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Modern bone replacements including xenogenic materials of animal origin for reconstructive medicine and dentistry

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Development of biochemistry and engineering allows not only for better reuse of biological material obtained from the patient but also allows for the development of new materials for bone replacement. The specific factors contributing to the growing trend of the reconstructive medicine market are: the growing geriatric population, the increasing number of procedures using bone

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reconstruction methods and the favourable approach of the research financing institutions. Most reconstruction materials are based on animal derived substances. Materials meeting the criteria for the use in reconstructive medicine for bone structures are divided into allogeneic, alloplastic and xenogeneic including xenogenic materials of animal origin. This paper contains a review of the materials currently used to reconstruct bone defects and materials modifying the existing bone structure by supporting the processes of bone tissue regeneration.

KEYWORDS: animal-derived bone substitutes / bone replacement materials / bovine / regeneration

Regenerative medicine is one of the fastest growing branches of medicine. It is estimated that the value of the regenerative medicine market in 2019 reached the value of 6.5 million dollars. After blood, which can't be produced artificially [Roberts *et al*. 2016], the bone tissue was ranked second in terms of the frequency of transplants [Sanches *et al*. 2020]. The bone tissue is used in the orthopaedic and neurosurgical procedures. Moreover, in recent years, a dynamic growth of reconstruction techniques in the craniofacial area has been observed [Parisi *et al*. 2020]. Undoubtedly, it can be attributed to the dynamically developing area of the dental implantology techniques stimulating maxillofacial surgery and periodontology [Aerts *et al.* 2020].

The variety of materials that can be used to achieve excellent clinical results is constantly increasing [Li *et al*. 2020]. Overall, the bone-replacement materials can be distinguished into 3 groups: allogeneic, synthetic and xenogeneic preparations. Application of implantological screws, sinus lift or treatment of bone pockets are today routine procedures. In dental implantology, the choice of materials is very wide, but due to the specific anatomical area it is not a problem. For instance, it has been possible to reconstruct damaged and lost jaw bones or periodontal structures [Li *et al.* 2020]. However, a significant group of patients qualified for the procedures are patients with advanced cancer in the craniofacial area [Yamada *et al*. 2020]. Every year there are about 600,000 new cases of head and neck cancer worldwide. Mechanical injuries, in turn, result from: traffic accidents, robberies, fights, accidents at work or sports injuries. The extent of cancer or mechanical tissue injury and the duration of the procedure significantly affects the reduction of the number of patients operated on a daily basis, which results in a longer waiting time of patients for the surgery, and thus the progress of the disease [Feng *et al*. 2020]. The problem is additionally hindered by the patient's condition, as most of the currently diagnosed neoplastic diseases are in an advanced stage of the disease requiring partial removal of the diseased bone.

For years, reconstruction with the use of the patient's bone from another part of the body, such as the forearm or lower leg, has been used [Cohen *et al*. 2020, Ocak *et al*. 2017]. However, not all patients are eligible for this type of treatment, and the procedure of bone collection and preparation lasts from 1.5 to 2 hours. Moreover, in a group of people who might have performed a bone extraction procedure, 20% of patient do not consent to its collection. Annually on average 400 maxillo-sitium reconstruction operations, 100 orbital reconstruction operations, 50 nasal reconstruction operations, 100 anterior cranial fossa reconstruction operations, including the wedge-sit ceiling

and 300 jaw reconstruction operations are performed globally [Mathew *et al*. 2020, Silva *et al*. 2020].

For such patients, there are very limited possibilities for the choice of bone reconstruction materials. First of all, it results from the specificity of the region of action, which is characterized by a very complex anatomical structure, and thus is characterized by high anatomical variability and details [Shokri *et al*. 2020]. This results in the fact that in surgical practice the bone taken from the patient undergoing treatment is most often used. Such action results in longer surgery time and full involvement of the operating team, and thus generates high costs such as the cost of anaesthesia or medications used [Xin *et al*. 2020]. The collection of bone or flap of free or bulbous soft tissues for transplantation is an additional 1.5 to 2 hours of surgery time. Not without significance is also the fact that there is an increased risk of complications, such as infections, both in the place where the material is collected and in the place where it is implanted, which increases the risk of death of the implanted structure, posing further risks to patients health [Xiong *et al*. 2020].

In order for the implanted structure to initiate proper tissue regeneration within the treatment area, it is necessary to meet certain conditions. The presence of osteogenic cells, osteoinductive signals transmitted by means of growth factors, appropriate structure and a supply of blood and nutrients [Cheng *et al*. 2020] is desirable. In the case of autologous bone implantation, i.e. when the donor and the recipient are genetically identical, all the above conditions are met. The use of autologous bone is the most advantageous from both the biological and immunological point of view and from the legal point of view [Mounir *et al*. 2020]. In most cases, this material is taken from a plate of hip bone or ribs. In surgical practice, the reconstruction of the craniofacial area after the removal of the tumour requires the performance of bone extraction operations from, among others, the hip plate, forearm, personalization of the shape of the collected bone, closure of the wound, i.e. the place where bone tissue was collected, opening of the new wound, i.e. the place where the tumour was detected, removal of cancer tissue, implantation of personalized bone tissue and closure of the wound [Chiapasco *et al*. 2020]. All these activities are extremely time and manpower demanding and pose a large risk to the patient's life. Such a situation also generates a risk of complications such as infections, hematoma, nerve damage, chronic postoperative pain at the place of collection and postoperative deformities of the donor point [Sethi *et al*. 2020].

Allogeneic materials

In the allogeneic transplants the grafted material is allogeneic bone, i.e. the material which shows identical genetic features between the donor and the recipient. The challenge of this type of transplant, apart from the possible postoperative complications described above, is the amount of collected material, which in many cases is not sufficient to perform a full-fledged procedure of reconstruction of the

damaged bone fragment [Lago *et al*. 2020, Francisco *et al*. 2020]. The solution in this case is to apply commercial bone replacement materials. The material for allgonene implants is subject to a strict procedure of collection. The donor's medical records are analyzed and serological samples are taken to exclude HIV, HBV, HCV and syphilis. Only the exclusion of all these diseases allows the collection of material which is then subjected to radiation sterilisation and low temperatures [D`Elia *et al.* 2020]. This preparation undergoes a freeze-drying process consisting of freezing and drying the material. What remains afterwards is a material that has the characteristics of a collagen scaffold with a high hydroxyapatite content and contains growth factors. This results in two types of cell-free material: demineralized freeze – dried bone allograft (DFDBA) or demineralized material, called demineralized bone matrix (DBM) and mineralized form of dried bone allograft (FDBA). Demineralised bone structure due to earlier processes has exposed collagen fibres and organic proteins and thus has better osteinductive properties [Laugisch *et al*. 2019, Kothiwale *et al*. 2019]. Such properties are obtained by using NHCL or EDTA material in the chemical preparation process. As much as 90% of the newly formed material is occupied by type I collagen, the remaining 10% are non-collagen proteins that determine the osteinductive potential. The resulting preparations, although deprived of cells, stimulate the process of remodeling and revascularization, which takes place much slower than in the autologous bone environment [Zhou *et al*. 2018, Fujioka-Koabayashi *et al*. 2017].

Synthetic materials

The second group of bone-replacement materials consists of alloplastic preparations described as synthetic. These are materials produced synthetically or from natural organic sources such as algae or corals or inorganic theses, which include hydroxyapatite or bioactive glass [Shi *et al.* 2020]. The oldest known alloplastic material is calcium sulphate, which is biocompatible and has a resorbability of 30 to 60 days [Hung *et al*. 2020, Saha *et al.* 2019]. Fast rate of its resorption does not allow for a complete development of a proper bone structure after its surgical introduction. Calcium sulphate, however, is an excellent carrier of drugs, which allows it to be used as a barrier membrane and additive to other, more modern preparations [Leventis *et al.* 2018].

Modern preparations are based on calcium phosphate salts, which can be divided into ceramics and cements. The differences are due to the different physico-chemical properties and, consequently, to the different manufacturing processes. Ceramics are packaged as compact blocks, which can be formed in any way depending on the defect, which is subject to reconstruction. Cements, on the other hand, are in the form of powders and pastes, which often harden during implantation after mixing with the patient's blood, adapting to the shape of the defect [Cheng *et al*. 2020].

Osteconductive materials serve as transplants and implants and are an inactive scaffold supporting the growth of active bone cells in the cavity area. They ensure appropriate optimal conditions for the growth of bone forming elements within the

treated lesion. The most popular are ceramic materials, which in turn are divided into biocompatible materials, i.e. without negative reactions of the organism, and bioactive materials, where their task is to stimulate the bone formation process [Singh *et al*. 2020, Cole *et al*. 2020]. Both in natural and synthetic materials the key role is played by calcium phosphate or hydroxywapatite. It is an active mineral compound found in normal human bones. Hydroxyapatite can be obtained in a synthetic form, or it can be of natural origin. An alternative to often used animal origin biomaterials (for example of beef origin) is material made from corals. Such material enriched with strontium and silicon is stronger than synthetic materials. At the same time, it is completely resorbable and free of the risk of negative reactions with the patient's tissue [Pountos *et al*. 2016]. As the bioceramics of hydroxywapatite is very brittle, it must be combined with other substances to allow its use in areas particularly exposed to mechanical stress [Sundarabharathi *et al.* 2020, Zo *et al*. 2020]. Tri-calcium phosphate has better properties than hydroxyapatite, which, being a bioceramic material, has better chemical solubility. Additionally, it is quickly resorbed and in place of its implementation, due to dynamic osteoconduction processes, proper bone structures are formed. However, this material shrinks during the bone formation process, resulting in a smaller final volume in relation to the amount of material applied. Therefore, this material is used as an additive to other materials [Suzuki *et al*. 2020].

Biologically active glasses are a hard and elastic material. Their chemical composition is based on 4 substances: silicates, sodium oxide, calcium oxide and phosphorus oxide. These are bioactive compounds, reacting to body fluids. At the moment of contact with them, as a result of many chemical reactions, hydroxywapatite is formed, which in turn is a connection with the patient's bone. Such a system is characterised by a relatively high mechanical strength [Shymon *et al.* 2020].

Modern synthetic preparations are polymers. Polylactide or polysulphone are one of the many examples that have found application in bone tissue regeneration. Polymers are not subject to corrosion caused by the biological environment, they are lightweight and resistant to mechanical deformation. The main application was in reconstruction techniques within the craniofacial area. Polymers, depending on their composition, may be resorbed in different ranges, but they have a disadvantage, which is a periodic change in properties. This is the so-called ageing process of polymers during which their mechanical strength is significantly reduced [Yao *et al*. 2020, Rufino *et al*. 2020]. Other modern materials such as titanium, aluminium oxide, water glass, zirconium oxide or porous ceramics are also used as bone substitutes [Moest *et al.* 2020, Beckmann *et al*. 2020].

Xenogeneic materials of animal origin

The third group of bone-replacement materials consists of xenogeneic preparations which are made from compact or spongy bone of animal origin, often of bovine, porcine, sheep, rabbit and ostrich origin [Linde *et al*. 1989, Thoma *et al.* 2010, Cooper

et al. 2008, Jiang *et al.* 2015, Leventis *et al.* 2018, Moest *et al.* 2020]. They are considered to be biocompatible with human recipients and have osteoconductive properties [Sheikh *et al*. 2015]. This material has been divided into two groups: de-inflated bone and de-mineralized bone [Mendoza -Azpur *et al.* 2019].

The de-inflated part is a scaffold with a structure similar to human bone. The initial material is subjected to strict physico-chemical processes. It is treated with strong-base baths, high temperature and radiation. As a result of the temperature, the material can be divided into un-sintered and sintered. Such preparations are available in form of granules and blocks showing only osteoconductive activity, also without greater mechanical strength, which disqualifies the preparation for use in places exposed to increased loads. However, these preparations make a significant contribution to bone density at the site of administration [Moussa *et al.* 2020]. The preparation obtained is almost pH-neutral, which in turn supports bone formation processes in the initial stage of development. In the past, bovine xenografts had failed due to graft rejection [Melcher *et al*. 1963], which was probably due to chemical detergent extraction techniques that left residual proteins and hence produced adverse reactions [Emmings *et al.* 1974]. An advantage of these graft materials is the higher osteoconductive potential compared with synthetically derived materials. Bovine-derived bone grafts (particulate and blocks) have successfully been used for the treatment of human intrabony defects and ridge augmentation [Valentini *et al.* 1997, Thoma *et al*. 2010].

Animal origin preparations made from porcine tissues are a biocompatible material with a structure similar to that of human bone [Linde *et al*. 1989]. Remaining organic components must be removed by heat treatment at high temperatures. Such a technological process produces a higher crystal structure and a significantly larger hydroxyapatite crystal [Gao *et al*. 2006, Nazirkar *et al*. 2014]. A comparison was made of the material produced from pig cells and the material made of the mineral part of bones of Australian cattle. The porcine product has been shown to offer a similar cellular response and bone regeneration as the bovine bone product. The micro-rough surfaces of both preparations are believed to promote the adhesion and proliferation of osteogenic cells [Eun-Bin *et al*. 2019]. The possibilities of using mesenchymal stem cells derived from equine synovial fluid (SFMSC) were also investigated [Cucchiarini *et al*. 2014]. It has been shown that SFMSC implantation is less aggressive compared to other techniques. Further research is planned [Zayed *et al.* 2018].

For material collected from sheep, a good biocompatibility has been demonstrated when tested *in vivo* with the New Zealand white rabbit. No toxicity, pyrogenic reaction, irritation or cytotoxicity of the materials was found [Xu *et al*. 2011]. The surface structure is important in the assisted bone formation techniques. In case of smooth surfaces, fibrin fibres with connective tissue and preosteoblasts may break away. This will lead to the formation of the bone structure at a certain distance from the implant. If the surface is rough enough, it will provide the newly formed structures with a sufficiently strong adhesion [Wang *et al*. 2020]. Thus, the bone will be formed on the surface of the implant. This phenomenon is used in dental implantology, where

the implant surface is shaped in a strictly defined way to create cavities of a size suitable for newly formed bone structures, thus ensuring optimal conditions for tissue growth [Park *et al*. 2020].

Stem cells and blood

In order to optimize and accelerate the processes of bone formation and regeneration of bone structures, stem cells and growth factors isolated from blood are used [Huang *et al*. 2020]. The therapy is based on both embryonic and non-embryonic cells [Yamada *et al*. 2020]. Embryonic stem cells are separated from the pulp of milk teeth and umbilical cord blood. The method based on the separation of platelet growth factors from the blood of an adult patient is becoming popular. The obtained platelet mass in ambulatory conditions is added to materials used in controlled tissue regeneration. In recent years, menzenchymal stem cells of fat tissue and bone marrow have gained significant importance [Wang *et al*. 2020]. Mesenchymal stem cells produce an anti-inflammatory local environment, thus inhibiting intensive proliferation of T and B lymphocytes, thus stimulating the body to rebuild damaged tissue structures. These cells also have the ability to induce the process of production of new bone structures by accelerating the proliferation of osteoblasts and mineralization of the produced bone matrix [Dompe *et al*. 2020].

Reconstruction materials – challenges and opportunities

Reconstructive procedures and especially those in the craniofacial area, due to its complex anatomical character, are subject to a particularly high risk. Despite many materials available in modern surgery, there is a lack of materials that can be used for reconstruction of the craniofacial area. There are risks associated with bone collection and implantation for the patient to restore the defect: infection, death of the transplanted lobe or bone, which destroys the work of surgeons and causes additional patient's suffer, deformation of the tissue in the area of its collection, visible deformation of the patient's face, which affects the perception of his person in society and the psyche, resulting in both a deterioration in health, but also a decrease in productivity and well-being. Reconstructions also generate large treatment costs for patients affected by cancer hospitalized due to craniofacial injury.

The increasing use of 3D printing technology is creating new opportunities. Until recently reserved for the precision industry, it now offers the possibility to print physiologically active tissue structures [Negreisros *et al*. 2020]. Currently, there are many material solutions available on the market that can be used in 3D printing technology [Wu *et al.* 2018]. The following systems should be mentioned here: FDM based on biodegradable and non-biodegradable materials such as ABS, PC, PC-ABS, PLA, nylon; SLS/SLM based on powders: metals (titanium alloys), ceramics and polymer (polyamide P12); SLM based on: metal powders; 3SP based on: resins; as

well as bioplotters based on: collagen, chondroitin sulphate, alginates [Liu *et al*. 2020]. Printing even the most complex shapes is possible with the use of SLS technology, however, the market lacks biodegradable and non-biodegradable materials that would be used in the area of SLS print [Li *et al*. 2018].

Since the medical market aims to be able to print highly accurate shapes from biodegradable and non-biodegradable materials that can accurately reproduce the resulting tissue loss, one of the main streams of current work is to focus on the development of powders from biodegradable and non-biodegradable composite materials suitable for SLS printing. The use of 3D printing technology for medical applications is very attractive. Acquisition of tissue scaffolding characterized by: high accuracy, proper porosity and physicochemical properties on which bone tissue (biodegradable materials) or tissue (non-biodegradable materials) will grow after implantation in combination with shape personalization is available thanks to the SLS technology [Doustkhah *et al.* 2019].

Currently, work is underway to develop a technology for obtaining personalized tissue scaffolding for use in reconstructive surgery [Wu *et al*. 2018]. The use of such a product will depend on the clinical case and the choice of the combination of project results will be made by the surgeon. The overarching result of the developed technology will be the creation of a completely new technology for the treatment of patients, consisting of the supply of both biodegradable and non-biodegradable tissue scaffolds to clinics, personalized in terms of their shape, which will be able to settle with the patient's target cells (osteoblasts) formed from the output cells: bone marrow stem cells (as a result of differentiation) or fat cells (as a result of transdifferentiation). As a result, the surgeon will have access to personalized tissue scaffolding in the operating room before the surgery, which will significantly accelerate the recovery of the patient. This will allow the surgeon to avoid the surgery of collecting the patient's bone tissue in order to supplement the defect formed after the resection of the area which was disintegrated as a result of the disease.

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