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The Use of Bone Substitutes in the Treatment of Bone Defects – the Clinical View and History

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Summary: Bone has the ability to regenerate and remodel itself. In the clinic circumstances appear when bone defects do not heal spontaneously. These situations frequently result from trauma, congenital abnormities, infection or tumor resection. Hence, filling of the resulting defect by bone transplantation is a common practise with an increasing value in the re-establishment of the musculoskeletal system to promote bone healing. Since decades, efforts have been put to improve the effectiveness of bone substitutes. Conventional approaches with the use of ivory, animal and also human bone were not satisfactory. Negative effects like allergic reactions, rejection reactions, inflammations and other problems occurred. These led to implant failure, non union and amputation, to only mention a few. The introduction of bone banks and the development of standards in bone transplantation brought up the false hope to find a final solution for the treatment of bone loss. Disease transmissions (HIV) by allografts caused critical discussions. Despite all efforts, transplantation of autogenous cancellous bone is still the ''gold standard'' to induce bone healing. However, autografts are only limited available and are accompanied with high morbidity and mortality during the harvest. The problems associated with autologous and allogenous bone grafts promoted the development of multiple organic and inorganic bone substitutes. Well established substitutes at the present are demineralised bone matrix (DBM), composites and calcium phosphates (hydroxyl apatite and tri-calcium phosphate). These osteoconductive substances have shown to improve new bone formation. Nevertheless, clinical application of these materials is merely successful in a good bony environment but does not induce large progress in critical bone defects.

Keywords: autologous bone; bone bank; bone defect; critical size defect; clinical use of bone substitutes; implant

History of Bone Transplantation

The goal of bone replacement is to bridge a bone defect over healing procedures stable and durable without having to accept new problems or complications.

First reproducible investigations for bone regeneration are present from the 18th century (1742) by Duhamel du Monceau.[1] He could show with silver wire loops wrapt around bones of young animals that the coil of wire came to lie more near to the marrow cave during the process of growth. He concluded this as a phenomenon similar to the growth of a tree. From the bone fragment a succus osseus becomes separated, that reshuffles itself in cartilage, in which then by change the bone new formation takes place. In the year 1821 von Walter described the successful clinical application

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of an autogenous bone transplant for the first time in Germany.[2]

Further investigations for bone new formation were accomplished by Ollier in the 19th century. Ollier placed the periosteum into the center of the meaning for the formation of new bone and represented the view that autogenous bones covered with periosteum can survive when being transplanted.[3] Wolff (1863) showed, in knowledge of the investigations of Ollier, in his own clinical studies that also periosteum free bone could in-heal.[4] Attempts with substitutes presented Gluck 1891, by filling bone defects with ivory cylinders.[5] The results of Ollier stepped Barth 1893 due to own investigations against at the end of the 19th century: he transplanted pieces of the skull, which he took by trepanation, and found in each form (autogenous, allogenic, xenogenic) in the healing process no fundamental difference.[6] In his experiments the piece of in healed bone always proved as necrotic. He concluded from it the general change of the material and the replacement by the surrounding tissue. 1894 he reported on attempts with sponges (bath sponges), with which he was able to show a good growth of bone through the sponges.[7]

The experimental work of George Axhausen (1909, 1911) led to the so-called classical osteoblast doctrine.[8,9] After his conception the dead bone substance is converted by the adhering survivor cells of the periosteum. Only in the second line of importance are after his conception the cells of the osseous layer. Lexer (1924) underlined the theory of Axhausen, by formulating for the first time differences between bony layers.[10] The osteoblast doctrine of George Axhausen faced in the discussion the induction doctrine. Their trailer represented the view, that the surrounding tissue becomes lively for bone new formation by the transplanted bone material (1934).[11]

The investigations of Matti from Berne in the 30's of the last century (1932) showed the paramount meaning of the autogenous cancellous bone in the field of bone transplantation. He drew the following conclusions: on the one hand can transferred autogenous cancellous bone remain vital and can favour the regeneration of a dissected area, on the other hand is of crucial importance the transplantation into a homogeneous, adequate environment, apart from the condition of the implant and the implant bed.[12] The first bone bank for the use of allogenic transplants was justified 1945 in New York by Bush and Garber.[13]

A connection of the osteoblast theory and the induction theory presented Wolfgang Axhausen 1952.[14] He postulated from his own experimental work that the bone new formation runs off in two phases: in the first phase survived bone cells contribute to the new formation of bone (osteoblast phase), in a second phase it comes to the induced osteogenesis by a differentiation of the storage cells to bone-forming tissue.

The problem of the antigen reaction caused by the transplanted allogenic material came up for discussion since the publications of Medawar (1944), Chalmers (1959) and Enneking (1962).[15–17]

Urist (1965) postulated the presence of a bone morphogenic protein (BMP) in the bone matrix.[18] He was also the first, who could reconstruct the step in the experiment and who proved the presence of effective substances with an osteoinductive activity, by being able to isolate the inducing substance, from him mentioned as Bone Morphogenic Protein (BMP) (1984).[19] Despite the fundamental realizations of this step the reproducible proof of the osteoinductive effect of a substitute did not succeed to the bone regeneration with humans until today.

H_2O_2-macerated bone, also mentioned as ''Kieler span'', was in 50's and 60's the subject of many experimental and clinical studies. The ''Kieler span'' presented of Maatz (1957),[20] in a special procedure deproteined and degreased, should lose its antigenity by processing, but the ability of bone regeneration should remain preserved. The use of the material as

Table 1.
Schweiberer (1970).

1. osteogenesis is primarily a performance of preterminated, osteoblastic bone tissue
2. osteogenesis by the influence of the dismantling bone basic substance on nonspecific mesenchym cells is proven (osteoinduction)
3. osteoinduction is not possible, if the bone basic substance by maceration, cooking, or glowing, the so called intercellular substances, which is the complex of the mucopolysaccharides, is extracted
4. the inorganic substances and the so called formed intercellular substance (collagene fibrils) are insignificant for the osteogenesis
5. the regeneration of the bone is not accelerated by the offer of a line stand. The bone generation follows the vessels originating from the camp and not the artificially offered guide bar
6. macerated bone basic substance is only hesitatingly diminished cellularly and halistericly; their fate is generally the "dead inhealing of a foreign body"

xenogenic bone replacement ended around 1986. Katthagen showed clearly that it comes to immunological defence reactions against proteins still existing in the transplants.[21]

Substantially Schweiberer (1970) contributed to understand the osteogenesis by its recapitulatory statements after his investigations of bone transplants with unchanged and with denatured bone basic substance[22] (Table 1.):

His terminal summary: "The autologous transplant remains the only really reliable transplantation material also in the future, if it applies to bring new bone formation in course or crucially support to bridge bone defects" can remain invariably further existing in this form until today (Schweiberer 1970).

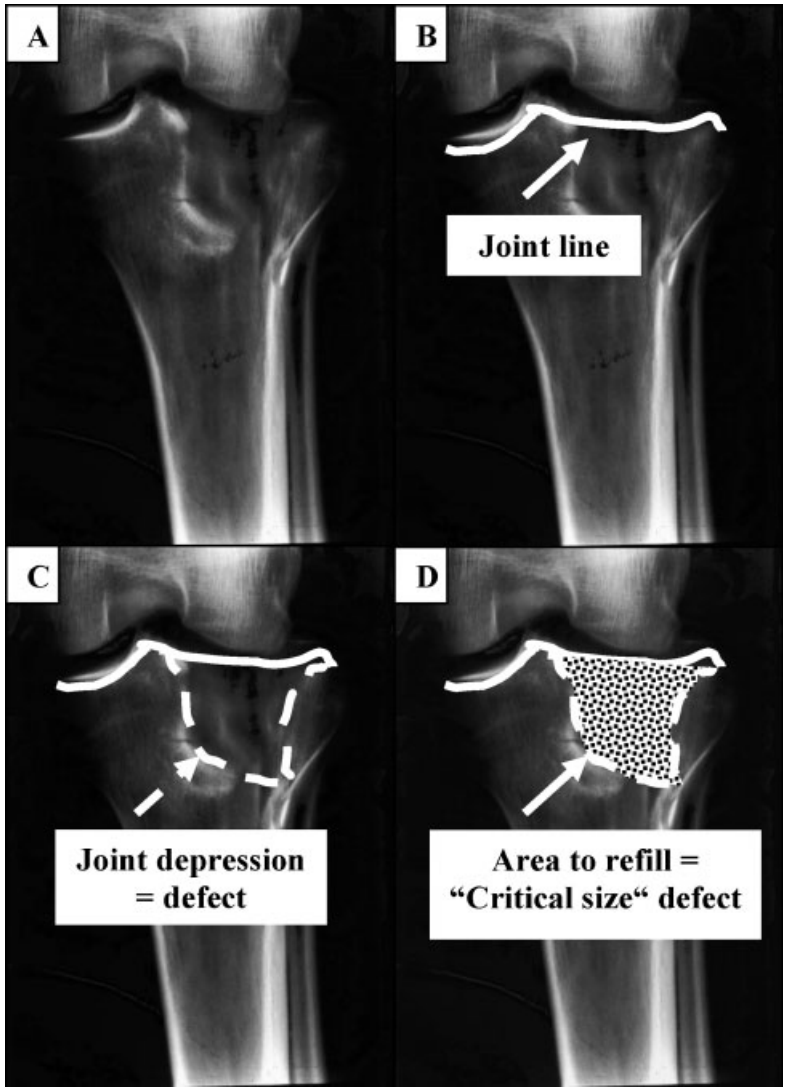
To conclude however from this that another material is not meaningful as bone transplant or substitute is wrong, since also the autogenous bone transplant has its indication borders. Autogenous material in arbitrary quantity is not available and the production of autogenous material always accompanies with injuries. This explains the high clinical relevance of alternative bone substitutes.

Autogenous Bone Transplantation

By the investigations of the Swiss surgeon Hermann Matti (1932) was proven the paramount meaning of the autogenous bone transplantion in experimental work as by clinical process observations.[12] Today, still the transplantation of autogenous bone is considered as gold standard for the bypass of bone defects[23,24] (Picture 1.) The autogenous bone brings by the transplantation of living osteoblasts the most reliable transplantation results.[25,26]

For the autogenous transplantation the tissue is available in different forms as cortical bone, bone chips and cancellous bone. The choice of the material depends on the requirements posed against the transplant. If initially against the transplant the demand of stability must be placed, pure cancellous bone is not indicated. In these cases bone chips should be used and bolted if necessary. Pure cancellous bone has its indication particularly during the defect replenishment in connection with an osteosynthesis and with infected bone defects (Figure 1.).

In discussion is the question of the storage ability of the autogenous materials from the withdrawal to the implanting stands.[8,27,28] Drenhaus indicates the fact that the cell density of the forerunner cells of the osteoblasts (bone lining cells) goes over 50% back already to a storage time from only 15 minutes.[28] As consequence of its investigations he demands the change of the expiration of operations with autogenous transplantations. A longer storage time should be avoided, therefore in principle withdrawal and implantations are accomplished in one operation. The high osteogenetic power makes the autogenous transplantation, despite more intensive and in the last years by the medical profession and industry for even still increased research after bone replacement materials, in its quality unsurpassed. The osteogenetic



Picture 1.
A. View of a tibial head fracture with typical joint depression. **B**. Marking of the anatomical joint line. **C**. Joint depression line and defect area. **D**. Defect area that has to be refilled with a bone graft substitute.

regeneration of bones is only made possible by the transfer of osteoblasts. The structure of the transplanted bone works as osteoconductive ''guide line''. At the same time also an osteoinduction is given by the fact that the mucopolysaccharid complex of the bone basic substance will be transfer intact and thus this part of the osteoreparation likewise remains (Figure 2.). Antigenity does not exist with autogenous transplants.

Some disadvantages face in addition, the advantages of the autogenous bone replacement: Limited offer of transplantation material, extension of the operation as well as the narcosis time, the additionally operational interference to withdrawal and from it the developing pain, partly continuing for a long time.[29] Additionally are still the possible early complications at the point of usage. Many cases of vascular injuries

Bone transplantation indications

- recent fractures (defects)
- delayed fracture healing
- pseudarthroses
- extension osteotomy
- necrosis of femoral head
- resection of benign bone tumors
- changing of endoprothesis
- treatment of osteomyelitis

Figure 1.
Bone transplant indications.

during the harvesting of bone grafts are reported[30] and can lead to injuries where the artery retract into the pelvis and may not be apparent until significant blood loss has occurred.[31] This is a reason why bleeding complications can be counted as another risk of autologous bone graft harvesting and as a reason to debate about the use of allografts.[32,33] A significant difference occurse by the comparison of harvested bone graft of the anterior or posterior iliac crest. Ahlmann et al. had an average blood loss by 108 patients of 232.47mL by anterior intervention and 169.14mL by posterior intervention.[34] Cockin reported 1971 on a subluxation of the hip after a relatively large amount

Definition of osteoinduction

- The new formation of bony tissue in a heterotopic locality. The substance inserted releases a sequence of events which leads to the formation of new bone

Definition of osteoconduction

- A " guide-line" effect; i.e. the capacity of a substance to promote the development of bony tissue

Figure 2.
Definition of osteoinduction and osteoconduction.

removal of the iliac crest. The occurrence of a postoperational haematoma ranges between 0 and 3%[35] mostly after harvesting at the anterior iliac.[36] The risk of infection is increased by haematoma formation. Fracture endangerment has been reported by Burchardt.[37] Donor site pain can delay the mobilization and represents the most common postoperative symptom.[33] Prolonged postoperative hospital stay and an increased operation and narcosis time are quoted widely as disadvantages of autologous bone grafts from the iliac crest.[32,33,35] Herniation is a possible complication that is only associated with tricortical harvest. Instability of the pelvic was reported by Chan et al. after bone graft harvest on nine female patients.[38] The differentiations between major and minor complications vary among series like also the acute and chronic complications. Major complications have been reported to be less common than minor ones.[32,34]

Already permanent damages (nerve damage) were described, above all a lesion of the N. cutaneus femoris lateralis (a sensory branch of the lumbar plexus).[39] Nerve injuries have been reported with the harvesting of bone graft both anterior and posterior iliac crest bones. Particularly in infancy the withdrawal possibilities are limited, whereby also still the possibility is given to damage the growth joint as additional danger. By the extended operation time, the increased morbidity and the resulting care for the withdrawal wound leave to costs, so that the argument that from autogenous materials no additional costs would the result does not appear correct.

Allogenous Bone Transplantation

The main advantage of allogenous bone material lies in the practically unlimited availability of the material and was for the first time successfully used and documented in the year 1879 by Sir William MacEwen.[40] The production of the transplant usually comes from organ donors. It is however also

possible to harvest allogenous material from femoral heads of patients who get an artificial joint substitute hip. Alone physiological aging limits the usefulness of allogenic bone substitutes.

The disadvantages of the allogenic bone in the comparison with the autogenous bone refer essentially to three points. On the one hand, the use of an allogenic bone substitute holds the danger to cause an immunological reaction with the receiver (Chalmers 1959), on the other hand to comes to a decrease or a biological reduction of the osteogenetic power of the material during the preservation procedures and every transplant of foreign material finally holds the danger of an infection.[16]

The bacterial contamination under sterile conditions with the withdrawal of allogenic material is described between 1% and 92%. This difference probably explains itself best with different kinds of tests, which find use during the different microbiological investigations. The bacterial infections usually are infections with bacterium tribes of low pathogenicity so that a contamination of the transplant doesn't inevitably lead to an infection of the receiver. Contamination of the allogenous transplant with a virus represents a by far more dangerous infection risk than a bacterial contamination. The Center for Disease Control (CDC) published already in the year 1988 the first case of an infection with the HI-Virus (Human Immunodeficiency Virus) by cryoconserved allogenous bone fabric.[41]

The requirements of a bone bank,[42] in which the allogenous material is kept in safe custody after the withdrawal up to the transplatation, have strongly changed in the last years. Since highly dangerous viral infectious diseases like AIDS or also hepatitis B and hepatitis C gain in importance and can be transferred with an allogenous transplant, body strange material is handled considerably more critically today, than a couple of years ago. Other viruses have a rather low risk value according and lead either to no chronic or serious infection or there exist such a large immunity in the population that it comes to no infection. By this development the existing guidelines for managing a bone bank have changed and were adapted to the science and to the technology state-of-the-art.[43] The scientific advisory council of the Federal Medical Society (Bundesärztekammer) regulated in the meantime the handling of allogenous materials in such a way that a sufficient protection exists for the patient, which however does not amount 100%. In contrast have the substantial basic conditions not changed, which already Bush described in the first bone bank 1945 in New York[13] (Table 2.)

Many hospitals in Germany possessed their own bone bank in and the 70's and 80's. These stored primarily cancellous bone. In the USA however bone banks were usually created on a commercial basis. Thus the costs of allogenous bone transplants can vary substantially from Federal State to Federal State and country to country. The new guidelines to manage a bone bank caused that the necessary logistic and infrastructure as well as the developing costs, which are raised by the adherence to the guidelines led to the fact that only a few hospitals could maintain their own bone bank.

Different conservation methods have been used. The freezing and the freeze drying achieved acceptance. Other procedures, like the keeping in saline solution with chloroform or toluol additive or sublimated spirit in alcohol as well as conservation in merthiolat, an organic mercury compound, or cialit have no more relevance. It has to be considered that the biomechanical characteristics of the grafts

Table 2.
Basic conditions for a bone bank (Bush 1945).

1. secure conditions, which ensure the sterility of the transplants
2. no change of the osteogenetic characteristics and the mechanical characteristics of the transplants by the preparation or storage of the bones
3. reduction or neutralization of the antigenicity of the transplants
4. visible financial expenditure for the operator of the bone bank

are changed by the different sterilization procedures in different ways.[44]

A further disadvantage is the antigenity of the allogenic bone, which becomes not equally apparent as by organ transplantations, which must be made responsible for the treatment failure however in some cases. Generally however rejection reactions have hardly clinical relevance with allogenic bone replacement. Friedlaender et al. showed 1976 that by a freezing drying process a reduction of the antigenity can be attained.[45] The antigenity is lowered by the deep freezing but is not set to zero. Antigens of the class MHC-I and MHC-II sit on the outside of the cell wall and cause an immune defense over the mechanism of the specific t-cell activation.[46] Also proteins of the bone matrix can have an antigenic effect.

First experimental works were accomplished, in order to decrease by immuno suppressant drugs the antigenity from bone allotransplants. Burchardt et al. could thereby reach no improvement of the healing.[37] On the imperative to consider also the Rh factor by transplantations with women Schmid-Schmidsfelden already reported 1954.[47] He presented the case of a child with a lethal process of an icterus gravis neonatorum, that caused to his anamnesis, as he formulated, on a "homoplastic knee joint transplantation". According to the biologically limited efficiency also the indication for an allogenic transplantation is reduced. Particularly a vascularised allograft is meaningful only by very special indications.

An increasing problem of the allogenic deep frozen bone transplantation is the fact that an infection from the donor to the receiver is possible. The highest risk have virus infections like the human immunodeficiency virus (HIV), the hepatitis B virus (HBV), and the hepatitis C virus. Schratt et al. reported four cases of HIV infection caused by bone allografts from one donor.[48] All transplantations were performed between November 1984 and January 1985. In all, 12 recipients had bone allografts from the HIV-infected donor, 7 of whom were HIV-negative and 4 HIV- positive. One of the patients died a natural death in her 10th decade. The donor was not been tested before the grafts were harvested, as HIV-antibody detection was not possible at the time (October 1984).

Increasing recognizing of the risk of the allogenic transplantation led to ever more expanded regulations for the transmission of deep frozen bone in the last decades. The guidelines of the scientific adviser of the Federal Medical Society (Bundesärztekammer) for leading a bone bank changed in the course of the years always again.[43] Even most diverse treatments of the transplant do not offer 100% protection. The better knowledge of the risk limited also the indication decision at the same time, since with not compelling indication for the transplantation a risk consideration is to be made. The demand for a declaration of consent to the transplantation before the interference both of donor and receiver, leads particularly in the area of the acute supply of the trauma surgery, with a high portion of emergency operations to not solvable logistic and legal problems. Also the justified necessity to repeat the HIV test with the donor after 3 months after the bone donation leads to the fact that a part of the taken samples after this period already because of missing documents would have to be rejected. So the number of the not used samples of approx. 5% rose with usual expenditure to in the meantime over 30% which led not least to a substantial raising of the price of the bone banks.[21]

The risk of a HIV infection can be lowered after investigations of Buck et al. (1989) under utilization of all techniques from 1:161 to 1:1.000.000. To demand then however are beside an accurate donor screening, lymph node investigations and a HIV antigenity test, HIV anti body test and the hepatitis and syphilis (lues) serology. Further investigations confirmed that the HIV virus is still provable both after deep freezing (in its work $-70\,^{\circ}C$) and after freezing drying process. From this problem a further substantial stimulus results to advance the search for suitable new substitutes.

Xenogenous Bone Transplantation

The first documented use of a xenogenic transplant originates from the 16th century. Job van Meékren from the Netherlands tried to fill a hole in the head of a soldier with a piece of a dog skull.[49] The Transplantation of unchanged xenogenic bone material did not win a signification. The transmission of released antigenicity reactions led to substantial treatment problems. Another material was the ''Kieler Span''. The material was processed in a special way to be deproteinated and to reduce the antigenicity. Despite initial positive reports the ''Kieler Span'' could not become generally accepted.[20] Schweiberer showed with his investigations (1970) that this material the bone regeneration does not demand, but even handicapped. Altogether the xenogenic bone transplantation won no clinical value because of the released immune reaction and additionally is missing bone induction.[22]

Autoclaved Bone Transplantation

The transplantation of allogenic autoclaved bone is a special kind of the allogenic transplantation. The cancellous bone is reduced to the bone basic substance by cell reduction. The heating and autoclaving of bones are in the scientific discussion since the beginning of the last century[50] and have won by the infection problem and the immunological risks of the frozen transplants and the complex preliminary investigations and finally also because of the extensive documentation requirements at topicality. For some years the method had moved into the background by the good results of the frozen and also the freeze dried cancellous bone graft, particularly since it could not be attributed osteoinductive characteristics and thus the priority lies under that of frozen cancellous bone.

The autoclaving had always a reduced value with the question of the reimplantation of bone sections seized with a tumor. The advantage of the safe sterility is bought by the loss of mechanical firmness, so that autoclaved bone, if at all, has a closely limited indication range, if the requirements to the transplant place not stability into the foreground and require no osteoinductive achievement. A clear relation could be found between the time of autoclaviculation and the temperature regarding to the structural changes developing at the bone. Above all an extension of the autoclaviculation time leads to a significant reduction of mechanical stability. Knaepler et al. examined 1991 the mechanical inherent stability, the firmness and the biological valence and found likewise a significant reduction for these parameters.[42] In agreement they reported that by the heat effect a safe sterility of the bone can be achieved. Schratt et al. described that by the autoclaving the antigenicity of the material is destroyed. An alternative can be seen in the extracorporale irradiation.[48]

The autoclaved bone can only serve as a guide bar for the in growing bone in the sense of osteoconductivity. About clinical application with good successes reports Johnston et al. (1983) by knee joint near tumors, with which they reimplanted autogenous local bone after autoclaving.[52] Wagner und Pesch (1989) report on good results with 83 hip prosthesis change operations, during them defects were filled out with autoclaved bone splinters and saw no postopeartive complications, which had to do with the autoclaving.[53]

Bone Substitutes

If after a defect body-own bone is again to take over the function in the long run, the

Definition of bone substitutes

- a synthetic, inorganic or biologically organic combination –"biomaterial" – which can be inserted for the treatment of a bone defect instead of autogenous or allogenous bone

Figure 3.
Definition of bone substitutes.

Table 3.
The 4 groups of biomaterial bone substitutes (Rueger 1992).

I.	**biological, organic substances**
	• demineralized bone matrix (DBM), growth factors (BMP)
II.	**synthetic, anorganic materials**
	• calciumphosphate, hydroxyapatite (ceramics)
III	**synthetic, organic substances**
	• polymere and their combinations (polyester)
IV.	**composites**
	• graft composites and combinations of groups I–III

following substitutes stand to disposal for a temporary replacement. They were experimentally examined and used partially clinically or are still in application. It concerns thereby collagen, glass ceramics, calcium phosphate, bone matrix and the combination of materials (Figure 3.). They are divided in 4 different groups after Rueger 1992 (Table 3.).[75]

Collagen

Bedacht (1969) has submitted animal experimental and clinical investigations over the use of allogenous collagen as implant into the marrow area of long tube bones.[54] He could show the gradual change of the primarily formed granulate tissue into newly formed bone and concluded from its investigations a positive influence of the collagen on the formation of new bone. Benfer und Struck (1972) saw in their investigations a faster formation of callus tissue after local collagen application.[55]

Similarly positive results published Springorum et al. (1977) after experimental investigations at the rabbit.[56]

Glass Ceramic

Glass ceramics were mostly used as surface coating of metal implants, because the material is inert and approves direct contact. Hench et al. (1972) and Blencke et al. (1979) as Harms and Männle (1980) could show that glass ceramics are bioactive and biocompatible, i.e. that they release a specific biological reaction after bringing into the bone.[57–59] After implantation of glass ceramics in the bone a direct contact without a connective tissue boundary layer results. A so-called free connective tissue group develops (Blencke et al. 1979). Due to the material condition glass ceramics are to be settled between the permanent implants and the temporary substitutes. They won no special meaning as substitutes, but being used in increasing measure in the surface coating of implants.

Calcium Phosphate

Beside water and collagen, which are counted to the organic matrix of the bone, are it the calcium phosphates, which count to the inorganic matrix, which constitute approximately 70% of the bone, the quantitatively substantial most important basic substance of the bone fabric. The inorganic bone matrix consists mainly of calcium apatite ($Ca_5(PO_4)_3$). Hydroxyl apatite ($Ca_5(OH)(PO_4)_3$) is the hydroxylated calcium apatite and possesses a very high degree of hardness. It forms the main part of the inorganic substance in bones and teeth, where it is partly replaced with fluorine apatite ($Ca_5(F)(PO_4)_3$). Hydroxyl apatite is soluble only in strongly acidic environment. In the bone the mineral phases amorphous calcium phosphate (ACP) and octacalcium phosphate (OCP) are also still present in smaller quantities (Osborn 1985). In the bone contained biological apatite consists of heavy soluble calcium phosphate, in its crystal structure it is a calcium hydroxyl apatite ($Ca_{10}(PO_4)_6(OH)_2$). As substitutes for the clinical employment hydroxyl apatites and tri calcium phosphates stand ($Ca_3(PO_4)_2$)

for disposal. They differ in their chemical structure slightly, in clinical application regarding absorbableness and mechanical stability however clearly.

First experimental work with calcium phosphates was submitted 1920 by Albee and Morrison.[59] They filled up a 0.5 cm large defect by rabbits, after resection at the radius, with a suspension from tri calcium and could by this show an accelerated bypass of the bone defect. An examination of their results in the following years led to different interpretations. 1934 Haldeman and Moore described likewise an accelerated type of fracture healing with local use of tri calcium phosphate in a radius defect with rabbits.[60] By the establishment of the first bone bank by Bush 1947 the scientiffic interest moved increasingly toward the allogenic bone replacement and its transplantation possibilities. Therefore only a few investigations to calcium phosphate as bone substitute were published.

1972 Little formulated the realization that after the removal of the antigenetic substances in the bone transplants also the groups, which were for the osteogenetic effect of importance, were also removed.[61] He pointed out hereby that a further use of antigen-free bone transplants is not to be aimed at necessarily, but instead of its also bone substitutes could be used. The search for a suitable substitute began. A new impulse for the employment of calcium containing substitutes was apart of the risks of the allogenous transplantations the increasing knowledge of the fact that bone has the condition, to go into a mechanical - a so-called ''bonding'' - connection, as well as the progresses in the production of the bioceramic implants. The investigation on the applicableness of synthetic calcium phosphates show on the one side increasingly the borders of this substitute and on the other side the great characteristics of the bone. Investigations with calcium phosphates, the hydroxyl apatites, increased in the years since 1970. As crucial characteristic proved here the mechanical stability and the biological reactions between the receiver bone and the implant, which could be pointed out by electron microscopic investigations. The high fabric compatibility of the calcium phosphates had already been confirmed.

In different experimental models (Getter et al. 1972, Köster et al. 1976, Holmes 1979, Jarcho 1981, Wagner et al. 1981, Katthagen 1986, Eggli et al. 1988, Pochon 1990, Meiss 1991) it could be shown that it comes, after placing a synthetically manufactured and calcium apatite containing implant, rapidly to an accumulation of natural apatite, which promotes again the protein absorption and the accumulation of bone cells.[21,62] This for the ''bone bonding'' mandatory pre-phase is a good reference for the fact that natural bone minerals can be incorporated more rapidly in bones, if they contain already natural apatite. Also clinical studies of Bucholz et al. (1988) with tibial plateau fractures confirm these findings.[63]

In contrast to the synthetic apatite, which is very close and rigid, natural from bone-won apatite offers the advantage to be similar to bones in a higher dimension and possesses fewer problems with ''bone bonding''. This applies particularly to the surface integrity and the porosity of the substance. West and Brustein (1985) submitted good results using hydroxy apatit formed ceramics formed of corals;[64] White and Shors (1986) could prove for corals a remodeling as with normal bone.[65] Klawitter and Hulbert (1971) showed in own investigations that for an ingrowth of mineralized bone fabric a minimum pore size of 100 μm is necessary.[66]

For the successful ossification of a bone substitute with newly formed bone the characteristic of the substitute is as guide bar, to work as osteoconductive graft, of crucial importance.

Crucial is also that the pores of the substance in itself form no final areas, but stay in connection among themselves, to open the possibility of a potential growth of the bone through the hole implant. In addition it is of decisive importance for the success of the long-term ossification that a resorption of the inserted material or a physiological remodelling runs off. The

exact factors which steer and control this remodelling are still unknown. Porous hydroxyl apatite shows if it is combined with marrow cells an osteogenic potential in vivo situations.[67,68] Newly formed bone is derived from donor cells.[69] In contrast to harvesting autogenous bone from patients, it is easy to obtain bone marrow cells from them.

Demineralised Bone Matrix

The fact that after heterotroph transplantation a bone new formation can be released by bone cells and bone matrix, led to the acceptance that in the matrix of bones so-called osteoinductive substances must be contained.[210] Already in 1940 Levander had emphasized the role of the bone marrow as the substance, which arranges mesenchyme to formate new bone.[71] In the consequence investigations with mineralized and demineralized matrix as well as with demineralized matrix excerpts or bone gel as a substitute and as a highly purified matrix excerpts and matrix factors were accomplished.

In various experimental works all these substances was certified more or less an osteoinductive effect.[72–74] The experimental proof of the effectiveness of the matrix substances is however in reproducible way so far only furnished with the rodent, so that in the year 1991 neither for matrix excerpts nor for the isolated matrix factors a clinical employment could be recommended.[75]

Composites - Combinations

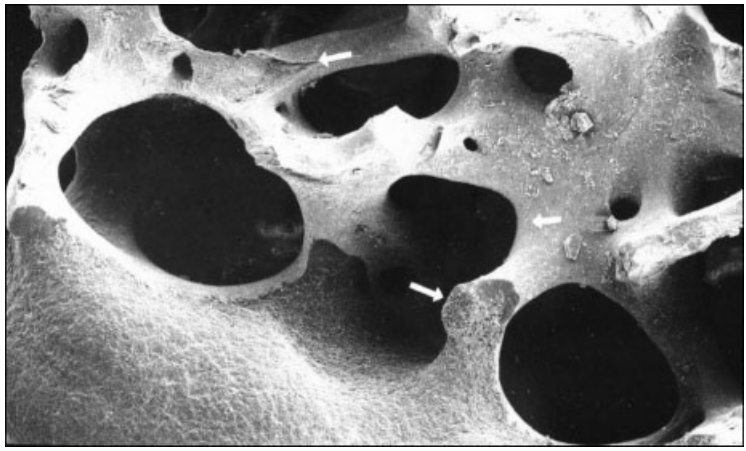
Mittelmeier developed 1977 the combination of collagen with hydroxy apatite as ''Collapat''.[76] The basic idea was to use the lyophilised netlike collagen fleece as fast resorbable support and the mineral particles as stimulation germs ''Stimulationskeime''.[77] Altogether for this 1984 a clinical experience of approximal 500 applications were present.[78] Similarly attempts were presented by Bösch et al. with the coating of cancellous bone[79] or the ''Kieler Span'' with fibrin gluten. Salama declared with autogenous marrow inoculation of ''Kieler Span'' good results.[80] Rueger et al. combined bone gel with tri calcium phosphate or hydroxy apatite.[81] Köhler und Kreicsberg (1987) enriched autoclaved bone with allogenic demineralized bone matrix (DABM).[82] Comparative investigations of a defect replenishment at the coney ulna with DABM augmentated and with not augmentated bone, showed a significantly higher rate of cultivation of bone after the augmentation. Furthermore the use of bone substitutes is discussed and being tested as carrier substance like antibiotics.

A further fundamental thought on the optimization of the results in the bone replacement is the combination of a carrier with a marrow inoculation, as described from Burwell to pair the the oeteoconductive effect of the carrier with the osteoinductive effect of the marrow.[83] Gupta et al. impregnated a xenograft with autogenous marrow.[84] Urist et al. (1984/1987) combined beta tri calcium phosphate (TCB) with bone morphogenetic protein (BMP).[19] W. Mittelmeier (1992) presented experimental investigations at the rabbit for bone new formation in the spareweak camp with a cancellous mineral bone substitute and autogenous marrow inoculation.[85]

Authors Experience

In own experimental and clinical investigations a natural bone mineral (Orthoss®) has been evaluated. The material is a biological apatite with less hydroxyl groups than synthetic substances. The inner surface with its interconnected pores is very similar to human bone (Picture 2.).

Small apatite crystals form a crystalline structure which simplifies the remodelling. In an animal study with rabbits the use of the natural bone mineral (Orthoss®) as a very effective osteoconductive material



Picture 2.
The electron microscope investigation of the natural bone mineral (Orthoss®) shows a similar structure like human bone.

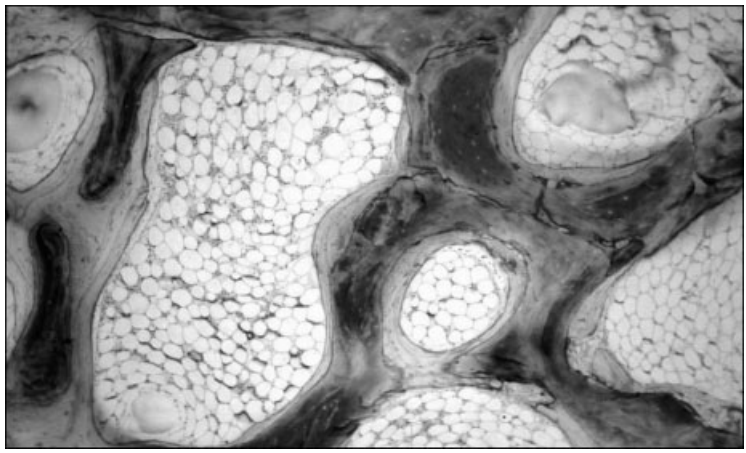
could be proven (Picture 3.). Clinical results show the effectiveness of the material filling bone defects in good bony layers.

Outlook

Bone transplantation has still an increasing value (Table 4.). Major progresses have been made in the field of bone regeneration in the clinical field in the last two centuries.

Material science technology has resulted in clear improvements. But still no adequate bone substitute has been developed which can solve the problems that bone transplantation has to deal with. It is still necessary to improve our knowledge about bone healing, materials and stem cell biology.

The concept to promote and to improve bone healing in the future will be a combi-



Picture 3.
6 month control of a ''critical-size'' defect in a rabbit treated with the natural mineral (Orthoss®). The material is totally covered by new formed bone.

Table 4.
Millenium Research Report of bone transpantations in Europe 2001.

Country	Total Grafting Procedures	Autograft Penetration	Autograft Procedures	Allograft (fresh frozen ex. Hospital) Penetration	Allograft (fresh frozen ex. Hospital) Procedures	Bone Graft Substitutes Penetration	Bone Graft Substitute Procedures
France	150'800	53.6%	80'900	21.9%	33'000	24.5%	36'900
Germany	233'400	51.7%	120'800	35.7%	83'200	12.6%	29'400
Italy	86'100	62.6%	53'900	28%	24'000	9.4%	8'100
Spain	56'700	60%	34'020	31%	17'577	9%	5'103
UK	56'700	60.6%	94'300	28.5%	44'400	10.8%	16'800
Rest of Europe	155'500	62%	36'456	32%	18'816	6%	3'528
Total	**741'300**	**56.7%**	**420'376**	**29.8%**	**220'993**	**13.5%**	**99'831**

nation of osteoconductive, osteoinductive and osteogenetic substances, in a biocompatible, bioresorbable, and cost-effective bone graft substitute to approve the advantages and to decrease the disadvantages of the concepts.[86]

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