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# Influence of Freeze-Drying and Irradiation on Mechanical Properties of Human Cancellous Bone: Application to Impaction Bone Grafting

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## 1. Introduction

The use of a solid bone graft to restore bone stock and insure implant stability in hip revision surgery was introduced by Harris et al. (1). Better results have been obtained on the femoral site than on the acetabulum where high failure rate has been reported at ten years (2-7). Progressively, a new technique for restoring the acetabulum emerged with the concept of bone impaction which was introduced by Slooff et al. and later extended to the femur by Ling et al. (8-9). The technique consists in impacting bone chips with a phantom into the contained femoral or acetabular defect to produce a layer of tightly impacted bone where an implant shall be inserted with the cement being pressurized into the graft during cementation. Clinical results of impaction bone grafting techniques were largely improved with re-revision rates comparable to those observed after primary arthroplasty (10-12). Acetabular reconstructions were described as requiring between one and three femoral heads (12) and femoral impactions two or more, based on the preoperative bone loss (13-14).

As bone impaction became a recognised modality for bone reconstruction, the demand for bone allograft sharply increased. Consequently, an existing shortfall in the supply of banked bone was predicted to increase (15-16). As the impaction technique had been set up with frozen material, most bone banks were facing difficulties to provide frozen femoral heads (17). The increase in the number of hip arthroplasties did not mirror a concurrent increase in banked femoral heads. Indeed, the rate of rejection remained high (16) whereas the formal 6-month visit to get out the quarantine was difficult to obtained.

Concerns were raised about the possibility of an occult pathology into a femoral head, which could not have been identified through careful history and when different authors reported an incidence of 5 to 8% (18-19). Bacterial contamination rate reported with cadaver bone harvesting was another concern that limited supply from another source of fresh frozen bone (20).

Bone processing which allows a complete removal of bone marrow and cell debris from the bone and the machining of the material represented a potential solution to overcome these problems. However, no study had been reported comparing the mechanical stability of a

femur and acetabulum restored with either impacted frozen bone morsels or freeze-dried ones. The bone marrow content was considered to be important for the graft stickiness which influenced the biological and mechanical properties in impaction bone grafting (21). Although each separate step of the bone process did not appear to influence the bone strength (22-27), surgeons reported that freeze-dried bone was brittle and hence, unsuitable for being fixed and trimmed during surgery (28). However, the cumulative effects of every applied treatment were known to impact the mechanical properties of the musculo-skeletal tissue (29-30).

This chapter will cover the influences of various parameters (bone processing, freeze-drying, irradiation, processing sequence and temperature during irradiation) on the mechanical properties of cancellous bone. Mechanical damage due to irradiation will be related to damage of the collagen protein. Benefits of defatting, freeze-drying and irradiation in terms of osteoconductivity and tissue safety will be further discussed.

Application of processed freeze-dried irradiated bone to impaction bone grafting technique will be considered. The embrittlement theory and the influence of particle sizes will be presented to explain how processed bone is suitable to meet the mechanical demand of hip revision surgery. Results will be discussed and compared in more realistic surgical situations by observing implant stability after frozen or freeze-dried irradiated bone impaction. Finally, bone graft remodelling will be discussed.

## 2. Influence of various parameters on cancellous bone: Bone processing, freeze-drying, irradiation, processing sequence and temperature during irradiation

### 2.1 Mechanical effects of drying, freeze-drying and defatting

The effect of drying and rewetting on the mechanical properties of cortical bone was thought to be negligible because changes of the mechanical properties were very limited and considered as insignificant (31). Prolonged storage of bone in frozen state or in ethanol solution did not change the bone stiffness of trabecular bone, and neither did several thawing and refreezing sequences (32). Defatting combined with dehydration made the bone stiffer and brittle (32). The importance of re-hydration of a bone that has been dried was further emphasized by Conrad et al., as non rehydrated dried bones appeared to be both stronger and stiffer than their rehydrated counterparts (33). After 24 hours rehydration, freeze-dried grafts compared with frozen grafts showed no significant difference in mean compressive strength. An average gain of 40 % of the compressive strength and stiffness was recovered after one-hour rehydration in vacuo. The same observation was done by Bright and Burchardt on cortical bone (23). Complete restorations of the mechanical parameters after rehydration were also reported by Pelker et al. and Thoren et al. (24, 27). These authors did not find a significant difference in the compressive strength of freeze-dried rehydrated bone compared with normal bone in a rat vertebral model (24) and did not observe difference in the biomechanical properties of rehydrated bone after lipid extraction with chloroform methanol (27).

In our experiments, defatting and freeze-drying caused just a slight reduction in the ultimate compressive strength and stiffness but did not affect the work to failure, due to a higher ductility (34). In contrast to the observations of Bright and Burstein and Conrad et al.

works (33, 35) who noted that 24h were required for regaining the natural mechanical properties of the bone, a short 30-minute period of rehydration was enough to make bone more resilient.

Slight influence of physical or chemical defatting of cancellous bone grafts was recently confirmed (36-37). Other authors investigated biomechanical properties of the cortical and trabecular bone after high pressure lavage. Young's modulus and ultimate strength did not decrease after exposure to 300 MPa. After pressure treatment at 600 MPa, Young's modulus and ultimate strength respectively remained almost unchanged in trabecular bone and were reduced about 15% in cortical bone (38).

## 2.2 Mechanical effects of irradiation and sequences of freeze-drying and irradiation

### 2.2.1 Irradiation of a frozen bone

Gamma irradiation at a dose of 25 kGy has no apparent detrimental effect on cancellous bone strength. The mean values obtained in our experiments were within the range of values commonly observed for human bone that has been exposed to as high as 50 kGy (39). Anderson et al. reported earlier a 60% reduction of compressive strength and modulus for doses at or above 60 kGy (40). Their data were in agreement with our observations that processed frozen irradiated bone under dry ice did not show any detrimental effect after a 30 kGy irradiation.

### 2.2.2 Irradiation at room temperature of a freeze-dried bone

However at a 25 kGy dose at room temperature, alteration in the mechanical properties of cortical bone in compression occurred in the plastic modulus whereas the elastic domain remained unchanged. The capacity to absorb work before failure was also decreased in a dose-dependent manner (41-42). Similarly torque resistance of the frozen bone was greatly impaired with gamma-irradiation at a dose of 25kGy (43).

Our data for freeze-dried irradiated at room temperature cancellous bone are similar to the observation from Currey et al. and Hamer et al. (34, 41-42). The quantification of the post yield parameters showed that irradiation of freeze-dried cancellous bone at 25 kGy and at room temperature mainly reduced the capacity for energy absorption by shrinking the post-yield strain. Whether bone brittleness was due to irradiation on freeze-dried bone alone or temperature during irradiation or to a synergetic effect of the freeze-drying-irradiation process could not be yet assessed. Therefore, an inverted sequence of the freeze-drying-irradiation process and irradiation under dry ice was also examined.

### 2.2.3 Sequence of order and irradiation under dry ice

Performing freeze-drying either before or after irradiation under dry ice decreased the ultimate stress from 30% and the work to failure from 40% and impaired the results obtained with irradiation or freeze-drying separately. Stiffness was more preserved when freeze-drying preceded irradiation. The plastic domain of the strain-stress curve was more adversely affected by the usual freeze-drying-irradiation at room temperature sequence. Performing freeze-drying after irradiation allowed strain preservation but work to failure was decreased due to the stiffness and stress drops (Figure 1).
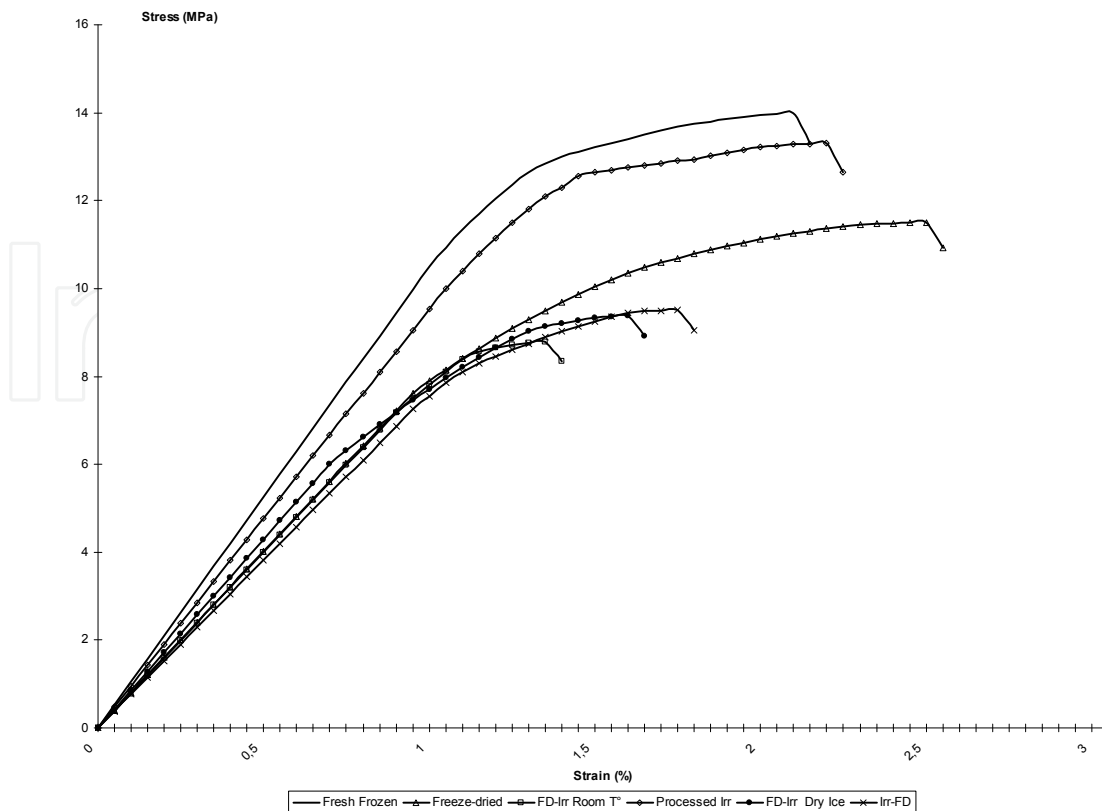
Compressive mechanical properties of cancellous bone are not influenced by irradiation under dry ice and supported moderate changes with freeze-drying. Negative synergetic effects of combined freeze-drying-irradiation processes are observed whatever the temperature during irradiation cycle. Irradiation cycle was performed within 3h00 at a 25 kGy dose rate. The curves were drawn proportionally to the observed mean values.

Fig. 1. Comparison of typical stress-strain curves. Strain-stress curves observed after freeze-drying, irradiation and sequence of both at two different temperatures.

Negative cumulative effect of freeze-drying and irradiation was already noted by Bright et al. and Triantafyllou et al. on cortical bone (25, 44). Preserving freeze-dried graft under dry ice during irradiation limited the damage compare to the same sequence at room temperature. These observations were consistent with the report of Hamer et al. (45), who found that cortical bone irradiated at –78°C was less brittle and had less collagen damage than when irradiated at room temperature.

## 2.3 Irradiation and collagen

Bright and Burchardt considered that a bone that has been freeze-dried and irradiated resembled to bone from old patient in term of mechanics. They thought that alterations were due to changes in the bone collagen cross-linking (23). Significant decrease in hydroxypyridinium cross-link density was reported after irradiation of bone tendon bone patellar allograft with significant correlation of dose dependant reduction of modulus properties (46). It was further suggested that gamma radiation might have less effect on the collagen structure in older bone because there were fewer reductible cross-links than in younger one (47).

Adding glucose, which in theory can initiate cross-link formation in collagen during exposure to gamma-irradiation, allowed collagen films containing glucose to have significantly greater mechanical properties and resistance to enzymatic degradation compared with controls. Nevertheless, gel electrophoresis showed that glucose did not prevent peptide fragmentation and therefore, the higher strength and stability in glucose-incorporated collagen films might be due to glucose-derived cross-links (48). Thiourea has been selected as a free radical scavenger and demonstrated a positive effect on the fracture energy of thiourea treated-irradiated bones than those of the irradiated bones. Irradiated specimens did not exhibit a noteworthy amount of intact alpha-chains whereas those irradiated in the presence of thiourea demonstrated intact alpha-chains. The damage occurred through the cleavage of the collagen backbone (49).

Drózdz et al. found a significant decrease in total collagen content resulting from the reduction of salt-soluble and acid-soluble collagen fractions (50). He estimated that an increased content of insoluble collagen fraction may confirm the opinion about stimulative gamma-rays influence upon cross-links formation. His observations were confirmed by Nguyen et al. who reported that irradiation caused release of free radicals resulting from radiolysis of water molecules and inducing cross-linking reactions in collagen molecules in wet specimens and split polypeptide chains (51). This hypothesis of the damaged first-order structure of the collagen macromolecule was also supported by Marzec et al. (52).

Differences in the mechanical behaviour after the different freeze-drying-irradiation-temperature sequences may be explained by the variation in active oxygen free radicals formation due to ionizing radiation. Free radicals are obtained by water radiolyis and their ability to move and interact with the material may be impaired when this one is frozen (53). The increased damages observed in absence of free water in pre-dried specimen may be due to direct damages to the proteins by irradiation, suggesting a higher sensitivity of freeze-dried proteins to irradiation than hydrated ones. This is supported by the observation of better osteoinductive properties of demineralised powder when irradiated in the hydrated frozen state (54). Collagen degradation by irradiation may account for the accelerate graft remodelling (54-55).

The good compressive mechanical performance of processed frozen irradiated cancellous bone shall be considered cautiously in regard with potential collagen damage. The impairment of the mechanical function of gamma radiation sterilized cortical allografts is even worse in fatigue and may increase the risk of fracture (47, 49, 56).

## 2.4 Osteoconductivity of defatted bone

Extraction of lipids from cancellous bone before implantation increased the ingrowth of cells from the host enhancing the osteoconductivity of the bone (57). In this situation, the graft provided the template to guide the repairing tissue. Along with the increased new bone formation, there was a concomitant decrease of the grafted bone that led to a net increase of new bone when bone was defatted before implantation (27). This means that the grafted bone is progressively removed as a result of osteoclastic action and new bone from the host is deposited into the graft. This process of bone removal and new bone deposited has been called creeping-substitution. The amount of unresorbed graft remnant was higher in the unprocessed bone grafts than in the washed ones whether or not subsequently irradiated (58). This observation is consistent with an accelerate bone remodelling after irradiation.

Another argument for defatting the bone before implantation is that the removal of fat will avoid the peroxidation of lipids during radiation sterilisation as reported by Moreau et al (59). They further demonstrated that peroxidated lipids had a cytotoxic effect on cultured cells. Peroxidation of marrow fat was further incriminated in increasing apoptosis of osteoblasts and decreasing activity of osteoclasts when they were cultured onto irradiated bone slices (51). Finally, when processing was not performed in an aseptic manner, bacterial by-products can persist after irradiation and induce inflammatory bone resorption following macrophage activation (51).

## 2.5 Tissue safety: Freeze-drying, irradiation and processing

### 2.5.1 Freeze-drying

Lyophilisation of tissues is usually performed without cryoprotective agent and consequently there is no cell survival in a freeze-dried tissue. The finding that only recipients of frozen bone from an infected seronegative donor contracted human immunodeficiency virus has led to speculation that freeze-drying may render a retroviral-infected tissue non infectious. However, it has been demonstrated in a feline-leukemia-virus infected allograft model that freeze-drying did not inactivate retrovirus (60).

### 2.5.2 Irradiation

While Campbell et al. firstly reported retrovirus inactivation with a standard 25 kGy dose in a HTLV-IIIB virus infected cortical allograft model (61), he pointed out that the virus was a relatively radio-resistant organism, a property common to most viruses. This irradiation resistance was recognized by many authors who estimated that irradiation at 25 kGy did not appear to be effective enough for HIV virus (62-64). Campbell et al. noted that an irradiation dose required to inactivate the HIV bioburden in allograft bone should be 35 kGy and the irradiation dose required to achieve a sterility assurance level of $10^{-6}$ was 89 kGy (65). If irradiation is applied to a frozen hydrated specimen, it may be beneficial from a mechanical and biological point of view, but sterilizing effect may be lowered. It has been shown that HIV inactivation was decreased when irradiation was performed at low temperature on frozen plasma (66).

The radiosensitivity of hepatitis viruses is higher and clinical data suggest that hepatitis C-contaminated tissues did not transmit the virus after irradiation (67, 68). While high inactivation rate have been achieved with 50 kGy doses in virus infected bone allografts model (69, 70), it is actually concluded that gamma irradiation should be disregarded as a significant isolated virus inactivation method for bone allografts.

Prions are strongly resistant to radiation (71- 72) and therefore irradiation is unable to inactivate this pathogenic agent.

A standard 25 kGy irradiation is appropriate for bacterial sterilisation when a bio-burden control or a process validation have been performed. We have reported 7 to 9 logarithms bacterial reductions after 25 kGy irradiation of highly contaminated cancellous bone blocks (73). Analysis of Clostridium sordellii inactivation kinetics indicated that a 16 log10 reduction was obtained after 50 kGy (70). Contamination during bone preparation shall be strongly limited to allow sterility assurance level.

### 2.5.3 Processing

Processes based on multiple steps of inactivating treatments offer a cumulative effect and a striking reduction of the risk of disease transmission. Steps may be chosen on their ability to specifically inactivate pathogenic agents. Pulse lavage decontaminates tissue from bacterial microorganisms with one decimal reduction (74-75), while virus elimination was also reported after mechanical lavage of bone (76). Demineralization process inactivated infectious retrovirus in infected cortical bone, thereby preventing disease transmission (76-77).

Detergents are able to remote or inactivate coated viruses (78), while sodium hydroxide and sodium hypochlorite are effective against transmissible spongiform encephalopathy agent (79). Hydrogen peroxide produces free radicals and is effective against viruses and bacteria. Hydrogen peroxide and prion inactivating steps adopted in our bank (two steps out of twelve) have been validated with five representative or inactivating-agent-resistant viruses in a cancellous bone blocks model. Cumulative seven logarithm reductions have been obtained for all tested viruses. Similar viral inactivation rates were obtained with a multiple step process by Fages et al. (80).

No bacterial growth were observed after each step of the chemical process developed by our bank, while largely contaminated bone blocks with pathogenic, sporulated and environment resistant microorganisms were processed (73).

## 3. Mechanical consideration in impaction bone grafting

### 3.1 Changes in stiffness and compactness during impaction

### 3.1.1 Embrittlement theory

Freeze-drying and irradiation at room temperature make cancellous bone brittle. How can a softer material give a stiffer reconstruction? In our experiments, freeze-dried irradiated bone appeared to get impacted faster than the frozen control whatever the particle size (81-83). During an impaction bone grafting procedure, the stress is applied at such high speed that the flow of liquids may play an important role (84) and the replacement of bone marrow by saline in the processed bone may accelerate the grafts compaction (85). The faster reduction in height observed in our second experiments with the processed allografts series (defatted, defatted and freeze-dried, defatted and freeze-dried irradiated) might account for a rapid expulsion of liquid but stiffness and bone density increased faster and to a higher value in the irradiated group (82). The embrittlement due to the freeze-drying-irradiation sequence might enhance the compaction rate while the higher ductility of the freeze-dried but non irradiated bone reduced brittleness and might account for the lower compaction, stiffness and density obtained with this material.

Freeze-dried irradiated large particles were stiffer after 30 impactions than any other morsels. Nevertheless, these series showed a reduction of their stiffness for higher impaction rate and tended to the same stiffness as freeze dried small particles. Under significant loading, these trabeculae might fail with a fracture that loosened the particle interlock. Such loosening explains the stiffness reduction of the large freeze-dried and irradiated particles. This was supported by the loss of height and the final density of these series which are comparable to those from small freeze-dried particles. Structurally damaged cancellous bone is known to have a much lower elastic modulus (86). The preservation of the plastic

mechanical properties as well as the presence of bone marrow in the frozen series may encounter of the lower compactness (85, 87-89) and the absence of collapsing in our models. The theory of bone embrittlement was further supported as compaction was faster when the grafts were morselised twice.

The mechanical properties of cancellous bone have been shown to be related to its apparent density, which depends on the porosity (90). Impacting freeze-dried irradiated bone created a layer that had a higher density and therefore a higher modulus, throughout the relationship between density and material properties cannot be fully applied to morselised bone, as the graft no longer has structural continuity (91).

### 3.1.2 Particle size influence

Particles sizing may also influence mechanical strength. For optimum shear strength, particles aggregate requires a mix of sizes represented by a logarithmic curve (92). While, none of the bone mills will produce an ideal profile, the particle size profile is larger when bigger particles are produced. In the clinical setting, an increase in the range size of particles has been obtained by putting bone through two different sizes of graters or passing some of the large graft morsels through the same mill twice.

3.1.2.1 The interlocking effect

Experimentally, we noted that preparation of well graded graft through a 1 mm beater mill (Retsch Cross beater mill SK100, Retsch GmbH, Haan, Germany) produced grafting material that was almost fluid. In our femoral model, these frozen particles did perform differently from those obtained with a rongeur, but not from those obtained with the Noviomagus bone mill. In the acetabular model, the particles were mechanically inferior in compaction and shear. These millimetric particles obtained from the Retsch mill was comparable to a fine powder, and filled a lower volume when placed in the impactor and showed higher density after few impactions but stiffness did not increase comparatively and remained lower than those from centimetric large particles. As suggested by authors, fresh frozen large particles got a higher stiffness than smaller morsels during impaction (92-95). This was due to the small size of the bone chips that did not allow an interlocking effect (96). The internal porosity of each morsel allowed deformation and causes them to interlock with each other during impaction (93).

This was coherent with our observation of an improved shear resistance of large particles. In our hands, acetabular reconstruction with ring reinforcement has been performed without significant complication when large particles obtained with a rongeur were used while some hardware failures have been observed with smaller morsels obtained with small rasps bone mills. These clinical observations found their explanation in the lower shear properties of the small particles.

3.1.2.2 The role of fluids

From the soil mechanic theory, it is known that the mechanical strength of a mixture is reduced when too much fluid is present with no drainage possibility, similarly to quicksand (97). The release of excessive fat and marrow that is captured in the closed system may prevent the compactive effort (87). The recoil of smaller bone chips was also significantly higher and increased after impaction with higher force than those from larger chips (95, 98).

This might explain why clinicians recommended larger particles rather than slurry (99). The advantage of large morsels on small ones might be tight to the preservation of a trabecular structure and to fat and marrow retention in the interstices (96).

Removing excessive and lubricating fluid of the graft material improved overall graft strength (100-101). While reducing the water content alone had some influence on properties, reducing the fat content improved both the static and dynamic behaviour (102). Processing bone particles with solvents, freeze-drying and irradiation improved the compaction properties and the shear strength of the reconstruction. The improvement in strength was due to an increase in the friction angle and a tighter graft compaction secondary to marrow tissue removal. Washed particles might have little lubrification at the contacts with other particles and therefore friction resistance was increased (103). On the other hand, graft stickiness was advocated to increase interparticulate cohesion (104). The combination of human bone marrow stromal cells with washed allograft to produce a living composite, offered a biological and mechanical advantage over the current gold standard of allograft alone and provided a higher shear strength when compared with allograft alone (105).

Improved results observed with freeze-dried irradiated bone may also be related to an incomplete rehydration. Stickiness between freeze-dried irradiated bone morsels and the impactor was observed during our experiments. Conrad et al found that rehydration could last for longer than one day (33). Impacted freeze-dried irradiated grafts could increase the interlocking effect by increasing their water content. In our study on femoral implant stability in a hip simulator, we observed that implant pull-out was extremely difficult in reconstruction with freeze-dried material compared with frozen one after 1 million cycles and did not result in a shear separation of the graft layers.

## 3.2 Implant stability

When the initial stability of femoral stem is compared in hip simulator models, cemented hip prosthesis stability within a normal medullar canal was higher than stability of femoral revision with impaction bone grafting (106). More subsidence was found in revision with the impaction technique than with a primary prosthesis (107). In our hip simulator model, a stable reconstruction was achieved with freeze-dried irradiated bone as filling material for impaction bone grafting (108). The stability was even greater than with frozen morsels and compared favourably with stability of primary stem cementation in the same model (109). Taylor considered that the initial mechanical demand was met when the graft was as strong as the endofemoral cancellous bone in a primary prosthesis (110).

The low subsidence registered in our study was about the same as that reported by Karrholm et al. in a clinical study of revisions with impaction technique and non polished stem (85). As in our experimental observation, a considerable amount of migration occurred during the first week after surgery, giving evidence of graft compaction due to patient activity (111). In the clinical cases, the lowest migration registered by radiostereometry was reported by a group who used a mechanical defatting method of the bone (85, 112). The importance of graft compaction has clearly a strong logical appeal and the lack of sufficient compaction is considered as the most likely explanation for substantial migration in clinical situation (85, 113-114).

If implant stability is the first goal, the impaction procedure must be done with energy until the impactors cannot be further driven into the bone (73, 115). This vigorous procedure exposes to fracture that remains one of the major complications of the impaction technique (72, 116-117). Three to five times fewer hammer shocks were needed to impact the freeze-dried irradiated bone correctly, and less energy was needed to compact the material due to its loss of ductility, reducing the risk of per-operative fracture. In our experiments, femoral fracture was associated with a higher subsidence and inducible displacement, which might further increase the risk of loosening. Recently, an innovative vibration-assisted bone-graft compaction system has been tested to reduce peak loads transmitted to the femoral cortex during graft compaction and prevent the risk of intra-operative fracture (118).

### 3.3 Impacted graft remodelling

Ling pointed out that the initial stability ensures later stability during the remodeling (119). Slooff et al. considered that morselised and impacted graft should be as resistant as a cortical graft and would remodel like a cancellous graft without transient mechanical weakening (120). The concept relies on the cancellous impacted grafts maintaining its volume during remodeling and not being resorbed.

The remodeling of cancellous graft includes a direct new bone deposition along the trabecula whereas resorption proceeds lately within the inner part of the trabecula with no net volume change (21, 23). The morselised and impacted graft is a porous structure and ingrowth of vessels was firstly thought not be impaired (121). In humans, biopsies often revealed mixed areas of living bone and non vital graft (122-127). Remodeled areas were mainly found in load bearing zones (128).

Tagil and Aspenberg demonstrated that impaction slowed down the remodeling (129). They noted that impaction reduced amounts of fat and marrow cells in the compacted graft which support the idea that the squeezing out of the bone marrow from bone will limit the availability of immunogenic cells and the immunogenicity of the impacted bone (72). This is consistent with the benefit of chemical lipid extraction reported in the same model (27). Removal of bone marrow from cancellous bone reduced the immunogenicity as bone marrow cells carry a wider range of transplantation antigens than osteocytes (91, 130).

New living bone is always limited in impacted bone and will appear only in revascularised area, leaving other areas with either non revascularisation or with only a fibrous recolonisation. Tagil and Aspenberg demonstrated that the mechanical properties of an impacted graft were enhanced by coexistence of fibrous tissue that embedded the particles (131). Nevertheless, Schimmel et al. demonstrated in a goat model that, when remodeling was completed and the interface revascularised, a fibrous membrane developed around the cement and the implant became loose (132). This implies that remodeling is not always beneficial and be hazardous for prosthesis longevity. Therefore, mechanical stability is probably more important and essential (133).

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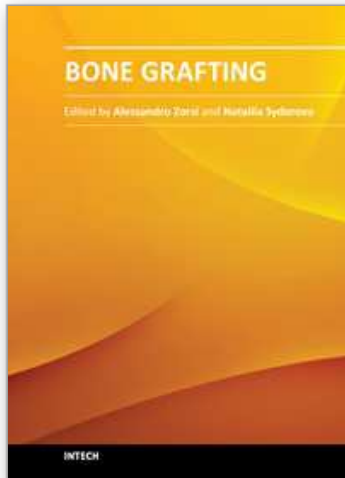
**Bone Grafting**

Edited by Dr Alessandro Zorzi

Bone grafting is the surgical procedure in which new bone (bone graft) or a replacement material (graft substitute), is placed into bone fractures or bone defects to aid in healing. Bone grafting is in the field of interest of many surgical specialties, such as: orthopedics, neurosurgery, dentistry, plastic surgery, head and neck surgery, otolaryngology and others. In common, all these specialties have to handle problems concerning the lack of bone tissue or impaired fracture healing. There is a myriad of surgical techniques nowadays involving some kind of bone graft or bone graft substitute. This book gathers authors from different continents, with different points of view and different experiences with bone grafting. Leading researchers of Asia, America and Europe have contributed as authors. In this book, the reader can find chapters from the ones on basic principles, devoted to students, to the ones on research results and description of new techniques, experts will find very beneficial.

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