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CREATING OSTEOPLASTIC MATERIALS TO REPAIR JAW BONES DEFECTS

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ABSTRACT — In the course of our research we have developed a technology for fabricating an osteoplastic material from natural bone tissue. The obtained preparation in the form of gel contains hydroxyapatite, calcium triphosphate compounds and stimulators for regeneration. The proposed experimental material produces a stimulating effect on the growth of animal cell types; it enables to produce bioactive materials with increased biocompatibility. Application of the experimental gel facilitates the process of bone-tissue regeneration in the laboratory animals, which, in its turn, confirms the optimal composition of the material. We have established that during the integration of our osteoinductive material the defect zone is completely replaced by the bone tissue.

KEYWORDS — bone defects, osteoplastic materials, bone powder, dental implantation.

INTRODUCTION

Today, osteoplastic materials are widely used to repair bone defects in maxillofacial surgery and dental surgery. Due to the bone resorption and alveolar bone atrophy the alveolar crest height decreases by 7 mm after one year with about 50% left from the original volume. That is why there is an acute need for replanting of an osteoplastic material aiming at performing a dental implant procedure. After the operation it is necessary to repair the dental bone defect of the jaw. The main problem of implant treatment is not only installation of a right-sized implant, but the build-up of the necessary volume of hard and soft tissues around it, what leads to an esthetic and functional result [1].

For this purpose, auto-, allo- and xenografts are used.

Currently available evidence has shown that the autogenous bone has the highest osteoinductive potential, but there are some disadvantages: a risk of resorption, a complicated preservation procedure, a necessity of inflicting an additional injury to a patient during sampling as well as a biological incompatibility of donor and recipient.

The use of allografts or xenograft entails the risk of immune conflict, which may result in rejection and attachment of secondary infection. [1, 2].

To date a limited line of bone replacement materials is brought to market which are applied in maxillofacial surgery, dental surgery and traumatology in general. Bio-Oss (Switzerland) and Osteo-Biol (Italy) are the leaders among the imported materials. These products have 98% of survival rate and higher, but they are several times more expensive than domestic analogues. Local osteoinductive materials are relatively inexpensive but they have significant disadvantages:

— lack of flexibility;
— low level of biocompatibility [3].

Therefore, it is highly desirable to produce an osteoplastic material which would be a mix of bioavailable components of bone matrix in combination with sources of energy and stimulation of osteocyte division and proliferation.

Aim of the work is to create and investigate a new internationally competitive material, fully bioinert and biocompatible with the body tissues, capable of replacing and compensating for jaw bones defects. For the first time, its composure will contain nanoparticles of silicon dioxide and minerals.

MATERIALS AND METHODS

Bone powder obtained by pretreatment of the tubular bones of slaughter bulls was used as components. The femoral diaphysis was stripped of muscles and ligaments and the cortical layers were sawed off, exposing the spongy bone. Spongy bone tissue was crushed in a hammer mill (Molot 200/800) to a particle size of 1–1,5 cm^3. Then the bone blocks were placed in a 2% solution of sodium chloride for 24 hours, washed with water, then placed for hydrolysis in a 0,4 n solution of sodium hydroxide for 24 hours; the blocks were washed with distilled water until complete neutralization of sodium hydroxide. After washing the blocks, they were degreased in chloroform and acetone for 48 hours, after which the blocks were washed again with purified water while constantly stirring on a magnetic stirrer. An intermediate control was performed: the blocks were stained with Sudan for the presence of fat and lipoproteins. Until a negative reaction result is obtained. Then the composition

was calcined in a muffle furnace (ELF 11/6), evenly increasing the temperature from 100 to 300° C for 5 hours. An intermediate control was performed — for Lowry-Barnstead protein. The negative reaction result served as a criterion for moving to the next technological process. Then an intermediate quality control was performed on the mineral component. It is carried out with a standard 1% H_2SO_4 solution. The obtained blocks were dissolved without residue and without sediment. The blocks of the bone mineral component obtained in this way are cream-colored blocks consisting of a compound of natural hydroxyapatite with phosphates and carbonates. The resulting blocks were crushed on an ultrasonic homogenizer (MEF93.1) to a powdery state and sterilized. As the most effective methods of sterilization of the obtained bone powder, the air method of sterilization (180° C — 30 minutes) in a drying cabinet ШС 35/250-250-П-Standard and microwave radiation in a microwave sterilizer LS-B701 were chosen. Sterilization control was performed using physical and chemical methods. The physical method of monitoring the operation of sterilizers was carried out by using control and measuring devices that record temperature, pressure and time. The chemical method consisted in the use of chemical tests and thermochemical indicators, which were placed in the sterilization chamber at control points for each tab of materials, both outside the packages and inside the packages. The effectiveness of sterilization was controlled by a bacteriological method, using biotests of sterilization, which are objects seeded with test microorganisms. The bacteria Bacillus licheniformis strain G VKM B-1711D in the amount of $n \cdot 10^6$ are used as test microorganisms. They are highly resistant to heat, so they are used to control sterilization. Based on the death of test microorganisms, a conclusion was made about the effectiveness of the process.

After sterilization, the powder was mixed with glucosamine and a premix containing minerals and vitamins (observing the rules of asepsis). To obtain a paste-like mass, a hyaluronic acid gel was added to the mixture with constant stirring, and mixed until a uniform cream-colored viscous-plastic mass was formed. For 3 months, 60 Wistar rats with an approximate weight of 120–130 g participated in the experiment. The animals were divided into two groups of 30 in each: group 1 was made an incision on the lower jaw under intra-abdominal chloral hydrate anesthesia at a dosage of 350 mg/kg of weight; then a defect in the form of a cavity of 0,5×0,5 mm was formed with a diamond (spherical) boron. The test sample of osteoplastic material was inserted into the formed defect. The wound was tightly sutured with monofilament suture material *Vicril*. The 2nd control group also consisted of 30 rats with similar defects. In the process of regeneration of this group we did not interfere.

RESULTS AND DISCUSSION

For material production powered bone mineral components and liquid hyaluronic acid solution were used [4]. The paste for filling the bone defect was made according to the developed technological scheme (Fig.1).

To obtain paste consistency, hyaluronic acid gels of various concentrations were used in the work. (1%, 1,5%, 2%). 1% gel had necessary rheological characteristics, stimulated tissue regeneration, accelerated scar resorption [4, 5]. In this regard, 1% hyaluronic acid gel was used in further work. The bone tissue material was placed in 10% neutral formalin and then placed in a decalcifying solution based on Trilon B. then paraffin blocks were made according to the standard histological method both with hematoxylin and eosin staining and according to Van Gieson. Evaluation of microphotographs was made with a microscope LeicaDM 100.

During the experiment, when integrating osteoinductive material, the defect zone was completely replaced by bone tissue, where the main components of the lower jaw bone were determined and represented by compact and spongy bone.

During an experimental morphological study of the biomedical characteristics of the osteoplastic material developed by us, it was found that when introduced into a bone wound, it contributed to the activation of reparative osteogenesis in the area of lower jaw injury in rats, which indicates a successful replacement of the bone defect.

CONCLUSIONS

1.Bone powder and glucosamine were a matrix for filling the bone pockets with a different number of existing limiting bone walls, as well as for restoring periodontically affected areas of root furcation. Implantation of bone powder along with glucosamine showed high biological compatibility with recipient tissues: practically there was no inflammatory reaction, as well as systemic and local toxicity. During implantation, the normal content of calcium and phosphates in blood serum was maintained due to the supply of calcium ions from bone meal to a specialized bone pool. In addition, hydroxyapatite resorption took place without the formation of a fibrous capsule around the implant.

2. Premix that contained microelements and vitamins induced the speeding up of the reparative osteogenesis.
3. One of the main differences in the technology of

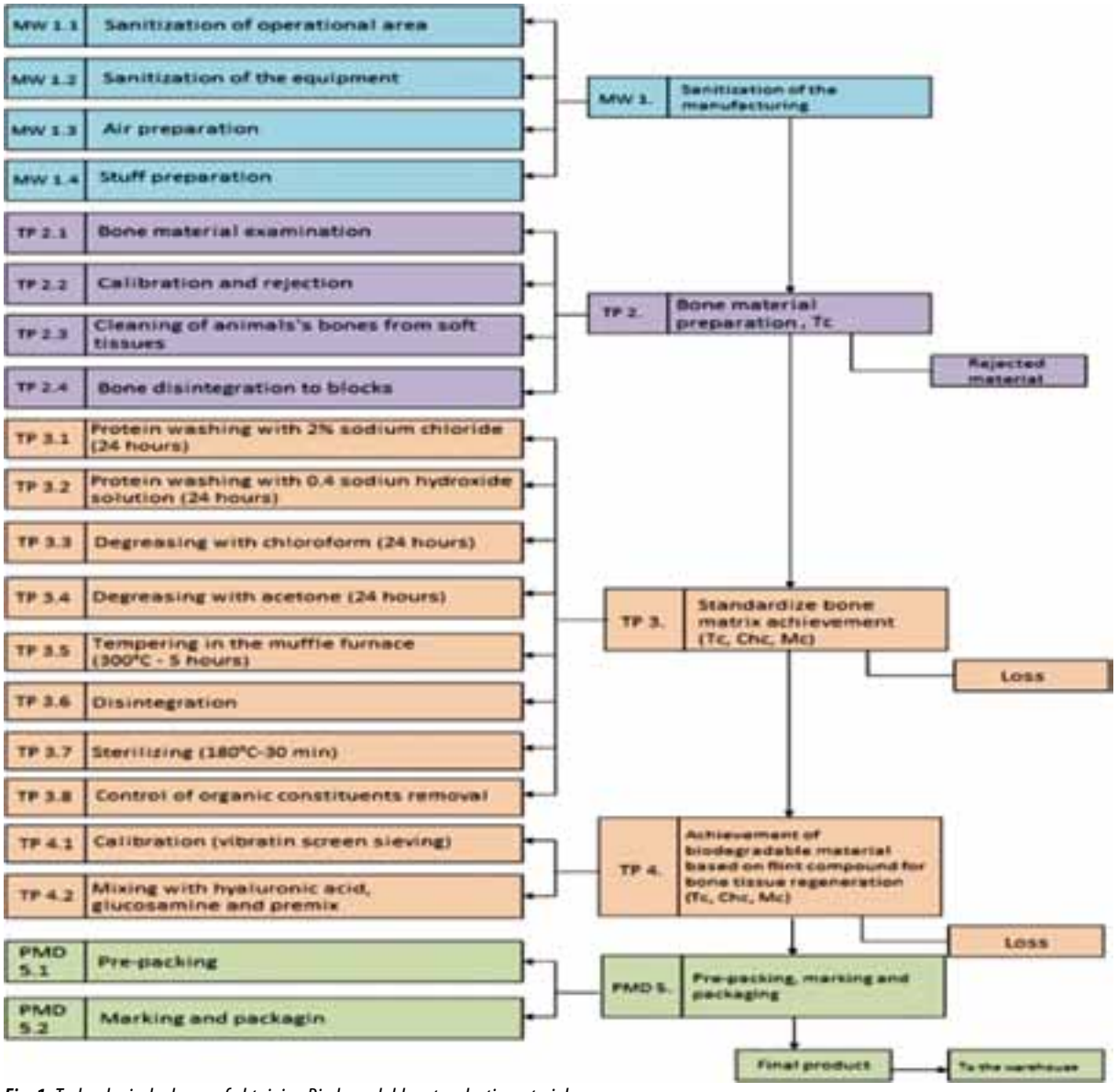

Fig. 1. *Technological scheme of obtaining Biodegradable osteoplastic material for bone tissue regeneration*

Table 1. *Ingredients of biodegradable osteoplastic material*

Ingredients	Content, %
Hyaluronic acid	10
Bone powder	75
Glucosamine	5
Premix*	15

** Premix containing silicon and magnesium oxides, zinc, manganese and copper sulphates, sodium borate and tricalcium phosphate, ascorbic acid, pyridoxine hydrochloride, cholecalciferol*

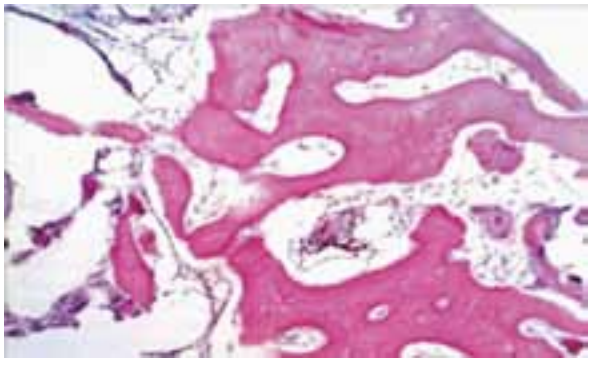

Fig. 2. *Formation of bone tissue after application of osteoplastic material. Staining with hematoxylin and eosin x100*

the developed bioactive osteoplastic preparation was the creation of a slowly metabolized spatially ordered system consisting of the material itself for osteofixation and energetically functional substrates actively involved in the metabolism and formation of bone tissue. All that, in our opinion, creates the possibility of including polysaccharides and vitamins that have a stimulating effect on the growth and proliferation of osteophytes, the formation of a collagen framework.

4. The proposed composition was slowly absorbed by the organism satisfying the local need of organism (in the place of bone tissue damage) for trophic, plastic and energy components.
5. The obtained osteoplastic material had the following properties:

— good tissue tolerance;
— porosity, ensuring bone germination;
— possibility of sterilization without quality changing;
— availability and low price (main components of domestic origin).

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