Incorporation of devitalised autografts in dogs

P. Böhm¹, M. A. Scherer²

¹ Orthopädische Klinik und Poliklinik, Eberhard-Karls-Universität Tübingen, Tübingen, Germany

² Sektion Unfallchirurgie der Chirurgischen Klinik und Poliklinik der Technischen Universität München, Germany

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Summary. *Segments of the tibial diaphysis from 24 adult dogs were reimplanted after extracorporeal autoclaving or γ -irradiation. Fixation was by interlocking intramedullary nailing, or plating. The dogs were killed after 36 weeks and the tibias examined. Bony union was seen in 46 of the 48 contact areas. Microangiography and Tc-perfusion scans demonstrated complete revascularisation of all the grafts. Biomechanical tests showed defective union with increased bending and decreased stiffness compared to normal controls. Fluorescent microscopy confirmed restitution of Haversian systems and slow replacement of the graft by lamellar bone containing viable osteocytes.*

Résumé. *Des segments diaphysaires du tibia gauche de 24 chiens (Beagle) furent réimplantés après avoir subi un traitement par autoclavage ou gamma-irradiations. Des clous centro-médullaires verrouillés ou des plaques furent utilisés comme moyen d'ostéosynthèse. Après 36 semaines, les animaux furent sacrifiés. On constata une union osseuse sur 46 des 48 zones de jonction. Les microangiographies et les scintigraphies au Technétium montrèrent une complète revascularisation de toutes les greffes. Les tests mécaniques montraient des anomalies de la consolidation avec augmentation de la courbure et diminution de la rigidité comparés au contrôle. La microscopie en fluorescence a confirmé la reconstitution d'un système haversien et un remplacement partiel de la greffe par de l'os lamellaire contenant des ostéocytes vivants.*

Introduction

The use of devitalised bone autografts in man has been reported rarely [10, 11, 12, 19, 23, 29, 34]. In this study we investigated the bony incorporation and revitalisation of tibial autotransplants devitalised by autoclaving or γ -irradiation. The study was approved by the Bavarian Laender State Government.

Materials and methods

Twenty-five mm long diaphyseal segments with periosteum were resected from the left tibia in 25 adult beagle dogs (6 ± 1.8 years old, mean body weight 15.3 ± 2.6 kg).

The specimens were either autoclaved at 134 °C for 3 min ($n = 12$), or γ -irradiated with 5000 Gy of ^{60}Co ($n = 13$), and then reimplanted orthotopically. In each group half the autografts were stabilised by plating (AP+IP), and the other half by interlocking nails (AN+IN). Six dogs which underwent reamed interlocking nailing after a transverse tibial osteotomy (group FN) are included for comparison (Fig. 3, 4).

During the 36 weeks follow up of the 4 subgroups AN, AP, IN and IP, clinical examination, anteroposterior and lateral radiographs of the left leg, 3-phase skeletal scintigraphy, and polychrome sequential fluorescent labelling [26] (Fig. 1) were carried out at regular intervals. Group FN was only assessed by scintigraphy. Finally, all the dogs were killed and the internal fixation removed.

Technetium scintiscans

The dogs were given a bolus injection of 15 MBq Tc-99m-MDP/kg/bw. Quantitative evaluation was performed by the region of interest (ROI) method [3]. Routine regions were the diaphyseal region proximal and distal to the transplant, the transplant itself, the corresponding topographical regions of the healthy control leg as well as the tail root, working as background-ROI of normal bone turnover. The results are given in left-to-right relation with values of x as 0, 0.1,

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Reprint requests to: P. Böhm, Orthopädische Universitätsklinik, Hoppe-Seyler-Strasse 3, D-72076 Tübingen, Germany

Fig. 1. Postoperative management: *Cl*, clinical examination; *R*, plain radiographs; *Sc*, 3-phase skeletal scintigraphy; *PSL*, polychrome sequential labelling

Fig. 2a, b. Development of bony union in (a) proximal and (b) distal osteotomy. Y-axis in arbitrary units (maximum 4 points). AN, autoclaved + nailing; AP, autoclaved + plating; IN, irradiated + nailing; IP, irradiated + plating

0.2...1+n, where the value of 1.0 is equivalent to an equal radiotracer uptake of both the operated and unoperated hind limb.

Polychrome sequential fluorescent labelling

This was made with the sequence: tetracycline – alizarin complexone – calcein-green – xylenol-orange – tetracycline.

Fig. 3. Tc scan. ROI transplant. Course of left to right ratio of the perfusion phase during 36 weeks

Fig. 4. Tc scan. ROI transplant. Course of left to right ratio of the bone phase during 36 weeks

Biomechanical tests

Stiffness and the extent of bending between 5N and 25N were tested by a nondestructive 3-point bending test at the middle of the transplant and at the proximal and distal osteotomy sites respectively. The speed of the testing stamp of the TZZ-707 Wolpert[®]-universal testing machine (Zwick, Ulm, Germany) was adjusted to 5 mm/min. The maximum load was limited to 25 N. There was a stable position of the bone without additional fixation because of its almost triangular shape, and the specimens were tested in anteroposterior, medio-lateral and latero-medial directions. Each load cycle was repeated 3 times and the 4th curve evaluated. The tibia of the unoperated side was used for comparison.

Bending in mm was defined as the distance of the force transducer position at 5 N compared with 25 N. Stiffness was the tangent to the maximum slope of the force-elongation curve (N/mm).

Microangiography

This was carried out in one animal in each subgroup using Rhinelander and Baragy's technique [27]. Undecalcified ground sections and contact microradiographs of the 500 μ thick specimens as well as the decalcified histology were examined in the other 20 dogs.

Table 1. Biomechanics: bending

		Antero-posterior	Medio-lateral	Latero-medial
AN	p	312 ± 228	295 ± 154	263 ± 64
	t	453 ± 505	291 ± 244	590 ± 656
	d	236 ± 103	257 ± 145	219 ± 129
AP	p	376 ± 337	449 ± 230	228 ± 60
	t	278 ± 205	185 ± 94	371 ± 235
	d	368 ± 163	259 ± 208	229 ± 143
IN	p	201 ± 43	283 ± 153	176 ± 76
	t	229 ± 109	132 ± 40	243 ± 90
	d	197 ± 86	241 ± 151	198 ± 157
IP	p	323 ± 215	395 ± 155	353 ± 186
	t	157 ± 98	138 ± 100	266 ± 142
	d	253 ± 165	293 ± 273	141 ± 65

Relative values in per cent to the non operated control side. Indicated is the mean value ± standard deviation:

(Bending operated side/Bending control side) × 100 ± standard deviation [%]

p = proximal osteotomy, t = transplant, d = distal osteotomy

Table 2. Biomechanics: stiffness

		Antero-posterior	Medio-lateral	Latero-medial
AN	p	57 ± 51	32 ± 11	34 ± 13
	t	35 ± 26	39 ± 34	22 ± 16
	d	41 ± 12	35 ± 14	45 ± 22
AP	p	47 ± 20	31 ± 12	47 ± 14
	t	42 ± 16	55 ± 31	36 ± 16
	d	34 ± 12	62 ± 51	49 ± 21
IN	p	49 ± 12	31 ± 11	51 ± 20
	t	45 ± 13	57 ± 11	38 ± 11
	d	46 ± 16	47 ± 26	56 ± 39
IP	p	50 ± 15	34 ± 18	34 ± 12
	t	86 ± 55	67 ± 31	43 ± 24
	d	58 ± 33	48 ± 18	76 ± 52

Relative values in per cent to the non operated control side.

Indicated is the mean value ± standard deviation:

(Stiffness operated side/Stiffness control side) × 100 ± standard deviation [%]

p = proximal osteotomy, t = transplant, d = distal osteotomy

The time course of incorporation of the autotransplants was recorded by plain radiographs. The microstructural state was analysed by microradiography and histology in which polychrome sequential labelling enabled a temporal assignment of bone turnover and remodelling. Radiological scores were used for semiquantitative evaluation, as well as for descriptive assessment. Modified histological scores were based on the work of Heiple et al. [13]. Two representative sections of each osteotomy with adjacent bone and the transplant were assessed separately for histological evaluation.

Results

Complications

One dog in subgroup IN died after an uneventful postoperative course from an anaesthetic complication during scintigraphy at 8 weeks. No acute or chronic infection occurred.

Radiography

The semiquantitative evaluation of healing between host bone and transplant, divided into proximal and distal osteotomy, and into the 4 subgroups is shown in Fig. 2.

Scintigraphy

In the arterial phase of radionuclide perfusion the maximum activity in all 4 subgroups was around the 4th postoperative week, and then declined until the end of observation (Fig. 3). Normal perfusion was restored after 6 months at the latest. The control group with simulated transverse fractures (FN) was at its highest 2 weeks earlier and showed

a slower decline of radiotracer perfusion. Perfusion in the IN group was significantly higher ($P < 0.01$, two-tailed test) than those of IP, AN, AP and FN 4 weeks after operation. No other statistically significant differences were found during this period.

The late static bone phase (Fig. 4) was sensitive to bone remodelling. The tracer uptake was significantly higher than that of normal bone throughout the observation period. Every difference between the operated and unoperated side was significant at the 1% level. The time course was similar to the perfusion phase, whereas the maximum activity was between the 4th and 8th postoperative week in the IP, AP and FN groups, and between the 8th to 12th postoperative week in the IN and AN groups, respectively. From the 12th to 36th week, irradiated transplants fixed with nails had higher ROI ratios compared with those which were plated. The differences between IN compared with IP and AP were significant at the 5% level.

The relative and absolute radioactivity peaks (counts/pixel) were highest in the late static phase. The course after a simple osteotomy fixed with a reamed nail (FN) was comparable with that of irradiated autografts (IN).

Biomechanical tests

The results of tests for bending and stiffness are shown in Tables 1 and 2.

Fig. 5. Microangiograph of dog AN6; section through the middle of the transplant

Fig. 6. Microangiographic cross-section of dog IP3 showing that vascularisation of the cortex is ensured by a new nutrient artery

Fig. 7. Contact microradiograph of the proximal osteotomy of dog AP3 (arrow, line of osteotomy). The autoclaved transplant in the lower half is more dense than the host bone in the upper half. The medulla is filled with new cancellous trabeculae

Fig. 8. Contact microradiograph of the distal osteotomy of dog IN3 (arrow, line of osteotomy). The irradiated transplant in the upper half is a little more porotic and the transition from transplant to host bone can be identified easily. The new bony lamellae surrounding the nail are clearly visible

Fig. 9. Histology of a polychrome labelled ground section of the distal osteotomy in dog AP4 (arrow, line of osteotomy) showing direct healing. The transplant is in the upper half (original $\times 25$)

Fig. 10. Polarised light micrograph of the distal osteotomy in dog IN3 with the periosteum on the left, after 36 weeks in plater. The callus remodelling is complete (original $\times 5$)

Microangiography

Intramedullary vessels were demonstrated in the proximal and distal host bone between nail and endosteum in the nailing subgroups (AN and IN). The cross section of the middle of the transplant showed thick dorsal callus criss-crossed

by a dense poorly organised vascular network (Fig. 5).

Microangiography from the plating subgroups (AP and IP) showed restitution of the nutrient artery in the medullary cavity. In axial section, the vascular supply of the cortical bone can be seen to

be provided by this vessel (Fig. 6). A longitudinal section shows one of these small vessels penetrating the anteromedial cortex as far as the plate. Some resorption cavities were seen in the cortical bone.

Contact microradiography

The results seen in plain radiographs were confirmed. In specimens which had been autoclaved, the autograft was less porotic than the neighbouring bone in subgroups AP and AN (Fig. 7), but the irradiated autografts were more porotic than the host bone. In subgroups where plating was used, the osteotomy could only be recognised with difficulty as the density of autograft and host bone were almost the same.

After nailing the transition from host bone to transplant was identified much more easily. The nails in both groups, AN and IN, were surrounded by newly formed bony lamellae simulating an inner limiting lamella (secondary medullary cavity) (Fig. 8). The space between this lamella and the cortex was filled with cancellous bone.

Histology

The connection between transplant and host bone

AN. In 2 specimens the distal part of the autograft and host was bridged by connective tissue only. In the remaining contact areas (6 proximal and 4 distal), the junction was easy to find and was filled with new lamellar or woven bone. The arrangement of most osteons was at right angles to the longitudinal axis, although occasional osteons penetrated from compact bone into the gap, bridging it longitudinally. Most of the boundary was irregularly serrated as a result of osteoclastic and osteoblastic activity. Polychrome labelling between the 4th and 8th week showed the first deposits of fluorochrome near the proximal osteotomy. These deposits did not occur until the 8th or 12th week in distal osteotomies where callus was formed in the dorsilateral area where there was good soft tissue cover. The structure was spongy lamellar bone which was not yet lined up longitudinally. In most cases the proximal and distal callus extended beyond the osteotomy. The periosteal and endosteal callus covered the transplant and the bone near it. Callus formation began in the 4th week, but a temporary delay occurred distally from the 8th to 12th week and endosteally from the 12th to 24th week.

AP. All 12 osteotomies showed a good bony incorporation (Fig. 9). Especially in the area of the medial cortex direct bone healing predominated whereas laterally sometimes indirect bone healing could be seen too. The intimate dovetailing of autograft and host bed resulted from penetrating of osteoclasts and from the resorption lacunae having been filled with bone. In the area of the distal osteotomy these processes of incorporation lagged behind. The woven bone, which had bridged the osteotomy gap during the initial stage, was replaced by lamellar bone particularly on the medial side. Using fluorescence microscopy we found few tetracycline- (4 weeks) and with increasing frequency alizarin- (8 weeks) and calcein-bands (12 weeks) running at right angles to the longitudinal axis of the tibia. Fluorochromes having been integrated after the 12th week increasingly were deposited in osteons longitudinally running along the tibia and in osteons that grew bridging the osteotomy gap.

IN. There was close connection between the mostly lamellar bone between host and autograft with the new osteons at right angles to the longitudinal axis. At the proximal osteotomy, the Haversian canals showed advanced transformation and were orientated longitudinally so that it was almost impossible to recognise the line of osteotomy. Fluorescence microscopy showed the first colour bands proximally from the 8th week and distally at the 8th to 12th week, with only the latter running longitudinally. The callus was almost completely integrated. Polychrome labelling dated the callus formation from within the 1st 12 weeks.

PI. In this subgroup the bone healing was far advanced and there were many new osteons running longitudinally across the width of the compact bone over the junction of transplant and host.

Internal bony repair

AN. Intense internal bony repair was present in the host bed. All colours in fluorochrome labelling indicated that the transformation extended over a long time. The revitalisation of the transplant resulted from ingrowth of vessels from the adjoining host bed and from the intramedullary and periosteal systems. Concentric bone formation developed around the Haversian canals.

AP. Bony repair was similar to AN.

IN. A relatively high-turn over of both intracortical, as well as periosteal and endosteal, bone occurred in the host bed. The transplant

showed an inner remodelling and was incorporated by periosteal and endosteal new bone. There was a tendency to increased cortical cancellisation.

IP. Bone repair extending over the whole period of observation was seen in the host bed in all subgroups. Formation of viable bone was concentrated around the radially arranged vessels from the nutrient artery in the area of the transplant. Active repair was seen near the screw holes. All colour bands were present in polychrome labelling.

Discussion

Experimental model. There is almost unanimous agreement that the bony reactions found in experimental studies in dogs are more applicable to man than in any other species. The only significant difference is that the repair process takes twice as long in man [4]. There is good correlation between this and the metabolic activity index (MAI) which is 1.5 for dogs and 1.0 for man [33]. The appositional bone formation rate is 0.7–1.5 µm/day for man; 1.5–2.0 µm/day in dogs; 4.6–10.1 µm/day in rats, and 62.7–69.5 µm/day in rabbits [28].

After removal of 2.5 cm of an osteoperiosteal fragment of a dog's tibia, the gap will not be bridged and there will be nonunion in every case [20, 24].

Technique of osteosynthesis. Fixation with a plate was successful and the 12 autografts incorporated at all the contact areas. Bone healing between devitalised graft and living host bone occurs the same way as in fractures.

Interlocking nails were satisfactory, but reaming should be minimal. Vascularity and mechanical fixation are decisive factors in fracture healing [35], so the conditions for healing are less favourable when the bone is alive on only one side of the fracture. In our model the radiological, histological and biomechanical results showed slight superiority of plate fixation over intramedullary nailing, but this could not be confirmed statistically. In clinical practice the internal repair must last for years and, since during this time mechanical stability is reduced, we prefer not to remove the metal. In this case, the intramedullary nail has an advantage and is used by most authors for the tibia and femur [5, 7, 31, 32, 36].

Method of devitalisation. Sterilisation of bone fragments of the size used in our experiments is achieved by autoclaving for 2 min at 131 °C [15]. With a sterilisation dose of 5 kGy, the assurance

level is 1:61 659. For medical devices a safety margin of 1:1 000 000 is demanded at a recommended dose of 25 kGy [25].

Autoclaving is fast and simple, and 134 °C for 3 minutes ensures sterilisation of small bone fragments in clinical use and destroys all tumour cells. Stiffness and resistance to fracture are only slightly reduced [15].

Irradiation was more time consuming and we used a 2 stage surgical procedure because of the time needed for transport. All tumour cells are destroyed by 250 Gy [29, 34], but a dose of 25 kGy is necessary for sterilisation [25]. There are no changes in protein structure or mechanical properties of the graft when using up to 10 kGy [29], and no measurable deterioration of these properties until a dose of 25 kGy is reached [9].

Incorporation. Calculating statistics from histological slides is vague as no normal distribution can be expected because of the relatively small number of dogs in each group ($n = 6$). Another series of experiments has shown that even if we had used 30 animals in each group, a normal distribution would not be obtained since not even the standard deviation of the mean changed when we increased the numbers from 10 to 78. Statistics based on semiquantitative analysis with arbitrary units and without proven reliability of more than 95% is uncertain and possibly invalid.

Bony incorporation was achieved in all contact areas after irradiation and in all but two after autoclaving. Nonunion has been reported in 8 out of 14 cases of replantation of a 20 mm segment of the ulna in rabbits [16]. Others have used treated and untreated allografts, and autoclaved autogenous cortical segments in adult mongrel dogs and found incorporation in 2 out of 18 contact areas with intramedullary nailing [14]. These authors were less optimistic about autoclaved grafts in spite of encouraging clinical results. In their control group nonunion occurred in 6 out of 18 dogs, so the method of fixation might be a factor. Polychrome sequential labelling demonstrated gap healing with primary bone formation orientated at right angles to the longitudinal axis.

In our model we expect bony incorporation under favourable conditions between the 8th and 12th week. In cases of indirect healing the period for consolidation is 12 to 24 weeks. This confirms the work of Ku et al. who reported delayed union in the femur of a sheep at the 24th week after replanting a 4 cm autoclaved segment with intramedullary nailing [17].

The perfusion phase of 3-phase skeletal scintigraphy correlates with the circulation in the operated limb [22], and uptake curves in our 4 subgroups are similar to the curve after a nailed transverse osteotomy (Fig. 3). The late static phase correlates with bone remodelling, and the living bone of our control group (FN) has the highest uptake in keeping with theoretical considerations. The order of ranking in the 4 subgroups is $IN > AN > IP > AP$, and it seems that the method of fixation, especially with intramedullary reaming, is of greater influence than the kind of devitalisation shown by scintigraphy. Bony repair seems to be more active after irradiation compared with autoclaving. This must be interpreted cautiously since a thin layer of new bone can produce scintigraphic activity, even when the transplant is not viable [1].

Revitalisation of dead grafts by revascularisation has been demonstrated by microangiography, and the restitution of the Haversian system by fluorescence microscopy. We conclude that the grafts were revascularised to a large extent by 36 weeks, but the substitution of dead bone by living osteons took longer. Nevertheless, the results of biopsies in clinical cases which showed replacement of grafts by lamellar bone were confirmed [12, 19]. Our results showed that repair was a little faster in irradiated compared with autoclaved autografts. Biopsies of allogeneic or autoclaved pelvic grafts have shown that creeping substitution starts at the junctions and later reaches the centre [12]. Connection to living bone is essential for revitalisation. Freiberg et al. could not find any viable osteocytes 10 years after autoclaving and replantation of a complete humerus [8].

Biomechanics. The high degree of standard deviation must be taken into account. Apart from incorporation of the reimplanted bone, adequate long term stability is important for clinical success. There will be reduced loadbearing capacity even if there is good incorporation. Irradiation is superior to autoclaving and plating to nailing in relation to the bending and stiffness at the middle of the graft.

Measured values are influenced by the primary weakening of the graft during revascularisation, the rigidity of internal fixation [18, 30] and the biomechanical changes which occur during remodelling. Enneking et al. found that there was minimal stability between 1.5 and 6 months after transplant of untreated autogenous grafts, and stability was almost achieved after one year [6]. Ku et al. found that in autoclaved transplants in

sheep, there was 50% stiffness after 12 weeks compared to the control, but after one year there was no difference between the two sides [17].

The temporary loss of stability is confirmed clinically and fractures were found in 16% of massive allografts, most occurring between the 2nd and 3rd postoperative year, and none after 4 years [2]. Fractures in this type of graft have been reported in 4 out of 6 patients followed for 22 to 36 years, and all occurred during the first 2 years [21].

Assessment of the radiological course, the histological results and biomechanical observations suggest that irradiation provided better incorporation and stability than autoclaving, but the results in all 4 subgroups were encouraging. Re-implantation of extracorporeally irradiated or autoclaved bone after excision of a tumour can be considered an alternative method to biological reconstruction with autogenous or allogeneic transplants.

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References

1. Berggren A, Weiland AJ, Östrop LT (1982) Bone scintigraphy in evaluating the viability of composite bone grafts revascularized by microvascular anastomoses, conventional autogenous bone grafts, and free non-revascularized periosteal grafts. *J Bone Joint Surg [Am]* 64: 799–809
2. Berrey BH, Lord CF, Gebhardt MC, Mankin HJ (1990) Fractures of allografts. *J Bone Joint Surg [Am]* 72: 825–833
3. Büll U, Keyl W, Meister P, Pfeifer JP, Hartel P (1981) Wertigkeit der "Region of Interest"-Technik in der skelettszintigraphischen Diagnostik primärer Knochentumoren. *Radiologe* 21: 46–51
4. Burchardt H (1989) Biology of cortical bone graft incorporation. In: Aebi M, Regazzoni P (eds) *Bone transplantation*. Springer, Berlin, pp 23–28
5. Campanacci M, Costa P (1979) Total resection of distal femur or proximal tibia for bone tumours. *J Bone Joint Surg [Br]* 61: 455–463
6. Enneking WF, Burchardt H, Puhl JJ, Piotrowsky G (1975) Physical and biological aspects of repair in dog cortical bone transplants. *J Bone Joint Surg [Am]* 67: 237–252
7. Enneking WF, Shirley PD (1977) Resection-arthrodesis for malignant and potentially malignant lesions about the knee using an intramedullary rod and local bone grafts. *J Bone Joint Surg [Am]* 59: 223–235
8. Freiberg AA, Saltzman C, Smith WS (1992) Replantation of an autoclaved, autogenous humerus in a patient who had chondrosarcoma. *J Bone Joint Surg [Am]* 74: 438–439
9. Früh HJ, Voggenreiter G, Ascherl R, Scherer MA, Siebels W (1990) Zur Biomechanik autoklavierter, bestrahlter und kältekonservierter Corticalis. *Hefte Unfallheilkd* 212: 681–682

10. Gallie WE (1918) The use of boiled bone in operative surgery. *Am J Orthop Surg* 16: 373–383
11. Harding RL (1957) Replantation of the mandible in cancer surgery. *Plast Reconstr Surg* 19: 373–383
12. Harrington KD (1992) The use of hemipelvic allografts or autoclaved grafts for reconstruction after wide resections of malignant tumors of the pelvis. *J Bone Joint Surg [Am]* 74: 331–341
13. Heiple KG, Chase SW, Herndon CH (1963) A comparative study of the healing process following different types of bone transplantation. *J Bone Joint Surg [Am]* 45: 1593–1612
14. Johnston JO, Harries TJ, Alexander CE, Alexander AH (1983) Limb salvage procedure for neoplasms about the knee by spherocentric total knee arthroplasty and autogenous autoclaved bone grafting. *Clin Orthop* 181: 137–145
15. Köhler P, Kreicbergs A, Strömberg L (1986) Physical properties of autoclaved bone. *Acta Orthop Scand* 57: 141–145
16. Kreicbergs A, Köhler P (1989) Reconstruction of large diaphyseal defects by autoclaved reimplanted bone: an experimental study in the rabbit. In: Aebi M, Regazzoni P (eds) *Bone transplantation*. Springer, Berlin, pp 198–208
17. Ku JL, Smith PA, Goldstein SA, Matthews LS (1985) An experimental investigation of the fate of autogenous autoclaved bone grafts. *Orthop Trans* 9: 340
18. Laftman P, Sigurdsson F, Strömberg L (1980) Recovery of diaphyseal bone strength after rigid internal plate fixation. *Acta Orthop Scand* 51: 215–222
19. Lauritzen C, Alberius P, Santanelli F, Vällfors B, Lilja J, Stephensen H (1991) Repositioning of craniofacial tumorous bone after autoclaving. *Scand J Plast Reconstr Hand Surg* 25: 161–165
20. Limeira dos Santos F, Volpon JB (1983) Experimental nonunion in dogs. *Clin Orthop* 187: 260–271
21. Musculo DL, Petracchi LJ, Ayerza MA, Calabrese ME (1992) Massive femoral allografts followed for 22 to 36 years. *J Bone Joint Surg [Br]* 74: 887–892
22. Nutton RW, Fitzgerald RH, Kelly PJ (1985) Early dynamic bone-imaging as an indicator of osseous blood flow and factors affecting the uptake of ^{99m}Tc hydroxymethylene diphosphonate in healing bone. *J Bone Joint Surg [Am]* 67: 763–770
23. Orell S (1937) Surgical bone grafting with “os purum”, “os novum”, and “boiled bone”. *J Bone Joint Surg* 19: 873–885
24. Paterson DC, Hillier TM, Carter RF, Ludbrook J, Maxwell GM, Savage JP (1977) Experimental delayed union of the dog tibia and its use in assessing the effect of an electrical bone growth stimulator. *Clin Orthop* 128: 340–350
25. Pfeiffer M (1991) Gamma-Sterilisation von medizinischen Einmalartikeln mit Dosen von 25 kGy aufgrund von Bioburden-Untersuchungen und unter Berücksichtigung extremer D10-Werte. *Hyg Med* 16: 245–252
26. Rahn BA, Perren SM (1975) Die mehrfarbige Fluoreszenzmarkierung des Knochenbaus. *Chem Rdsch* 28: 12–15
27. Rhinelander FW, Baragry RA (1962) Microangiography in bone healing. *J Bone Joint Surg [Am]* 44: 1273–1298
28. Rueger JM (1992) Knochenersatzmittel. *Hefte Unfallheilkd* 213: 1–298
29. Spira E, Brenner HT, Lubin E (1970) Extracorporeal irradiation of malignant bone tumors. In: Chapchal G (ed) *Operative treatment of bone tumors*. Thieme, Stuttgart, pp 136–140
30. Strömberg L (1975) Diaphyseal bone in rigid internal plate fixation. *Acta Chir Scand [Suppl 465]*: 1–34
31. Thompson RC, Pickvance EA, Garry D (1993) Fractures in large-segment allografts. *J Bone Joint Surg [Am]* 75: 1663–1673
32. Tomeno B, Gerber C (1987) Resection-reconstruction for tumours of the shafts of long bones. *French J Orthop Surg* 1: 69–74
33. Urist MR (1989) Introduction to update on osteochondral allograft surgery. In: Aebi M, Regazzoni P (eds) *Bone transplantation*. Springer, Berlin, pp 1–6
34. Uyttendaele D, de Schryver A, Claessens H, Roels H, Berkvens P, Mondelaers W (1988) Limb conservation in primary bone tumours by resection, extracorporeal irradiation and re-implantation. *J Bone Joint Surg [Br]* 70: 348–353
35. Willenegger H (1975) Verplattung und Marknagelung bei Femur- und Tibiaschaftfrakturen. *Chirurg* 46: 144–151
36. Wilson PD, Lance EM (1965) Surgical reconstruction of the skeleton following segmental resection for bone tumors. *J Bone Joint Surg* 47: 1629–1656