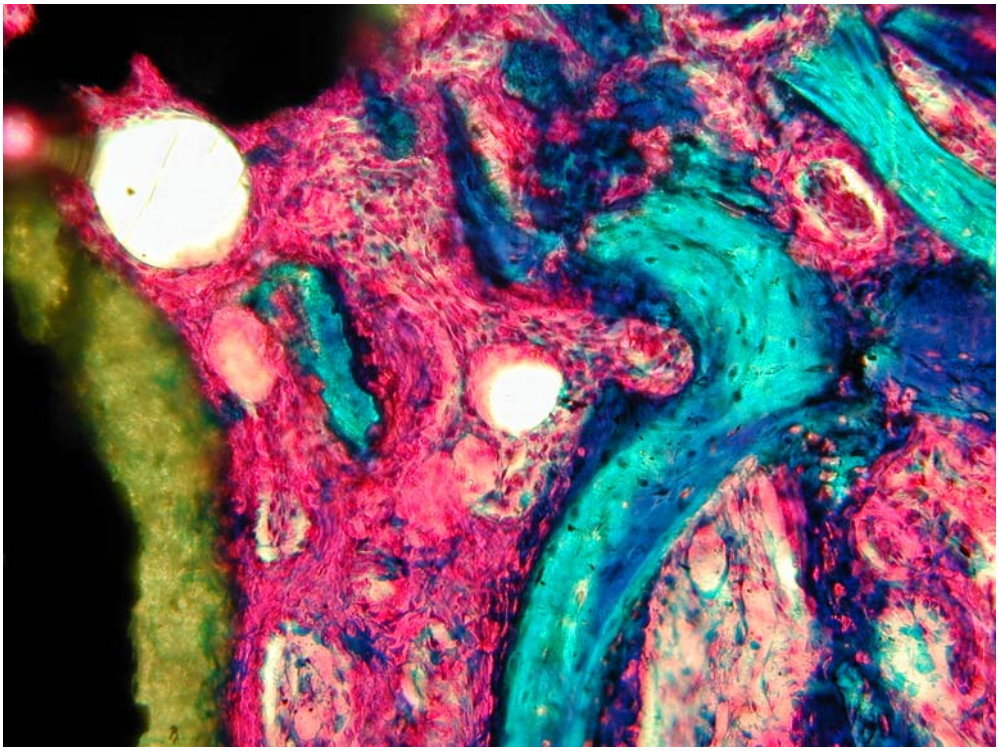

Fig. 3 Gap grafted with fresh-frozen bone allograft without platelet-rich plasma (PRP) (staining light green and basic fuscin, 100× magnification). A part of the titanium (Ti) implant is seen at the *lower left corner* coated with a thin layer of hydroxyapatite (HA). All mineralized tissue is stained green/blue. A bone allograft chip is localized at the *center* of the section. It can be distinguished from the newly formed bone by its lamellar structure, lighter color, and spindle-formed osteocytes. Newly formed bone is formed on the surface of the allograft. It is darker with the structure of woven bone.

Table 3 Bone ingrowth and volume fractions of woven bone, bone graft, and nonmineralized tissue in percentages of total area [median (range)]. *n* Final number of implants in each treatment group, *PRP* platelet-rich plasma

	Bone ingrowth	Woven bone		Graft		Nonmineralized tissue	
		Zone 1	Zone 2	Zone 1	Zone 2	Zone 1	Zone 2
Fresh-frozen allograft, *n*=6	35 (10–64)	13 (6–19)	18 (12–23)	32 (24–38)	24 (16–33)	55 (52–58)	58 (51–69)
Processed allograft, *n*=7	22 (8–46)	11 (6–20)	19 (9–28)	32 (27–39)	22 (14–29)	57 (50–56)	59 (50–67)
Fresh-frozen allograft + PRP, *n*=7	30 (12–49)	15 (8–21)	21 (17–29)	28 (16–38)	22 (11–31)	58 (51–64)	57 (41–66)
Processed allograft + PRP, *n*=7	23 (0–58)	11 (0–19)	25 (15–33)	29 (20–40)	22 (13–32)	59 (51–69)	52 (40–63)

None of the differences in any of the parameters were statistically significant

Discussion

Loosening of joint replacements is often associated with massive resorption of bone. The use of impacted bone allograft to reconstruct the bone bed on the femoral and acetabular side is now well described [12]. However, impacted graft is often not incorporated by bone [1], and massive subsidence of the femoral stem has been reported [6].

Bone allograft is being used fresh-frozen or processed. The main reasons for processing bone allograft is the risk of infections, immunological reactions, and handling. We processed the bone graft by sonication, ethanol lipid extraction, freeze-drying, and irradiation in accordance with clinical guidelines [3]. The influence of such processing of bone allograft on the biological and biomechanical properties is not clear. Growth factors contained in the graft are hypothesized to be liberated during remodeling of the graft and play an important role in bone incorporation [1]. Theoretically, bone-graft processing could inactivate the growth factors and thereby delay ingrowth of bone, but previous studies have not been conclusive. Aspenberg et al. [1] found only little change in bone incorporation of graft when growth factors were inactivated. In contrast, one study questioned the use of irradiated bone allograft around prostheses because of impaired biological activity [17].

Removal of cells and cellular debris by defatting and lavage might decrease the antigenicity of the graft and thereby decrease the host immunological reaction and possibly thereby increase bone ingrowth [21]. We found a decrease in bone formation and implant fixation in the groups grafted with processed bone allograft with or without PRP compared with fresh-frozen bone allograft. Unfortunately, the biological variation in this study was relatively large, and the differences were not statistically significant. Decreased biological activity would be in accordance with critical reports using processed bone allograft around hip arthroplasties [17]. One argument in favor of the use of freeze-dried bone allograft is that it can be stored at room temperature in appropriate shapes (e.g., granules, cubes). De Roeck and Drabu [4] preferred fresh-frozen bone allograft from freeze-dried bone allograft chips in terms of handling properties in the revision of hip arthroplasties.

Platelets are essential in fracture repair. Platelets are activated by collagen exposure as an immediate response to fracture, leading to fibrin clotting and platelet aggregation. Platelet α-granules contain growth factors of which PDGF, TGF-β, IGF, and epidermal growth factor (EGF) are stimulators of bone-forming cells. High levels of platelets in PRP seem to be correlated with a high level of TGF-β [23].

The use of PRP was first described by R.E. Marx who increased bone incorporation of autograft in mandibular defects in humans. In rabbits, PRP increases gap healing in combination with bovine cancellous bone in a calvarial defect model [18], and PRP accelerates bone incorporation of HA granules in bone chambers [18].

We found no effect of PRP, and since the activity of PRP was not tested in the present study, we cannot conclude if the negative result is due to failure in the processing of PRP or if a potential PRP stimulation of bone formation cannot be detected using our implant model. We prepared the concentrate according to previous studies, and we found a high level of platelets and leucocytes. Several studies have found a positive correlation between platelets and level of growth factors [5]. We reached an average platelet count of $1{,}884\times10^9$ platelets/l in PRP, which is more than the counts in our previous studies [8] and more than the level of 785×10^9 platelets/l in the Marx study. Marx used PRP mixed with bone graft to reconstruct large mandibular defects. In contrast, we applied PRP mixed with allograft in a relatively small gap in young dogs with good bone-healing potential. In the present study, we further optimized the gap-healing potential by coating the implants with HA, which is a very potent stimulator of gap healing [19, 20]. The border of the drill hole creates a large surface from which growth factors can leak and is a source of bone-forming cells. Under such optimal gap healing conditions, it might be difficult to further improve bone formation and fixation of allografted implants. We have recently shown that platelet concentrates prepared using a commercialized kit increase fixation of non-HA-coated implants in a similar model [8].

In conclusion, we have demonstrated a method of isolating PRP from a small volume of blood. This method proved to give an inexpensive uniform high level of platelets. However, we found no significant effect of PRP on gap healing or fixation of HA-coated implants in our experimental implant model. Since a number of commercial preparation kits are available but only limited studies proving the effect of PRP exist, more research should be done.

Acknowledgements The authors thank Biomet Inc. for delivering the implants. Jane Pauli and Annette Milton kindly assisted at the operations and prepared the sections. Thanks to Søren Ladefoged, Biochemical Department, Århus Kommune Hospital, for analyzing the blood samples. The study was financially supported by the Danish Rheumatism Association, the Danish Medical Research Council, Institute of Experimental Clinical Research, and the University of Århus.

In memory of Dr. Lai-Martine Schiøtz (1972–2000)

References

1. Aspenberg P, Tagil M, Kristensson C, Lidin S (1996) Bone graft proteins influence osteoconduction. A titanium chamber study in rats. Acta Orthop Scand 67:377–382
2. 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
3. Cornu O, Banse X, Docquier PL, Luyckx S, Delloye C (2000) Effect of freeze-drying and gamma irradiation on the mechanical properties of human cancellous bone. J Orthop Res 18:426–431
4. De Roeck NJ, Drabu KJ (2001) Impaction bone grafting using freeze-dried allograft in revision hip arthroplasty. J Arthroplast 16:201–206
5. Dugrillon A, Eichler H, Kern S, Kluter H (2002) Autologous concentrated platelet-rich plasma (cPRP) for local application in bone regeneration. Int J Oral Maxillofac Surg 31:615–619
6. Eldridge JD, Smith EJ, Hubble MJ, Whitehouse SL, Learmonth ID (1997) Massive early subsidence following femoral impaction grafting. J Arthroplast 12:535–540
7. Gotfredsen K, Budtz-Jørgensen E, Jensen LL (1989) Preparation and staining of sections containing titanium-implants. Stain Technol 64:121–127
8. Jensen TB, Bechtold J, Chen X, Elmengaard B, Soballe K (2003) Platelet concentrate improves fixation achieved with morselized bone allograft around non-cemented implants. Transactions 49th Annual Meeting Orthopaedic Research
9. Jensen TB, Rahbek O, Overgaard S, Soballe K (2004) Platelet rich plasma and fresh-frozen bone allograft as enhancement of implant fixation. An experimental study in dogs. J Orthop Res 22:653–658
10. Jensen TB, Overgaard S, Lind M, Rahbek O, Bunger C, Soballe K (2002) Osteogenic protein 1 device increases bone formation and bone graft resorption around cementless implants. Acta Orthop Scand 73:31–39
11. Lind M (1996) Growth factors: possible new clinical tools. A review. Acta Orthop Scand 67:407–417
12. Lind M, Krarup N, Mikkelsen S, Horlyck E (2002) Exchange impaction allografting for femoral revision hip arthroplasty: results in 87 cases after 3.6 years' follow-up. J Arthroplast 17:158–164
13. Kim ES, Park EJ, Choung PH (2001) Platelet concentration and its effect on bone formation in calvarial defects: an experimental study in rabbits. J Prosthet Dent 86:428–433
14. Marthy S, Richter M (1998) Human immunodeficiency virus activity in rib allografts. J Oral Maxillofac Surg 56:474–476
15. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR (1998) Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endo 85:638–646
16. Nelissen RG, Bauer TW, Weidenhielm LR, LeGolvan DP, Mikhail WE (1995) Revision hip arthroplasty with the use of cement and impaction grafting. Histological analysis of four cases. J Bone Joint Surg Am 77:412–422
17. Robinson DE, Lee MB, Smith EJ, Learmonth ID (2002) Femoral impaction grafting in revision hip arthroplasty with irradiated bone. J Arthroplast 17:834–840
18. Siebrecht MA, De Rooij PP, Arm DM, Olsson ML, Aspenberg P (2002) Platelet concentrate increases bone ingrowth into porous hydroxyapatite. Orthopedics 25:169–172
19. Soballe K, Hansen ES, Brockstedt Rasmussen H, Pedersen CM, Bunger C (1992) Bone graft incorporation around titanium-alloy- and hydroxyapatite-coated implants in dogs. Clin Orthop 274:282–293
20. Soballe K, Hansen ES, Brockstedt Rasmussen H, Bunger C (1993) Hydroxyapatite coating converts fibrous tissue to bone around loaded implants. J Bone Joint Surg Br 75:270–278
21. Thoren K, Aspenberg P, Thorngren KG (1995) Lipid extracted bank bone. Bone conductive and mechanical properties. Clin Orthop 311:232–246
22. Weibrich G, Kleis WK, Hafner G, Hitzler WE (2002) Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. J Craniomaxillofac Surg 30:97–102
23. Weibrich G, Kleis WK, Hafner G (2002) Growth factor levels in the platelet-rich plasma produced by 2 different methods: curasan-type PRP kit versus PCCS PRP system. Int J Oral Maxillofac Implants 17:184–190