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BONE GRAFTS

Tarasenko S.V.[1], Diachkova E.Yu.[2], Azarovskaya E.A.[3], Sevan S.M.[4], Alieva M.N.[5], Levonyan E.A.[6]

[1]DDS, PhD., professor, Chief of department of Dental Surgery of Sechenov First Moscow State Medical University (Sechenov University), Russia, Moscow, Mojaiskii val, h.11, 121059, email.: prof_Tarasenko@rambler.ru

[2]PhD, associate professor of department of Dental Surgery of Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia, Mojaiskii val, h.11, 121059, email.: secu2003@mail.ru

[3]Student of Institute of Dentistry of Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia, Mojaiskii val, h.11, 121059, email.: 79163135788@yandex.ru

[4]Student of Institute of Dentistry of Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia, Mojaiskii val, h.11, 121059, email.: sooz-sawan@mail.ru

[5]Student of Institute of Dentistry of Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia, Mojaiskii val, h.11, 121059, email.: piruzaalieva@mail.ru

[6]Student of Institute of Dentistry of Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia, Mojaiskii val, h.11, 121059, email.: Ledward96@mail.ru

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Abstract

Today, the analysis of several studies has proven the safety and consistency of bone healing after enucleation and simple closure of jaw cysts without the use of bone grafts or implants even in the case of the formation of large defects. Nevertheless, the use of different types of bone grafts is important. What concerns the autografts the authors have concluded the use of grafts of the ilium substantia spongiosa leads to significant acceleration of defect ossification and lowers the risk of infections and fractures in the lower jaw. Allogenic implants can be successfully used to treat patients with defects of the upper jaw and serve as an alternative to the use of autografts. When using embryonic (fetal) tissues the clinical effect is difficult to predict, because by the time it is used, the bone tissue does not yet reach the required maturity. In addition, abortion material is a risk category, so at present it is not used for bone grafting. Some authors recommend to use the stimulating effect of placenta in periodontal practice and at filling of cavity bone defects. In the past years scientists developed the xenogeneic materials which contain deproteinized bovine bone mineral (DBBM, Bio Oss ®) or coral derivatives (Algipore®) and phytogenic (Interpore®) hydroxyapatite, which are successfully applied for augmentation of the lower wall of the maxillary sinus. These materials, according to manufacturers, must have osteoconductive properties. In addition, it has been found that natural corals have osteoinductive properties for multipotent mesenchymal stromal cells, inducing osteodifferentiation gene expression in them. While implantation of synthetic materials can accelerate osteoconduction and osteointegration. The idea of using these osteoconductive materials is to stabilize the blood clot in a defect in order to prevent the development of an infectious process and accelerate bone tissue regeneration by increasing the migration of osteoprogenator cells In recent times it has become very popular to use collagen for the stabilization of the blood clot.

Keywords: *bone graft, autograft, allogenic graft, osteoconducion, osteoinduction, bone regeneration*

Corresponding author:
Diachkova E.Yu.
PhD, associate professor of department of Dental Surgery,
Sechenov University,
Moscow, Russia, Mojaiskii val, h.11, 121059,
email.: secu2003@mail.ru , telephone number: +7-926-519-93-42

QR code

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INTRODUCTION:

The variety of currently developed methods of osteoplastic operations provides the possibility of choosingthe optimal bone graft, which allows to achieve a favorable clinical result depending on the specific situation.

Impacts on bone tissue regeneration are classified as follows:
1. Osteoblastic osteogenesis
2. Osteoconduction
3. Osteoinduction
4. Osteostimulation

Two other methods that perform the process of osteoreparation, which are also aimed at achieving the type of the restored bone are:

1. Distraction osteogenesis
2. Guided Tissue Regeneration

The type of impact on reparative osteogenesis is determined by the choice of biomaterial and the method of bone grafting.

Classification of the main types of materials used for bone grafting (1)

1)Autografts
- Autogenic regional stem cells
- Cancellous bone autografts
- Cortical bone autografts
- Bone-cartilaginous autografts
- Vascularized autografts

2) Allogenic grafts
1. According to the anatomy:
- Cortical
- Cancellous
- Bone-cartilaginous
2. According to the method of conservation:
- Inorganic bone
- Frozen
- Formalized
- Lyophilized, non-demineralized
- Demineralized
3. According to the method of sterilization
- In the process of manufacturing (chemical or crioconservation)
- Radiation
- Gas (ethylene oxide)
4. According to therelease form
- Bone flour
- Bone scraping
- Gel
- Paste
- Chips
- Mini-blocks
- Blocks

3) Brefogenic implants
- Lyophilized, non-demineralized
- Demineralized

4)Xenogenicimplants
- Inorganic bone

The main requirements for osteoplastic materials are:
- Biological compatibility
- Patient safety
- Adequacy of the organ or its part that is to be restored, ensuring organotypic bone regeneration
- Stability of the anatomical, functional and cosmetic results achieved (2)

The use of osteotropic materials most commonly used in bone grafting of the maxillofacial area can be illustrated by comparing methods for filling defects of the jaws after a cysectomy operation, since cysts of the jaws are one of the most frequent causes of bone defects.

Odontogenic jaw cysts are a common lesion of the maxillofacial region.The preferred treatment for these lesions is enucleation of the cyst and the primary closure of the defect tightly, which Partsch described as "cystectomy" (Partsch II) in 1910.(3)Cysts located in the jaws represent a bone defect after enucleation, since in most cases they are completely surrounded by bone outside the operative site.This fact contributes to the formation of a stable blood clot, which leads to a permanent and safe healing process. Enucleation of various sized cyst of jaws with complete wound closure remains today the "golden standard".(4)

Partsch was convinced that cystectomy is applicable to entities no more than 2 cm, because of case of using the method for large cysts the infectious complications may develop. V. Schulte in the 1960s stated that with an increase in the size of the cyst, the risk of wound infection increases due to the retraction of the blood clot from the cyst wall. For this reason, he placed a gelatin sponge in combination with thrombin to stabilize the blood clot.(5)

In recent decades, there have been numerous studies in the treatment of lesions of the jaws using autogenic and allogenicgrafts, xenogenic implants or alloplastic (synthetic) implants as the filling materials. In addition to reducing the risk of infection during treatment, these materials could provide the acceleration of bone tissue regeneration, prevent soft tissue from "settling" inside the defect, and increase bone strength.(4)

Enucleation and primary closure of the defect without additional bone tissue substitutes – cystectomy:

Today, the analysis of several studies has proven the safety and consistency of bone healing after enucleation and simple closure of jaw cysts without the use of bone grafts or implants even in the case of the formation of large defects. (6)

The frequency of complications during cyst enucleation and primary closurewith the prescription of antibiotics is less than 5%, even in the case of defects greater than 3 cm. (4) Development of infectious processes is associated with suppuration of the cyst and divergence of the wound edges, which are the main complications. However, the detection of suppuration in the cyst area at the time of the operation does not necessarily lead to the elimination of the process. The optimal incision during surgical access — for example, along the edge of the bone — ensures the safe closure of a defect over intact bone, the presence of which is necessary for the smooth organization of a blood clot. (6)

Fractures after enucleation of lower jaw cysts are extremely rare. The highest risk exists when it is localized in the angle of the mandible. Bolouri et al. reported about the fracture rate of 3.1% in a study with 160 patients with follicular cysts in the mandible angle with an average size of lesions 31.5 mm.(7)

Concerning the regeneration of bone tissue after cystectomy with extensive defects up to 3-4 cm in diameter, it can be expected no earlier than after 12 months. IhanHren and Miljavec evaluated spontaneous bone healing with large defects of the mandible in 33 patients using computerized X-ray analysis. (8) The average bone density increase is indicated: 7%, 27%, and 46% at 2, 6, and 12 months, respectively. In small defects (up to 2-3 cm in diameter), the achievement of the final bone density, 97%, relative to the normal surrounding bone tissue, is observed after 12 months.Similar results were obtained by Yim and Lee in 74 patients after enucleation of a jaw cyst. In this study, an analysis of panoramic X-rays showed the restoration of X-ray contrast by more than 97% with defects not exceeding 3 × 4 cm, 12 months after the operation. It should be noted that the study included only patients without divergence of the wound edges.(9) If there are defects in the lower jaw, more than 3 cm in diameter, Hren and Miljavec, found that bone density is restored by 84% compared to the intact surrounding only after 12 months.(8)In another study, Chiapasco et al. evaluated spontaneous bone regeneration after enucleation of 27 cysts larger than 4 cm. A computer analysis of panoramic radiography showed a decrease in defectsize by an average of 12.3%, 43.5% and 81.3% after 6, 12 and 24 months, respectively. An increase in bone density was noted by 37.0%, 48.2% and 91.0% at 6, 12 and 24 months, respectively. This study showed that the almost complete healing of bone defects bigger than 4 cm in diameter occurs after 24 months. (10)

In young patients, bone healing processes are better than older ones, as well as them on cortical defects regenerate more quickly if both plates are affected (8)(9). In addition, the shape of the bone defect is a more important component of the healing process than its volume.Hren and Miljavec noted that the minimum lesion diameter is a key parameter; in addition, for some reason, elliptical defects show better bone healing results than round defects of similar volume. The same research group described the superiority of bone regeneration of lower jaw defects in the area of the angle and symphysis compared with defects in the body area.(8)Laffers and Zimmer published the results of a study of radiographs of 38 patients after cystectomy: the bone healing process was most favorable in the anterior mandible and least favorable in the anterior maxilla (11).

According to foreign authors, special attention should be paid to the preservation of the periosteum, which has great potential for regeneration osteogenesis.The periosteum probably has a higher potential than the adjacent intact bone areas.(12)(13)Guided bone regeneration using absorbable or non-absorbable membranes did not demonstrate clear advantages over the primary periosteal closure of monocortical mandible defects in humans .Even the additional use of the ileal spongy bone graft did not increase the rate of regeneration of the mandible bone tissue in the area of segmental defects with an intact periosteum in mongrel dogs, from which the authors concluded that resorption and replacement of bone grafts inhibits the healing process. However, if the periosteum is absent, spontaneous bone regeneration can be stimulated by osteoconductive and osteogenic spongy autografts protected by macroporous nets to prevent the 13466 relapsed of surrounding soft tissue. (12) (14)

Autografts

Many authors have repeatedly reported on autologous bone transplantation, today it represents the "gold standard" of bone grafting (15).The iliac crest is the most frequently chosen extraoralsite of donor graft intake, especially in the iliac spongy bone, which has osteoconductive, osteoinductive and osteogenic properties. Holtgrave and Spiessl described a homogeneous radiographic ossification of large jaw cysts in 3–13 months after the use of an autologous iliac spongy transplant. In a cohort of 14 patients with cysts of the upper and lower jaw, only two wounds were secondary healed, while in the remaining 12 cases there were no signs of the infection.

The authors have concluded compared with simple enucleation, the use of grafts of the ilium substantia spongiosa is safe, and leads to significant acceleration of defect ossification, and lowers the risk of infections and fractures in the lower jaw. As shown in the work, viable osteoblasts of substantia spongiosa feed through diffusion and early revascularization at the bone defect edges, and immediately start filling all the volume and producing bone tissue.(16) The use of an autological bone during the experiments on mini pigs with standard defects of the lower jaw (average size 10 × 5 mm) have demonstrated the greatest osteogenic properties, increased rate of regeneration at the beginning of the healing process, compared with simple adhesion to a blood clot and substances containing type I collagen, β-tricalcium phosphate (β-TCP), hydroxyapatite (HA), biphasic calcium phosphate (BCP), and demineralized freeze-dried bone allografts (DFDBA).(17)In 12 months neither the density, nor the quality of the newly formed bone changed significantly, when the autografts group was compared with the spontaneous regeneration group without application of any filler material. Osteogenic functions of a spongeous ilium may not demonstrate excellent results in clinical practice, if early progenitor cells do not survive during transplantation. (18) Moreover, osteogenic influence of substantia spongiosa is reduced with the patients' age increase. Besides damage to the place of extraction of donor graft, other flaws of the method include the need of hospitalization and general anesthesia. A cortical plate and the necessary periosteum on the defect edge may be an important factor indicating the need to use additional autografts. When it comes to defects where more than one cortical plate is missing or periosteum does not provide full coverage, autografts may prevent surrounding soft tissues from getting into the cut, contain the volume of the defect, and provide the necessary number of osteogenic cells and growth factors for full regeneration of the bone tissue(12).

Allogenic implants

Allogenic implants may be fresh or frozen, freeze-dried, mineralized and demineralized; may come in the form of chips, cortical granules, wedges or spongy powder.(19)(20) Fresh allogenic implants have high osteoconductive and osteoinductive potential, however, they may induce immune response or transmit diseases.Frozen or freeze-dried implants are less immunogenic, besides, they have osteoconductive properties but they lose the osteoinductive ones.(20)Previously some authors used allogenic freeze-dried bones to fill defects after enucleation of jaw cysts, and in 17-25% of cases researchers reported about complications. Bodner compared the influence of decalcified freeze-dried bone allogenic implant (DFDBA, Dembone ®) on jaw defect osteogenesis (3-8 cm in diameter) after enucleation of a cyst with the control group in which absorbable gelatin sponges were applied (Gelfoam ®).

Radiographically, the height of the alveolar process and the relative density of the regenerating bone structure at 6 and 12 months were higher in the DFDBA group. After 24 months, bone density did not differ in both groups. The author has considered that osteogenesis of bone tissue was more intense and that the alveolar part of the lower jaw was not lost in height when using DFDBA.(21) In the available sources we have not found information on comparative studies of the use of DFDBA in simple enucleation and the management of a wound under a blood clot. A.Acocella and other authors(19) conducted clinical studies in 16 patients with atrophy of the alveolar process of the upper jaw after reconstruction using fresh frozen blocks of human tibial bone. After 4, 6, 9 months a part of the bone block was taken for histological and histomorphometry analysis. Each patient was assigned 1-2 blocks. During the operation, all the implants were well vascularized, they fit the volume of the defect. The observation period ranged from 18 to 30 months. Histological examination showed the formation of bone tissue, like the normal, and compact layer surrounded by bone marrow.

According to the authors, allogenic implants can be successfully used to treat patients with defects of the upper jaw and serve as an alternative to the use of autografts.

Embryonic (fetal) tissue

Embryonic (fetal) tissues are a separate group in the series of materials for bone grafting. In the 80s of XX century for the treatment of fractures, false joints, filling of bone cavities after sequestrectomy and cystectomy, embryoplastic tissue preserved in weak solutions of formalin was used. By this time, the bone tissue does not yet reach the required maturity, and the clinical effect is difficult to predict. In addition, abortion material is a risk category, so at present it is not used for bone grafting.

In the dental practice, a composition of fetal bone and cartilage has been developed, which during mix forms a homogeneous plastic mass, convenient for filling the bone defects - brefosteoplast.(22) The collection of material for brefoplastic operations is associated with significant difficulties typical for the transplant of allogenic bone in general. Placenta transplantation is used as a non-specific stimulator of the regeneration process. The umbilical cord, along with the amniotic membranes, is a biological complex that is formed during the initial stages of pregnancy. In addition to collagen, which consists of the stroma of the villus of the placenta, it includes a large number of low and medium molecular protein fractions, which are nonspecific growth factors.(23)Many authors considered the placenta as a source of GAG (glucose aminoglycans), which play an important role in the formation of the organic matrix of bone tissue.

For moral, ethical and religious reasons, in several countries of the world the grafting tissues of human fetus is prohibited. In our country, the legal framework on this issue is currently not defined.

Some authors recommend to use the stimulating effect of placenta in periodontal practice and at filling of cavity bone defects. For clinical practice prompted compositions with brefobone.

Xenogenic implants

One of the first xenogenic materials used for bone defects after cyst enucleation was the so-called “Keel bone” derived from the bone of the calf. Weiss described 14 cases of filling cavities after removal of cysts with bone keel, resulting in bone implant removal in 3 (21%) cases due to the development of an infectious process. He established the basics of accelerating bone regeneration and stabilizing the blood clot.(24)Structural features of collagen provide the drug expressed reparative and hemostatic properties: significantly reduces the duration of bleeding, swelling and pain, accelerates the healing process of wounds. The mechanism of this action is associated with the regulation of the number of proteolytic enzymes, which creates a basis for the growth of cells of certain tissues, their further organization and proliferation. In order to stabilize the blood clot and prevent infection, Schulte used a gelatin sponge in combination with thrombin in 1959.(5) After filling the jaw with defects of a minimum size of 20 mm with collagen, Buser and Berthold established an average bone regeneration time for the upper and lower jaw - 15 and 8.5 months respectively. In addition, the effect on the acceleration of healing processes has been proven. (25)The results of comparative studies of filling defects with collagen and simple healing under a blood clot after cyst enucleation are ambiguous. Joos described the initial stages of bone regeneration and found that 81.1% of his patients had a complete restoration of bone structure in 2 years after filling the defects with collagen compared to the group of conventional healing, in which after the same period of time the bone recovered in 61.8%. The sizes of defects in both groups were comparable (up to 4.32 sq cm and 4.08 sq cm, respectively)(26).In another study conducted by Mitchell in 100 patients with jaw defects, a 3-month reduction in bone loss was observed in the collagen filling group compared to the control group in which no materials were implanted. After 6-12 months, bone healing did not differ significantly in both groups. Mitchell suggested that collagen acts as a biological space filler and does not have osteoinductive properties, so the bone healing process can occur only together with the biodegradation of the material (27).

Carter and others described successful regeneration of a big bone defect of the lower jaw after enaculation of a cyst and filling with recombinant human morphogenetic protein (rhBMP-2), absorbed on collagen sponges(28). In the past years scientists developed the xenogenic materials which contain deproteinized bovine bone mineral (DBBM, Bio Oss

®) or coral derivatives (Algipore®) and phytogenic (Interpore®) hydroxyapatite, which are successfully applied for augmentation of the lower wall of the maxillary sinus(29).These materials, according to manufacturers, must have osteoconductive properties. Nevertheless, they are considered and estimated as non-absorbable materials with a various degree of the body response to a foreign body.(17) No reports on the use of such materials for filling defects after enucleation of a cyst have been found in the foreign literature. I. Myslevtsev and others developed osteoplastic material based on the skeleton of natural corals from the Acroporidae family and experimented on big and small laboratory animals. It has been found that natural corals have osteoinductive properties for multipotent mesenchymal stromal cells, inducing osteodifferentiation gene expression in them. A clinical study in patients (n = 10) with various skeletal bone formations bearing axial load, compared with the control group (n = 10), proved a high resorption rate of natural corals in the implantation zone, comparable to the rate of bone tissue repair (18-24 months). In the comparison group, by 18-24 months after surgery, bone tissue replacement of implanted β-TCP in the area of the defect was slower and was mainly carried out due to the increased connective tissue, which may indicate the low osteoinductive properties of these calcium phosphate materials.(30)

Alloplastic synthetic implants

Implantation of synthetic materials can accelerate osteoconduction and osteointegration. They must have biocompatibility when receiving minimal fibrous changes in the area of placement. Available synthetic materials include bioactive glass, glass ionomers, aluminum oxide, calcium sulfate, calcium phosphate, α- and β-tricalcium phosphate (TCP) and synthetic hydroxyapatite (HA).(20)The main disadvantages of these materials are that under clinical conditions they may show unsatisfactory results or have an unpredictable rate of resorption, as well as cause inflammatory reactions to foreign bodies(31).Regarding the treatment of defects of the jaws after enucleation of a cyst, there is accumulated clinical experience using tricalcium phosphate and hydroxyapatite(32).The idea of using these osteoconductive materials is to stabilize the blood clot in a defect in order to prevent the development of an infectious process and accelerate bone tissue regeneration by increasing the migration of osteoprogenator cells(32). While traditional hydroxyapatite is generally regarded as a non-absorbable material(17), resorption presumably occurs in the region of the new nano-hydroxyapatite due to its high solubility.

Traditional hydroxyapatite is a non-absorbable material,(17)but resorption still occurs in the area of new nano-hydroxyapatite due to high solubility. In preclinical studies on domestic pigs the nanoparticles of hydroxyapatite Ostim ® were compared with autogenous bone, as well as with a combination of Ostim ® with 25% of autogenous bone when filling bone defects of at least 1 cm in size. Microradiography and histology showed similar mineralization processes in all three groups. Complete resorption of hydroxyapatite nanoparticles was observed after 12 weeks .The authors recommend Ostim ® because of its low percentage of complications and safety of its resorption, which is proven by x-ray examination. Upon closer examination of the radiographs after 12 months, the remains of the implant are still visible, although the authors describe complete ossification in the area of the defect. This may indicate a delay or even lack of resorption of hydroxyapatite. In the study of V. M. Bezrukov and co-authors the cavity which was formed after cyst removal was filled with 33% Ostim-100 ® incombination with lincomycin (n = 49) and compared with simple enucleation (n = 43). The authors reported a higher rate of regeneration and a lower incidence of complications in the group with a use of Ostim ®.(33)

Tricalcium phosphate (TCP), especially its pure phase (β-TCP), is considered as a biocompatible, osteoconductive and absorbable material(25)(32). Until recently, Cerasorb was widely used to fill bone defects after cyst enucleation(32). In all these studies subsequent resorption of β-TCP was shown after 12 months. In a recent clinical study Horch and co-authors filled the defects with Cerasorb with blood, when the diameter of the cyst was less than 2 cm, and they were using Cerasorb in combination with regional spone bone (a 1:1 ratio) if the size of the cyst was less than 2 cm. Bone tissue restoration was revealed in 9.2% of cases with partial loss of β-TCP granules in 5.9% of cases, and in 2% of cases filling of the defect with material taken from the iliac crest was required. In x-ray examination after 12 months, a loss of 65% of the density of the β-TCP implant without sponge bone was visualized and a loss of 85% of the density was diagnosed in the case of a combination of β-TCP with sponge bone,that indicates accelerated biodegradation when β-TCP is mixed with autogenous bone.(32)

According to comparative preclinical studies in animals with bone defects like cysts of the jaws, the use of β-TCP leads to accelerated bone regeneration in comparison with the use of implants based on hydroxyapatite. This is due to the rapid resorption of β-TCP and its complete replacement with a new bone.(17) The results of combining osteoconductive and osteoinductive functions in experimental studies of bone tissue regeneration in the lower jaw in the case of use of spongy β-TCPcombined with stromal bone marrow cells can be considered promising(34).

A combination of hydroxyapatite and β-tricalcium phosphate, known as biphasic calcium phosphate, has also been studied in various ratios as a bone substitute. Such combinations should combine all kinds of osteoconductive and osteogenic functionof both materials and should be used in the zone of defects with different morphology when rapid adaptive resorption is needed(31).

It is difficult to objectively assess the difference between cystectomy with simple closure of the cavity and the procedure involved the use of additional bone grafts and implants, since clinical comparative studies are rare and the criteria for the occurrence of complications, bone regeneration are determined inconsistently. Most estimates are based on x-ray methods for measuring bone density, but it should be considered that some bone implants are not radiopaque, which makes it difficult to analyze the images and evaluate the newly formed bone tissue. It is difficult to distinguish between the physiological regenerating of jaw bone and the newly formed tissue at the site of the material using radiographs.(4)

Primary closure of defects with muco-periosteal flap and simultaneous use of antibiotics leads to complications in less than 5% of cases, even with defects much larger than 3 cm in diameter(8)(10). The infectious process can lead to such complication as a jaw fracture, which occurs in a maximum of 3% of cases, especially if it is in the angle of the lower jaw .(7) Currently, there is no convincing clinical evidence that the additional use of autografts, allogenic implants, xenogenic implants (gelatin, collagen) or synthetic implants (HA, β-TCP) significantly reduce the risk of infection during primary wound healing. There are reports of the development of infection when using modern synthetic materials, for example, rapidly resorbed β-TCP, which are comparable in this indicator with simple healing under a blood clot or with collagen(32).

Given bone regeneration, complete ossification of defects of the jaw up to 3 cm takes about 12 months after enucleation and simple closure with a muco-periosteal flap. For larger defects, complete healing of the bone defect can be expected no earlier than 24 months(8)(9)(10)). Preservation of the periosteum and bone walls is the most important factor for normal bone regeneration(12)(13)(14). Spontaneous bone regeneration may be delayed in elderly patients and in the case of bicortical or circular defects or defects in the anterior maxilla(9)(11).

Autologous sponge bone shows a high level of ossification during the initial healing period in the placement zone, but ultimately, in terms of bone density and its structure, the result is similar to those after simple healing of defects under a blood clot after 12 months (16).Stable membranes additionally prevent the prolapse of surrounding soft tissues into the wound(12) (14). Currently, there are no comparative clinical trials of transplantation of sponge bone of the ilium and simple enucleation of large cysts of the jaws. The autologous bone from the iliac crest is still the material of choice for very large defects with partial absence of the periosteum or the loss of more than one cortical plate, although donor morbidity, need for hospitalization and general anesthesia should be considered.

In recent times it has become very popular to use collagen for the stabilization of the blood clot. The risk of infection and bone regeneration seem comparable to simple enucleation, although the literature is inconsistent(25)(26)(27). The use of collagen is justified under certain circumstances, for example, for patients at high risk of bleeding. In the future, the results of its clinical application can be improved by combining it with growth factors or osteoblasts obtained by tissue bioengineering.(15)

CONCLUSION:

Today, surgeon dentists and maxillofacial surgeons in their practice use a huge amount of materials for bone grafting both natural and artificial origin. All implants or transplants must have a number of properties to ensure the desired clinical outcome, with predictable behavior in the bone wound and safety for the patient.

Defects in the bones of the facial skull are different in origin, size and shape. To eliminate them, a great variety of materials is used, information about which is not always available or contradictory. In such a situation, the surgeon would like to have a universal material with predictable effectiveness that meets the criteria for osteotropic drugs, has sufficient evidence base and is available in everyday practice. Opinions about what material meets the requirements vary widely, so additional research and clinical trials are needed.

Abbreviations TCP - Tricalcium phosphate
β-TCP - β-tricalcium phosphate
HA - hydroxyapatite
BCP - biphasic calcium phosphate
DFDBA - demineralized freeze-dried bone allografts
GAG - glucose aminoglycans
DBBM - deproteinized bovine bone mineral
rhBMP-2 - recombinant human morphogenetic protein

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