Materials and methods. As the object of this study served rat livers (n=9) which were subjected to decellularization with sodium dodecyl sulfate solution (SDS) 0.1 and 0.5% and the combination of sodium dodecyl sulfate 0.1% to 0.5% and anticoagulant. Subsequently, the extraction of nucleic acids was performed according to the protocol QIAamp Blood Mini Kit (2003).

Results. After the liver tissue decellularization we obtained the liver matrix. The quantification of nucleic acids revealed the existence of a small amount of DNA 1.04 ± 0.43 ng/ μ l, * p<0,05 in decellularised matrix with SDS solution and anticoagulant. In case of decellularization by SDS exclusively, we obtained a larger amount of nucleic acids which revealed a less efficient decellularization 5.2 ± 2.19 ng/ μ l, * p <0.05.

Conclusions. The use of detergent SDS with anticoagulant for decelularisation is more effective method in comparision with only SDS solution, which was proved by quantification of nucleic acids content in decellularised matrix. A more efficient decellularized liver tissue represent a 3D bioconstruction for future recellularisation.

Key words: decellularization, recellularisation, liver matrix.

267. OBTAINING OF A SUITABLE OSTEOCHONDRAL GRAFT FOR ARTICULAR CARTILAGE ENGINEERING

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Introduction. Chondral injuries are common following a knee trauma. There are numerous studies with different ways to obtain a suitable graft for articular cartilage regeneration, but without imposing results.

Material and methods. From two freshly sacrificed rabbits the distal femurs were harvested and frozen at -84°C for one week. From each distal femur all tissues except cartilage and subcondral bone were removed and small pieces of normal osteochondral tissue (NOCT) were taken. The remaining osteochondral tissue has been demineralized in 0,6M HCl (Chem-Lab, Belgium) over night and again small pieces of demineralized osteochondral tissue (OCDT) were cutted with a scalpel and placed in a PBS solution for 24 hours. The remaining OCDT were separated in 4 groups. Two groups were decellularized in 0,5% and 1% SDS (Sigma, UK) and another two in 0,5% and 1% Triton X-100 (HiMedia, India). The decellularization lasted for 24 hours. At the next day the decellularized and demineralized osteochondral tissues (OCDDT) were washed with distilled water and PBS for 24 hours. All tissues were dessicated through centrifugation at 4000 rpm for 10 min (Hettich, Germany). From all types of OCT were cutted from three to nine pieces 20 mg each and quantification of DNA was performed with GeneJET Genomic DNA Purification Kit (Thermo Fisher, Lithuania). The results were read with spectrophotometer NanoDrop 2000c at wavelength of 260 nm (Thermo Fisher, USA). The best decellularized tissue and OCDT were tested for cytotoxicity with MTT test (ISO 10993-5) with mesenchymal stem cells and chondrocytes.

Results. The average of DNA content in a rabbit NOCT is 36 ng/ μ l, in OCDT 4,23 ng/ μ l, OCDDT with 0,5% and 1% SDS is 3,23 ng/ μ l and 2,16 ng/ μ l respectively and in OCDDT with 0,5% and 1% TritonX-100 is 1,96 ng/ μ l and 0,96 ng/ μ l. At the MTT assay with mesenchymal stem cells and chondrocytes on the OCDT and OCDDT with 1% TritonX-100, we obtained a higher cell viability in both cases more than 80%.

Conclusions. Obtaining a suitable osteochondral tissue for cartilaginous tissue engineering is very difficult because this process involves utilisation of a very toxic chemicals that harm this tissue. A shorter exposure period to chemical agents and preliminary modeling of the graft is mandatory. Also the OCDDT with 1% TritonX-100 shows the best results compared to others.

Key words: graft, osteochondral, demineralized, decellularized

268. THE VOLUME OF THE DENTAL PULP CHAMBER DETERMINED BY USING CONE-BEAM COMPUTED TOMOGRAPHY

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Introduction. Cone-beam computed tomographic (CBCT) imaging is a valuable tool in dental practice. It is widely used in endodontic treatment for the root canal morphology examination. Therefore, the purpose of this study was to use CBCT to calculate the volume of the pulp chamber at different tooth groups.

Aim of the study. of this study was to verify whether clinical use of CBCT imaging can accurately acquire parameters concerning molar pulp chamber landmarks, which are important data to help start a successful way to calculate the number of stem cells in the dental pulp.

Material and methods. This study conforms to protocols approved and in accordance with the ethics committee's requirements, informed consent was obtained from each patient. Morphologic measurements of 120 maxillary and 120 mandibular molars (from 40 patients, aged 18–45 years) were included in this study. CBCT images were taken using a Kodak 9500 (Dental Systems, Carestream Health) operated at 90 kVp with a voxel size of 300 mm and a field of view of 90 150 mm. All scans were taken following the manufacturer's recommendation protocol. According to the examination requirements, C-shaped roots, single-rooted molars, crowned teeth, and teeth with caries and/ or restorations violating the pulp chamber were excluded. All measurements were taken on the coronal plane view.

Results and disscution. In the present study, we used CBCT imaging to gather information regarding pulp chamber volume. With the scanned 3-dimensional images, we were able to clinically determine the pulp chamber parameters using a standardized and defined spatial approach.

Conclusions. The data we collected here serve as a proof of principle for the analysis of dental landmarks before colecting stem cells. In this particular study, existing CBCT scans were used to provide useful information that can be used as a guide for determine volume of the pulp chamber.

Key words: stem cells, cone-beam computed tomographic imaging, pulp chamber

269. GRAFTS OF THE CORNEA IN PEDIATRICS

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Introduction. Transplantation of the cornea in pediatrics remains a challenge. In 2008, Edward Wilson, from South Carolina, relates that the keratoplasty with the stem cell transplantation around the cornea induces the immune modulation and allows only a part of the cornea to be grafted and being more beneficial in the adults. All these advances improve the transplantation of