IN VIVO EXPERIMENTAL PIG MODEL WITH INDUCED CARTILAGE INJURIES TO ELABORATE TREATMENT OF CARTILAGE AND BONE DEFECTS WITH COMBINED USE OF NEW GENERATION BIOMATERIALS AND STEM CELL FRACTIONS

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Introduction

Civilization diseases such as diabetes and obesity are serious problems because of numerous and severe complications significantly impacting on the quality of life of patients [1]. One of the serious complications in the course of diabetes and obesity is osteoarthritis leading to degeneration of cartilage tissue [2]. Articular cartilage represents connective tissue with limited regenerative capacity, low level of metabolic activity and poor vascularization. Although methods of treating cartilage defects have been tried to be optimally developed for many years, new and effective methods of treatment are still required [3]. Such novel therapies may include combined use of stem cell fractions settled on new types of biomaterial scaffolds.

Thus, the aim of the research is to develop and use biocompatible materials based on innovative polymers and graphene modifications as scaffolds for human mesenchymal stem cells (MSCs) and ultimately their use in the regeneration of cartilage and bone tissue in patients with osteoarthritis (osteoarthrosis). Thus, an original research model was created on large animals - pigs, which represents one of the most suitable preclinical models translating new treatment procedures to clinic. Pigs as a research model are readily used in medical preclinical studies due to the similarities in both anatomical structure and physiology with human organism. In this project, we used transgenic pigs with a modified immune system allowing for xenotransplantations such as application of human cells into pig tissues with limited risk of immune rejection. This system allows simultaneous examination of new generation biomaterials based on polymer and graphene modifications as scaffolds for human mesenchymal stem cells (MSCs) of various origin. An original surgical model of the production of cartilage defects in pigs for experimental treatment of created injuries was developed.

Materials and Methods

In this project, nine (N=9) transgenic pigs with knockout of α 1,3-galactosyltransferase gene, were used as a research model. The animals were subjected to general anesthesia, supplemented with local anesthesia. The animals were placed laterally on the left side, providing free access to the right knee joint. At the height of the patella on the anterolateral side, skin was cut at a length of about 12 cm. Removal of connective tissue provided access to the knee joint. After cutting the knee capsule, the patella, lateral base of the femur and the base of the tibia bone were visible. Two cartilage injuries, each 6 mm in diameter and 3 - 4 mm deep, were made on the side and medial femoral condyles of the femur. After making the defects, the knee joint was closed with absorbable suture (PLGA 2/0). Subcutaneous tissue was sutured with two layers of absorbable sutures (PLGA 2/0). The skin was sutured with the intradermal hiatus suture, the PLGA 1 sutured suture. After the treatment the animals were provided with painkillers and antibiotic cover for 10 days.

Results and Discussion

The *in vivo* large animal model was developed using transgenic pigs as animals on which the process of healing cartilage defects using human tissues and stem cells can be studied. Cartilage injuries were made in knees of 9 transgenic pigs according to the procedure presented. Obtained easy and quick access to the knee joint, with the possibility of defect in cartilage. The difficulty of the procedure results from the anatomical structure of the knee joint. In the case of the assessment of defect and healing of the cartilage tissue, it is necessary to perform a cavity in the place of the highest tissue load, i.e. on the lateral and medial condyles of the distal femur. This is where the biggest burdens occur. Using the described procedure, access to femoral condyles was obtained with the possibility to perform injuries of any shape and size. Taking into account the size of the femur, it was estimated that a loss of 6 mm in diameter and 3-4 mm in depth would be optimal throughout the thickness of cartilage. During the procedure no difficulties were found in the performance of the cavity. Only one complication was observed in the form of moderate bleeding to the knee joint from the injury site. The bleeds were left for about 3-4 minutes for self-cure. The resulting clots were removed entirely from the knee joint after the bleeding had ceased. The postoperative wound was sutured with four layers of absorbable sutures.

Conclusions

The proposed method of surgical procedure allows quick, efficient access to the knee joint and the performance of surgical cartilage injuries. The use of transgenic pigs with modified immunological system will allow the study of new methods of treatment of cartilage defects in humans. The interspecific system of preclinical trials with use of human stem cells in animal without the risk of rejection seems to be the best for that kind of study.

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