DECELLULARIZATION TECHNIQUES OF DENTAL PULP

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Background. Endodontic regeneration shows promise in treating dental pulp diseases, however, no suitable scaffolds exist for pulp regeneration. Acellular natural extracellular matrix is a favorable scaffold for tissue regeneration. This study investigated the characteristics of decellularized dental pulp from extracted wisdom teeth and evaluated whether it could mediate pulp regeneration. Decellularized dental pulp represents a suitable scaffold for improving clinical outcomes and functions of teeth with dental pulp diseases.
Materials and methods. The excised dental pulp was mounted to the perfusion system and washed with 1-1.5 liters of distilled water and subsequently decellularized by two methods: with decellularization agent – 0.25% or 0.5% sodium dodecyl sulfate (SDS) and the second method, in which we provided perfusion of the dental pulp with anticuagulant solution (citrate phosphate dextrose) before decellularization and then – decellularization with 0.25% or 0.5% SDS. Subsequently, the dental pulp was perfused with 1-1.5 L of 1% PBS solution. The segments of the intact and decelularized dental pulp were fixed in 10% formalin and subsequently was performed histological samples preparation and H-E staining. The extraction of nucleic acids was performed according to the QIAamp Blood Mini Kit extraction protocol (2003). Each sample was quantified by a spectrophotometer (Nano Drop 200 C Thermo Scientific). The hydroxyproline content was determined on a spectrophotometer based on the oxidation of hydroxyproline in pyrrole under the action of chloramine B.
Results. The macroscopic evaluation of the dental pulp at the experiment beginning, showed that after its washing with distilled water (first method) and with distilled water and citrate dextrose phosphate (second method) in the second one the dental pulp became to be more pale in comparision to the first one. However, visual differences in perfusion liquid color were observed: the perfusion liquid obtained after washing using the distilled and anticoagulant mixture has more intense brown color, which is explained by the more efficient removal of the blood clots.
Conclusions. The comparative evaluating of the DNA tissues content after decellularization by those two methods in relation to intact tissue proves that the method of decellularization of the dental pulp with SDS and washing with phosphate citrate dextrose shows better results.
The maintaining of connective architecture and collagen fibers, the higher content of hydroxyproline in ECM obtained by our method demonstrated their high feasibility for the subsequent use as bioengineering structure for recellularization process.
Keywords - decellularization, recellularization, dental pulp.