MORPHOLOGICAL EVALUATION OF THE RESULTS OF USING THE OSTEOPLASTIC MATERIAL AND THE SHELL OF THE UMBILICAL CORD IN THE EXPERIMENT

There is a large variety of different resorbable and non-resorbable membranes. Use of softer materials is found more expedient as it is well adapted to the shape of the defect. Experimental studies were performed in 20 rabbits. There were created 3 defects on the lower jaw of rabbit. The first defect was left open, the second one - filled with granules of osteoplastic material “Collapan-L”, the third one - with “Collapan-L” followed by covering with flap of umbilical cord membrane taken from fetal rabbit. The animals were taken from the experiment in 1 and 3 months. Use of “Collapan-L” and umbilical cord flap caused (comparing to the first and second groups of animals) formation of thick bone trabeculae in intermediate callus by the 1st and 3rd months after the operation. The newly formed bone was formed directly on the surface of the “Collapan-L”. Interlayer of connective tissue between the bone trabeculae and implanted material were not revealed.

The use of “Collapan-L” and the umbilical cord as a membrane is very effective due to strengthening of osteoconductive and osteoinductive properties of the osteoplastic material “Collapan-L”. Accessibility and low cost of raw materials, ease of logging technology allow wide use of the umbilical cord flap it in clinical practice.

Keywords: Osteoplastic material "Collapan-L", shell of umbilical cord, membrane

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Background

There is a large variety of different resorbable and non-resorbable membranes. Use of softer materials remains more expedient as they are well adapted to the shape of the defect (Nicholskiy, 2007). For guided bone regeneration there were used various allogenic soft materials such as acellular dermal matrix (Novaes, Grisi, Molina, Souza et al., 2001), the dura mater (Busschop and De Boever, 1983; Zaner, Yukna, and Malinin, 1989). Umbilical tissue was successfully applied in various fields of medicine (Shamatov, 2002; Nadzhimutdinova, 2007), as it is characterized by a low content of cellular elements, including histamine producing cells and immature immune structure that determines its weak antigenicity (Onopriyenko, Berezhnoy, and Lavrishcheva, 1993).

It is known that tooth loss always entails the deformation of the jaws. Due to the lack of the support function the jaw gradually atrophies, that requires its augmenting during the fixing dental implants (Losev, Dmitriev, and Zharkov, 2003). One of the perspective methods of recovering the capacity of atrophied bone is augmenting it with the osteoplastic materials in combination with the membrane (Micsh, 1987). Today, a variety of membranes are widely used in practice. Even though all of them meet requirements, an important disadvantage of the factory produced membranes is their high cost. Therefore, a search for effective and affordable plastic means can be considered as the important issue.
The shell of umbilical cord as membrane has previously been used in various fields of medicine (Shamatov, 2002; Nadzhimutdinova, 2007). It contains a small number of cellular elements, and the immaturity of the immune structures which explains weak antigenicity of transplant (Onopriyenko, Berezhnoy, and Lavrishcheva, 1993). In the stroma and the epithelium of the umbilical cord contains a large number of glycosaminoglycans (GAG) that contribute modulation, differentiation and cell migration. In addition GAG regulates the organization processes and metabolism of the intercellular substance and provides anti-inflammatory and regenerative effect (Matching, Potapov, and Stroitelev, 2002). The shell of umbilical cord conserved in 0.5% formalin solution and 10% glycerol does not lose its plastic properties for a long time (Rahmatullin, 2005).

The aim of our research is to study the effects of umbilical shell used in conjunction with osteoplastic materials on the process of regeneration of bone wounds of the jaws.

Material and methods

The experimental part of the work was carried out in the Central Research Laboratory of Tashkent Medical Academy. The tissue of umbilical cord was obtained from the Research Institute of Obstetrics and Gynecology of the Republic of Uzbekistan.

Experimental studies were performed on 20 rabbits. Under general anesthesia using the drug “Ketamine” it was created the same channel-bone defects with a diameter of 3 mm and depth of 5 mm in the lower jaw of rabbit. 3 channels were created in the lower jaw of all animals. The first channel was left free, the second one - filled with granules of osteoplastic material “Collapan-L” (Intermediappatit, Russia), the third one - with “Collapan-L” followed by covering with flap of umbilical cord membrane (conserved method is from Khamrayev, Asilova, and Azizov, 1982) taken from fetal rabbit. The wound was sutured tightly. The animals were taken from the experiment in 1 and 3 months after experiment commencing.

The surgery carried segments of the rabbit jaw were resected together with the adjacent tissues and then divided into 3 fragments. Samples were decalcified with “Biodek” then embedded in paraffin. From the received blocks there were prepared serial 4-5 micron thick sections which were stained with hematoxylin and eosin. Part of the material was embedded in Epon-Araldite mixture to prepare blocks with further making of ultramicrotome semi-thin sections. The sections were stained with methylene blue and magenta. Preparations were viewed and photographed by a microscope “Karl Zeiss” (Germany).

Results

In the control group at 1 month after surgery in the bone wound the elements of osteochondral callus were determined. It should be noted that basically intermediate and endosteal calluses were existing, while the periosteal callus was poorly developed.

In certain animals there were noted the formation of cartilaginous tissue such as hyaline cartilage in cortical bone plate. In some cases in intracortical callus there were positioned cartilage tissue mixed with loose connective tissue. The area of endosteal bone-cartilage callus revealed newly formed bone trabeculae formed primarily by enchondral way. This is evidenced by the presence of newly formed bone trabeculae among of a large number of chondroid cells. The last ones were located by small groups and were surrounded by a small amount of primary intermediate substance. Distribution of newly formed bone trabeculae is denser in the field of bone wound compared with a close to wound unaffected areas. Hematopoietic elements were detected between bone trabeculae.

Elements of primary callus were determined 1 month after the operation and implantation of a “Collapan-L” in the field of bone wound. Intermediate callus was represented by separate thin newly formed bone trabeculae developing on the surface of the cortical bone sawdust. Centrally located area in the field of intermediate callus had prevalence of loose
connective tissue of varying maturity degree. Areas containing a large number of cellular elements interspersed with areas with the predominant fibrous tissue and where the cellular elements consisted principally maturing and mature connective tissue cells.

Elements of implanted material located in intermediate callus were surrounded by developing on its surface thin layers of cells and groups of hyaline cartilage. Some of the layers were formed within the implanted material. In some cases, the surface of the defect at a considerable length was followed by connective tissue, which in some places was rich of cellular elements. These elements were mostly fibroblast and osteoblast-like cells and histiocytes. Pronounced signs of inflammatory reaction caused by the implanted material were not observed.

The primary callus connecting the damaged bone sawdust was determined on the 30th day after the operation in the field of bone wound with introduction of “Collapan-L”. On the surface of sawdust cortical plate cancellous bone was formed, among the elements it was noted the presence of hematopoietic bone marrow. Directly sawdust was connected with plate of immature newly formed bone tissue. Inside separate trabeculae there were determined cavities filled with elements of “Collapan-L”. Umbilical cord tissue in sites of bone damage were not found, apparently they were resorbed.

It should be noted that compared to the second group of animals the use of “Collapan-L” and the umbilical cord flap in intermediate callus caused development of thick bone trabeculae. In the area of endosteal callus there were present lacunas of various sizes that were surrounded by newly formed and relatively thin bone trabeculae (Figure 1). The newly formed bone is formed directly on the surface of the “Collapan-L”. Interlayers of connective tissue between the bone trabeculae and an implanted material were not revealed. We determined the areas where the fibrous connective tissue attaches to the implant surface. Several newly formed bone tissues were found in the area of endosteal callus compared to the second group. Elements of hematopoietic bone marrow were determined between the lacunas covered with thin bone trabeculae. Signs of intra membranous bone formation were dominated as well as in the second group.

**Figure 1. Thin bony trabeculae. Stained with haematoxylin and eosin. Eyepiece - 10, the lens - 40**

3 months after the operation in the first control group some animals showed “soldering” of sawdust of cortical plate. Intermediate callus was replaced by bone acquiring lamellar structure in which osteons gradually oriented along the axis of the bone. Disappearance of
end plate and the restoration of the lumen of the medullary canal were noted in the area of endosteal callus in some cases. As a result of bone remodeling decreasing in the number of bone trabeculae was observed. Between these bone trabeculae there were located elements of hematopoietic and fat bone marrow. Some animals had a maintained end plate developed through the interposition of soft adjacent tissues to the area of bone wound at the time of injury.

In the second group of animals, in the field of intermediate and endosteal callus, there were observed further slow replacement of connective tissue and injected new bone material. At the same time a considerable amount of newly formed bone was formed.

The largest volume of newly formed bone was formed in the third group of animals. While in the second group in the bone defect among the newly formed bone mass there were major lacunas of cartilage, in the third group the newly formed bone occupied a large area and was more mature and there were determined only narrow strips of remaining cartilage (Figure 2).

**Figure 2. Narrow strips of remaining cartilage. Stained with haematoxylin and eosin. Eyepiece 10, the lens 20**

It should be noted that during all stages of the study the dissolution of investigated calcium-containing material and shell of umbilical cord occurred without visible involvement of histiocytes and polynuclear osteoclast cells.

**Discussion**

Studies on the effect of bio-implants for bone repair processes in the experiment showed that the studied materials in varying degrees provided a stimulating effect. Pronounced signs of an inflammatory reaction caused by the implanted material involving histiocytes and multi-osteoclast cells were no observed in all cases. This is apparently due to the presence of lincomycin hydrochloride “Collapan-L” and formalin in umbilical cord tissue. Filling the defect with “Collapan-L” and closing with the membrane of the umbilical cord made the callus developing more mature than in the control and the second groups. In some cases, loose connective tissue rich in cellular elements attached to the surface of granules of osteoplastic material for a considerable distance.

Filling the bone defect with “Collapan-L” showed different morphology from the first group of animals. Bone trabeculae were formed directly on the surface of osteoplastic
material without interlayer of connective tissue. Primary callus in the bone wound was located already in the early stages; it was dominated by signs of inter-membranous bone formation. However, in contrast to use the only “Collapan-L”, the implantation of osteoplastic material together with the membrane of the umbilical cord flap provided development of the relatively wide layer of more mature newly formed bone tissue. It can be explained by stimulating effect of umbilical cord tissue.

Conclusion

According to the morphological study it became known that the use of “Collapan-L” and the umbilical cord as a membrane is very effective as it conditions strengthening of osteoconductive and osteoinductive properties of the osteoplastic material. Accessibility and low cost of raw materials, simplicity of logging technology allows widely using the umbilical cord flap in clinical practice. The use for the conservation of umbilical cord 0.5% formalin solution with 10% glycerin solution eliminates the development of post-operative complications associated with infection of the graft.

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