EVALUATION OF REPAIR OF OSTEOCHONDRAL DEFECTS IN RABBITS USING NEW COMPOSITE BIOMATERIALS DURING THREE-MONTH OBSERVATION

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Abstract

In this work nanocomposite porous pins were implanted into rabbit knees. The pins were made of biodegradable synthetic poly-L/DL-lactide (80:20) which was modified with nanometric hydroksyapatite particles (HAp) and covered with biopolymer layer of sodium alginate (NA). In vivo experiments were conducted on a 4-element group of the New Zealand rabbits. Osteogenesis was observed using scanning electron microscope (SEM/EDS) after 12 weeks of implantation.

Key words: *osteochondral defects, regenerative medicine, scaffolds, hydroksyapatite, alginate*

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Introduction

Repair of osteochondral defects is the major clinical problem in orthopedics and still an unsolved issue. Articular cartilage is avascular and relatively acellular with low level of mitotic activity. Thus it displays a poor capacity for self-repair. Fibrocartilaginous substitute tissue which spontaneously fills osteochondral defects has completely different molecular composition and biomechanical properties. With time it tends to degenerate and fails to withstand loading during the joint movement. Nowadays, materials for tissue engineering and materials support for regenerative medicine are the most promising and alternative in the treatment of osteochondral defect. The main goal is to develop a new biomaterial that will enhance and encourage repair of such defects so the newly formed tissue would mimic in the largest extent the precise composition of the native one which is vital in regaining and maintaining a fully functional joint. An ideal scaffold should possess suitable stability, biocompatibility, permeability, porosity and three-dimensional structure. It should also have osteoconductive and chondoconductive properties to promote proliferation and migration of cells and to enhance the tissue ingrowth.

In this regard, increasing attention has been given to biodegradable polymers such as PLDLA, PCL, PGLA and biopolymers such as alginates, hyaluronan, chitosan (CS) and its compositions. Biopolymers could improve osteoblasts and chondrocyte attachment to poly(L-lactic acid) (PLLA), and stimulated of increase cell adhesion, proliferation and cells metabolism activity [1-2]. The intra-articular injection of chitosan has shown an increase of epiphyseal cartilage on tibial and femoral joints with simultaneous activation of chondrocyte proliferation. In order to increase cellular adhesiveness of chitosan Some authors shown that chitosan/alginate-hyaluronan complexes with or without covalent attachment of RGD-containing proteins could stimulated cells grown [3]. Implantation of chondrocyte-seeded scaffolds into the rabbit knee with cartilage defects caused their partial repair. Another authors modified three-dimensional biodegradable PLA-alginate scaffold by TGF-β1 to support the attachment/retention of osteoblasts and for chondrogenic differentiation of MSCs, while conferring mechanical stability to the construct [4]. Marijnissen et al. compared demineralized bone matrix to a PLA-PGA fleece, both used in conjunction with alginate gel, in their capacity to support the chondrocytic phenotype in vivo. Structural homogeneity as well as the number of type II collagen positive cells was found to be higher in the PLA-PGA-alginate constructs, once again confirming the well-suited applicability of such biodegradable polymers to the repair of cartilage and bone defects [5]. The most promising materials for stimulated bone tissue regeneration are polymeric nanocomposites containing such ceramic nanoparticles as; HA, TCP or SiO_2. A small amount of the nanoparticles improves bioactivity of the material and causes its better osteointegration with the surrounded bone tissue [6]. Nanocomposite materials produced and investigated during this study combined two properties of traditional biomaterials i.e.; bioactivity which supports osteoblast proliferatin and biopolymer structure which is suitable for adhesion and proliferation of chondrocytes.

Materials and methods

Nanocomposite sponges formed in pin shape were used in our research. These implants were made of poly (L/DL) lactide (PLDLA; 80:20, Purasorb, PURAC) enriched in bioactive nanoparticles of hydroksyapatite (3-60 nm, HA, Sigma-Aldrich) and sodium alginate (NA). The materials were obtained by a modified solvent casting/salt particulate leaching method using sodium chloride as pore former leading to pores of 100-300 μm diameter. Cylindrical pins were obtained with dimensions of 4×6 mm. Finally they were immersed in 1% solution of NA (FGM Biopolimer, Norway) and dried in vacuum (35oC/48h). In vivo experiment was conducted on the four New Zealand rabbits. Under general anaesthesia (using xylazine 5 mg/kg and ketamine 25 mg/kg) a lateral approach to the stifle joint under sterile conditions was made. After lateral arthrotomy and medial patellar luxation the femoral trochlea was visualized. Using a drill a cylindrical hole (4x5 mm) was created in trochlear groove imitating osteochondral defect (FIG.1). Then the defect was filled with the prepared biomaterial using press-fitting method. The joint capsule, fascia and skin were closed in a routine manner. After the operation all rabbits were allowed to move freely in cages without any splints. The animals were sacrificed at 3 months after implantation. The femoral

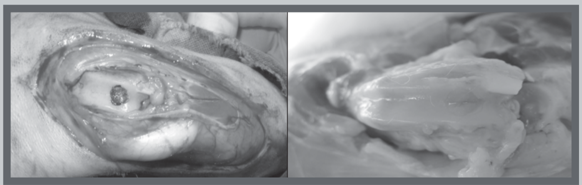

FIG. 1. Macroscoping appearance of tissue defect after 3 months from surgery (a). Intraoperative view of a defect in trochlear groove filled with the implant material (b).

trochleas were harvested, fixed in 4% paraformaldehyde solution and submitted to further analysis. The specimens were adequately cut and underwent dehydration using ethanol in different concentrations (20, 40 i 97%) for 24h. Scanning electron microscope (Nova NanoSEM, FEI) observation of multilevel microstructure was performed.

Results

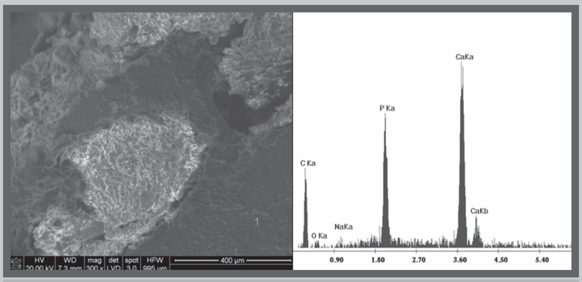

FIG. 2. SEM microphotograph of the implant surrounded by the bone tissue (a), EDS point analysis of newly created tissue (point 1).

Preliminary studies in vitro condition showed that biomaterials were biocompatible [7]. High porosity of these sponges (diameter of pores was 100-300 um) and a system of intercontective pores makes them suitable for cells migration, deposition and blood vessels ingrowth. Three dimensional structure mimics internal arrangement of bone and cartilage extracellular matrix. This is conducive to interactions between cells and matrix components. In the organism's environment the sodium ions from NA are exchanged with the calcium ions. Newly formed calcium-sodium alginate is more solid and enhances faster defect consolidation. SEM microphotographs showed complete integration between implant and bone surfaces (FIG.2a-3a). By means of EDS analysis (Genesis) the elemental composition of specimen was confirmed. As elements to detect were chose phosphorus, calcium and sodium. Punctual EDS analysis in the region of biomaterial – appears as smooth place on the picture – showed increased concentration of Na, Ca and P. In the region of bone – rough surface on the picture - minimal amount of Na and increased ratio of Ca:P were detected. Increased Ca and P concentration compared to carbon confirms the presence of bone. The linear EDS analysis also detected characteristic distribution of elements depending on particular regions of specimen. In the segment that was relevant to implant sodium concentration was high. On the contrary – in the section of bone sodium concentration was minimal. The average EDS analysis of the whole region of implantation showed on FIG.3b confirmed high concentrations of Ca and P which indicates good integration and colonization of material by new bone tissue. It was proved that the tested composite biomaterials because of its porosity, composition and bioactivity enhance cells migration and deposition; support cells organization and thus promote effective repair of osteochondral defects.

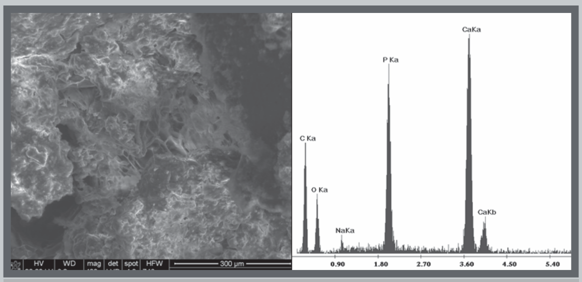

FIG. 3. SEM microphotograph of joint between the implant and the bone tissue (a), Average EDS analysis of the implant cover by tissue (b).

Conclusion

It was shown that the nanocomposite polymer implant covered with the biopolymer stimulated regeneration of osteo tissue. Bioactive, resorbable polymeric nanocomposite materials containing ceramic nanoparticles are interesting implant materials which fulfil requirements of the regenerative medicine. In the next step of analysis of the osteointegration and stimulation of osteo-tissue regeneration process histochemical studies ought to be performed.

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