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Morpho-compositional study of the ageing biomaterial in bone implants

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The restoration of the missing structure for damaged or destroyed tooth, are achieved with a variety of treatments and advanced biomaterials. Each case needs appropriate biomaterials, especially in direct implants where defects of human skeleton may occur. The biomaterials behavior related to a specific human skeleton is the most important issue in the restorative dentistry. This contribution reports few aspects referring to morphology and composition behavior of the few biomaterials used for bone recovering in dental surgery.

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Keywords: Dental addition biomaterial, Osteogenesys, Biocompatibility, ESEM

1. Introduction

The restoration of the bone defects of the human skeleton based on new advanced biomaterials with increased biocompatibility is of great interest in the restorative therapy. In order to obtain biocompatible materials with improved properties, it is noticed a major progress concerning techniques and reconstructive surgery in orthopedics, traumatology, oral -maxillo - facial surgery and periodontology [1-11].

The purpose of this study is to enlarge the investigations of the long-term properties, in peculiar resumption of the addition of biomaterials used in the stomatological surgery [12, 13].

The study is based on correlation between clinical observations and characterization of the biomaterials addition, to establish ways and methods for improved properties. We conclude with the correctness of indications and contra-indications, the correlation between biological response and clinical symptomatology, the appropriate methods of new osteogenesis and causes of the failures, comparing with autogenous material.

2. Materials and methods

Biomaterials as:

- Hydroxiapatite (NANOBONE ®) [14],
- Ceramic-based β -Tricalciumphosfat (CERASORB ®) [15],
- Cortical spongy bone collagen (OSTEOBIOL ®), and well – known autogenous bone as reference for comparison [16] were used.

Experimental support: animal house

A rabbit in the race New Zeeland, white male. Age of animals at the beginning of the experiment was five months.

Methods:

Rabbits weight along the experiment was: rabbit slaughtered in 30 days - 2.30 kg, rabbit slaughtered in 60 days - 2.25 kg, rabbit slaughtered in 90 days - 2.35 kg.

The medication administrated before surgery was Acepromazina 3 mg/body kg as tranquilizer and ketamin 100 mg/body kg as anesthetic.

Schedule insertion for augmentation biomaterials was the same for each animal:

- right tibia: defective education - autogenous bone - witness the test, lower defect - test sample; Cerasorb ® Curasan, Germany 50-150 μm.
- left tibia: - higher defect Osteobiol ® Gen-Os mix 0.5 g Tecnoss, Italy-test study; lower defect - Nanobone ® 0.6 mm, Artoss, Germany - test sample.

The defects were surgically created by milling with an auger diameter of 5 mm [17]. Autogenous material was collected at the moment of the defect creation (Fig. 1).

Maintenance conditions were standard during the experiment: 18-24 °C temperature, relative humidity 45-65%, natural ventilation, conventional farming system. Rabbits were housed in individual cages with dimensions in accordance with European standards.

Feeding was done with granulated fodder mixed with standard product to the laboratory fodder, Bucharest Cantacuzino Institute. During the experiment, we used feed from the same batch. Water was always available for the animals, automatic watering. After administration of the implants, the health of the animals was good and were not registered post-operative problems. Treatments with antibiotics and vaccination were not conducted during observations.

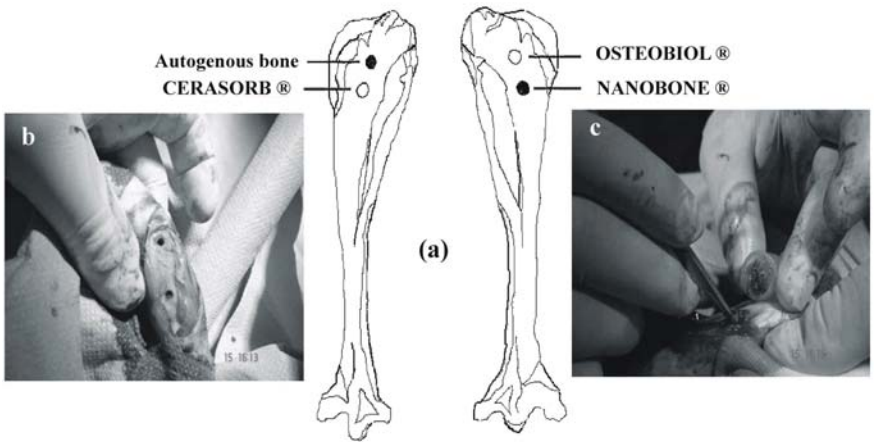

Fig.1. a) Schedule insertion materials. b) Bone defects created by milling. c) Application of additional material at the bone defect.

Weight of the rabbits at the end of the experiment was: rabbit sacrificed 30 days - 2.52 kg, the rabbit sacrificed at 60 days - 2.83 kg, the rabbit sacrificed at 90 days - 3.11 kg.

Bone fragments were collected after the euthanasia of the rabbits. The collection of pieces took place at intervals of 30, 60 and 90 days. Rabbits' euthanasia was conducted by administration of carbon dioxide mixed with oxygen. The experiment was conducted following ethical rules of protection and welfare of laboratory animals imposed by the European and national legislation.

Characterizations: The micrograph and local composition of the implants were analyzed by environmental scanning electron microscopy (ESEM) coupled with energy dispersive X-ray analysis (EDAX) for local composition measurements.

3. Experimental results

SEM morphologic analyses of the biological samples were collected 30 days after implantation (Fig. 2)

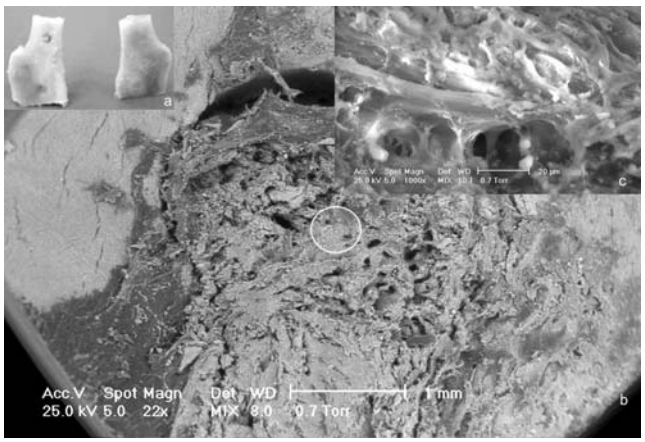

Fig.2. a) The pieces of bone harvested in order to be analyzed by SEM. b) Autogenous bone implant after 30 days (x22). c) Autogenous bone implant after 30 days(x1000).

30 days after implantation of the autogenous bone, the image on electron microscope appears as a homogeneous mass which fill the defect (figure 2b). There were noticed the edges of the top items fibro genetics. The fibril network is extremely rich, invading the implanted autogenous bone tissue as detailed in Fig. 2c.

The autogenous bone around of implants of different biomaterials, is developing with the same morphology and structure when biocompatibility is appropriate. Morphology and structures is observed at 30, 60 and 90 days time.

30 days after implant insertion of ceramic-based β-tricalciumphosfate (CERASORB ®), the image of electron microscopy appears as a scratchy mass, net bounded to the defect (Fig. 3a, b). Neo differentiated fibril network at the edge of the defect is stronger to the implanted material similar with other reports [18].

30 days after implant insertion of cortical bone - spongy collagen (OSTEOBIOL ®), it presents on electron – microscopy investigation as scratchy, with particles of different grain in a homogeneous matrix of fibrous tissue, in intimate contact to the defect walls (figure 3 c, d)

30 days after implant insertion, ceramic hydroxiapatite (NANOBONE ®), it is found that it does not adhere at the damaged walls, the collagen fibers being developed thin and immature (Fig.3e, f)

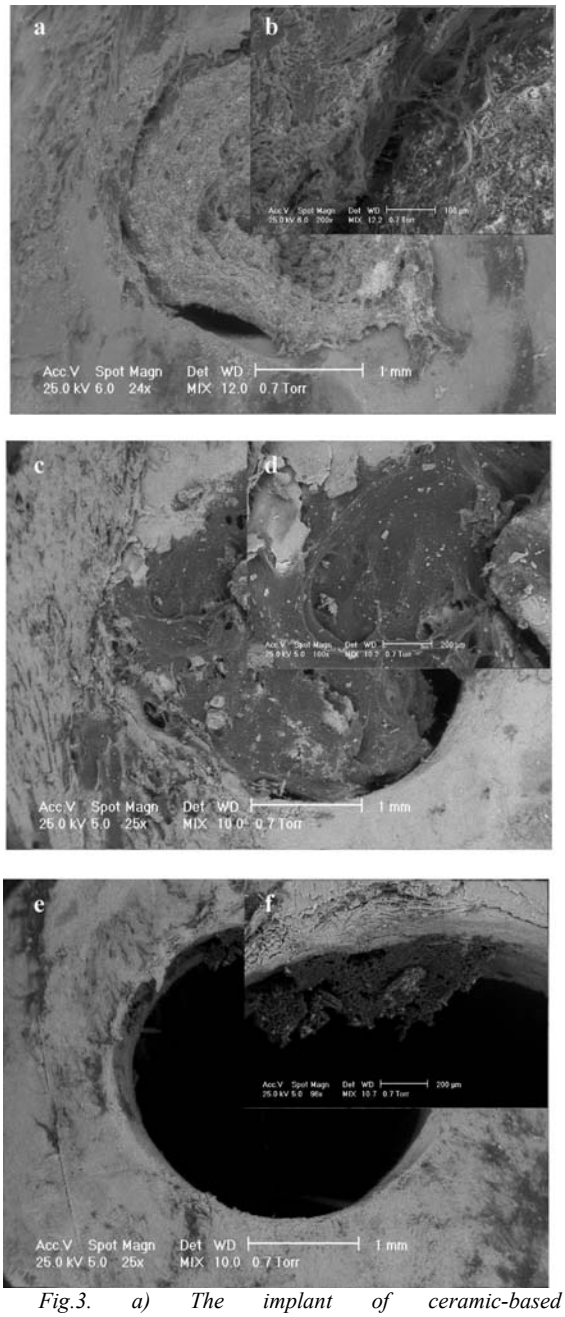

Fig.3. a) The implant of ceramic-based β-tricalciumphosfate (CERASORB ®) after 30 days (x24). b) The implant of ceramic-based β-tricalciumphosphatee (CERASORB ®) after 30 days (x200). c) ESEM image of cortical bone implant - spongy collagen (OSTEOBIOL ®) after 30 days (x25). d) ESEM image of cortical bone implant - spongy collagen (OSTEOBIOL ®) after 30 days (x100). e) The implant ceramic hydroxiapatite (NANOBONE ®) after 30 days (x25). f) The implant ceramic hydroxiapatite (NANOBONE ®) after 30 days (x96).

After a morphological analysis using ESEM electronic microscopy of all biological samples collected at 30 days after the insertion of materials, in addition to receiving of bone bed, we noticed that:

• For any of the investigated materials, we did not find any evidence of intense fagocitary biological process, or intolerability of the implanted material;
• Although the process of local recovery started with this stage (30 days),time variations depending on the type of material used for augmentation addition defect may appear; all materials used for space replacement are invaded in this tissue of the grain and a transformation into a fibrous tissue was observed.
• Speed of the recovery and quality design of the repairing tissue is on descending order with autogenous bone, cortical bone - spongy collagen (OSTEOBIOL ®), ceramic - based β - tricalciumphosfate (CERASORB ®), ceramics hidroxiapatite (NANOBONE ®).

SEM morphologic analysis of biological samples was collected at 60 days after implantation.

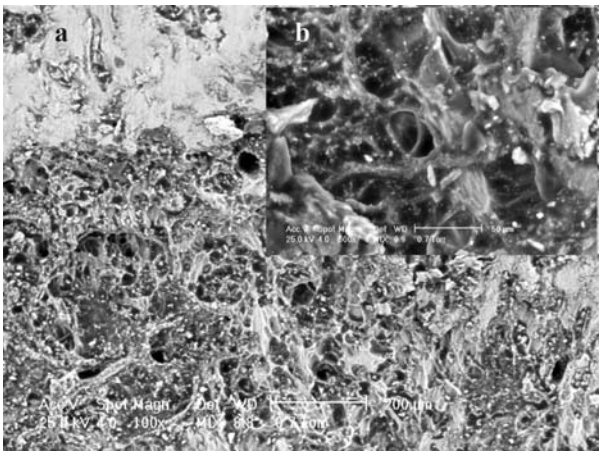

Fig.4. a) ESEM image of the implant autogenous bone after 60 days (x100). b) ESEM image of the implant autogenous bone after 60 days (x500).

60 days after implantation of the autogenous bone, the electron microscopy image of the probe appears as a mass of scratchy, well-developed network fibril process as the result of intense mineralization phenomena.

60 days after implant insertion of ceramic-based β - tricalciumphosfate (CERASORB ®), it appears scratchy in the image of electron microscopy, distinguishing the particles of tricalciumphosfate covered entirely by fibrous tissue of mature stage, result of pre-mineralization process.

60 days time after implant insertion of the cortical bone - spongy collagen (OSTEOBIOL ®),it is microscopically observed an intense mineralized fibrous tissue and neo differentiated channels into the vascular bone as well.

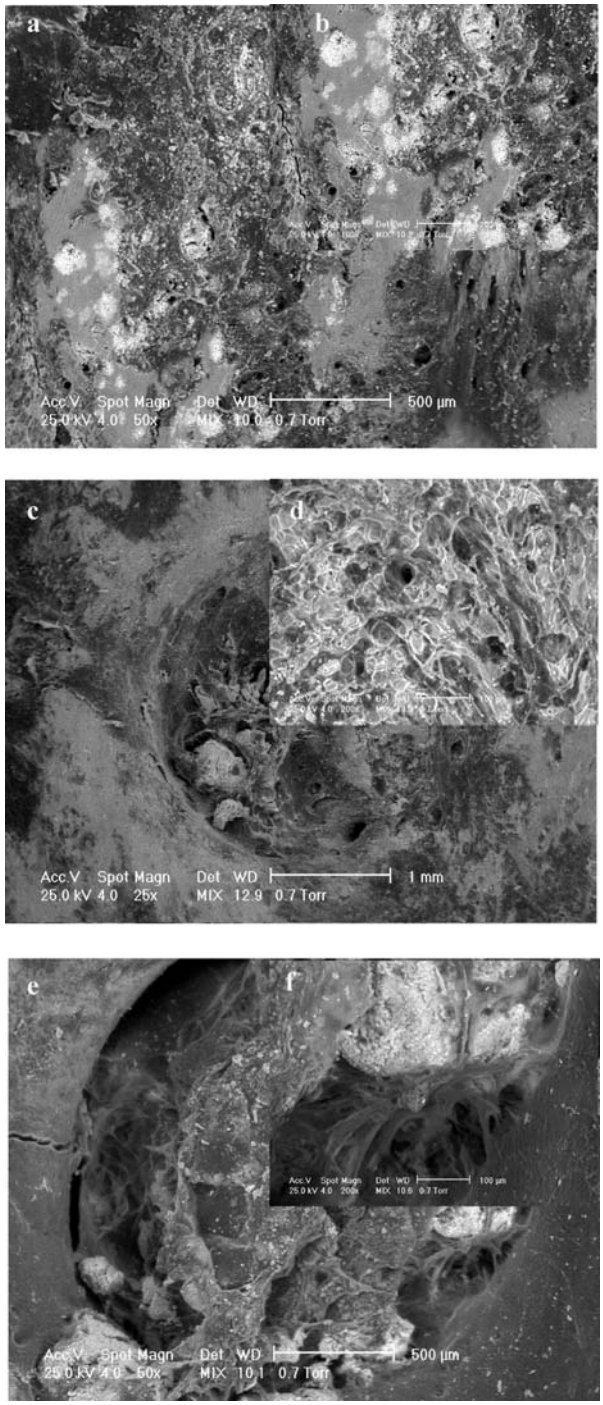

Fig.5. a) ESEM image of the implant of ceramic based β-tricalciumphosfate (CERASORB ®) after 60 days (x50). b) ESEM image of the implant of ceramic-based β-tricalciumphosfate (CERASORB ®) after 60 days (x100). c) ESEM image of cortical bone implant - spongy collagen (OSTEOBIOL ®) after 60 days (x25). d) ESEM image of cortical bone implant - spongy collagen (OSTEOBIOL ®) after 60 days (x200). e) ESEM image of the implant ceramic hydroxiapatite (NANOBONE ®) after 60 days (x50). f) ESEM image of the implant ceramic hydroxiapatite (NANOBONE ®) after 60 days (x200).

60 days time after implant insertion of the ceramic hydroxiapatite (NANOBONE ®), it presents a scratchy mass clearly marqued (figure 5e, f), with a weak adherence to the wall defect, the collagen fibers at the site of implantation - poorly differentiated and extremely thin. A process of reduced neo differentiation bone was identified. Morphological analysis using ESEM electronic microscopy of all biological samples collected from 60 days time of inserted material in addition to receive bone bed, are resumed such as:

• as evidence for the case taken after 30 days time from implantation, for all implanted materials used, no biological processes of rejection being observed;

• regarding the type of additional material used on the sites of implantation, , there was observed a maturation of the fibrous tissue and differentiated for neo differentiation bone;

• the neo transformation speed and quality design for the neo differentiated bone tissue was on descending order, autogenous bone, bone cortical - spongy collagen (OSTEOBIOL ®), ceramic - based β - tricalciumphosfate (CERASORB ®), ceramics hydroxiapatite (NANOBONE ®).

SEM morphologic analysis of biological samples collected at 90 days time after implantation.

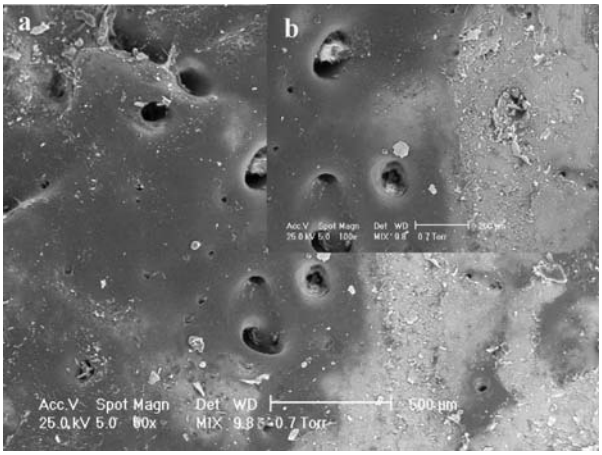

Fig. 6 a) ESEM image of the autogenous bone implant after 90 days (x25). b) ESEM image of the autogenous bone implant after 90 days (x100).

90 days time after the implantation of the autogenous bone, it is a neo differentiated bone that fills the whole defect, presenting a rich vascular network in a bone matrix, well differentiated stage of functional remodeling.

90 days time after the implant insertion of ceramic based β-tricalciumphosphate (CERASORB ®), the electron-microscope images show (Fig. 6 a, b) that the samples are covered by a new differentiated bone with small periostium defects and the granular material is almost entirely recovered.

After 90 days from cortical – spongy collagenate bone insertion (OSTEOBIOL®), to the level of inserted implant, a new differentiated bone tissue is observed in a process of very active recovery, very similar to the case of autogenous bone implant.

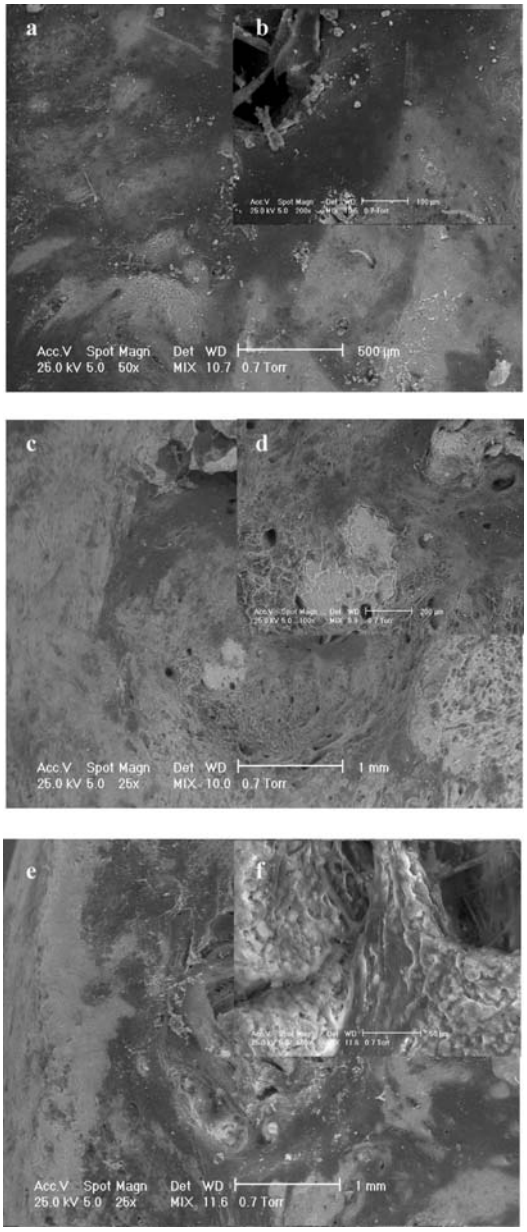

Fig.7 a) ESEM image of the implant of ceramic - based β - tricalciumphosphate (CERASORB ®) after 90 days (x50). b) ESEM image of the implant of ceramic - based β - tricalciumphosphate (CERASORB ®) after 90 days (x200). c) ESEM Image of cortical-spongy collagenated bone implant (OSTEOBIOL®) after 90 days (x25). d) ESEM Image of cortical-spongy collagenated bone implant (OSTEOBIOL®) after 90 days (x100). e) ESEM image of the implant ceramic hydroxiapatite (NANOBONE ®) after 90 days (x25). f) ESEM image of the implant ceramic hydroxiapatite (NANOBONE ®) after 90 days (x500).

90 days time after implant insertion of ceramic hydroxiapatite (NANOBONE ®), granules of material are covered by the addition of an intimate capsule of fibrous tissue with a slight trend of mineralization. A rift of new differentiated bone was observed between the capsules. Granular material does not appear to still suffer a process of macro phagocyte lessees.

Morphological analysis using ESEM electron microscopy for all biological samples collected at 90 days after insertion of the material, in addition to receive bone bed, conducted us to the following conclusions:
• All added materials investigated in this study were biologically integrated in the bone tissue;
• regarding the material augmentation: in the area of implantation, 90 days time after the implant, it is noticed the new differentiated bone tissue;
• the stage of the new differentiation and maturation of bone tissue noticed that 90 days time after implantation was autogenous bone, bone cortical - spongy collagen (OSTEOBIOL ®), ceramic - based β - tricalciumphosfate (CERASORB ®), ceramics hydroxiapatite (NANOBONE ®).

4. Discussion and conclusions

Resuming results of clinical and electron-microscopy investigation, we reached the conclusion that all the investigated augmentation materials are biocompatible and do not generate adverse reactions, i.e. rejections, during the time of the experiment. The augmentation materials succeeded in integration to the level of reception site, confirming the results obtained in other studies [1, 12, 18].

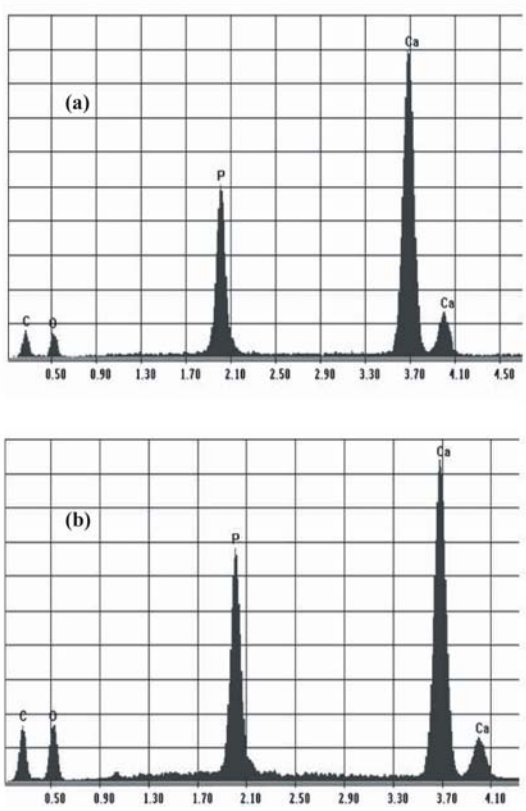

Fig.8 a) X-ray scattering spectra of the implant ceramic hydroxiapatite (NANOBONE ®). b) X-ray scattering spectra of the autogenous bone.

It was noticed a good compatibility of the insertion materials, due to the structure and composition similarities between them and the autogenous bone. It was confirmed by quantitative analysis from one of the addition material (NANOBONE ®) respectively, from the autogenous bone, figure 8. A simple qualitative interpretation of that spectra, indicates that both materials consist in C, O P and Ca as specimen elements, which are very common for life tissues.

It is very important to understand and to improve the materials' biocompatibility, not only to the local level, but also to the level of the entire biological system. Local, as it was expected, especially to the end of the experiment, the material implanted resembled with per implant tissue, demonstrating a physiological integration function, peculiar for loading conditions.

The new tissue succeeds in adaptation, resisting to the physiological forces. There were not noticed any areas of low bone resistance, like cracks or fractures.

The density, orientation and geometry of the bone reflect the adaptation to the biomechanical conditions, and also the morphology of the trabecular and cortical bone. The regeneration process is more evident to the bone-implant interface.

The autogenous bone was again the model for bio - integration.

The process of new bone formation must be understood, following the model of autogenous bone implant, [19], that assures the best conditions for morphological and functional rehabilitation.

The bone regeneration must be understood like an integral process, or as partial tissue reproduction with the restoration of initial functions.

The present study was based on the electron-microscope observations for the biological samples obtained from the following materials: autogenous bone, ceramics based on β-tricalciumphosphate (CERASORB®), cortical-spongios colagenated bone (OSTEOBIOL®), hidroxiapatite ceramics (NANOBONE ®), inserted in rabbit tibiae bone and retrieved after 30, 60 and 90 days .

5. Conclusions

1. as augmentation material, it may be obtained an optimal healing from qualitative and quantitative point of view from autogenous bone [20];

2. using of cortical - spongy collagen bone (OSTEOBIOL®), the healing process is almost the same to that of using of autogenous bone; by using this material we can successfully avoid a secondary surgery for retrieving the autogenousous bone;

3. 90 days after insertion of the ceramics implant based on β-tricalciumfosfat (CERASORB®), it was completely resorbed and the defect was partially filled with new formatted bone;

4. 90 days after the insertion of the hidroxiapatite ceramics implant (NANOBONE ®), we noticed that the granules of this material are not involved in the resorbtion processes or replaced with new bone formation. They are still surrounded by a fibrous matrix that isolate them from new formatted neighbors;

5. a quantitative investigation for the new differentiated bone to the level of those defects filled with these added bio - materials, may be performed in the future work, based on mechanical tests, surface study, adherence and resistance applied to biological samples.

The relevance of this study is quite important, in order to avoid difficulties and side effects, to improve biocompatibility for dental restoration works [21, 22].

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