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ESTABLISHMENT OF A PROTOCOL FOR RAT SKIN DECELLULARIZATION FOR USE AS A SKIN SUBSTITUTE MATRIX

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Introduction: One of the main bioengineering goals is the production of biomaterials able to replace damaged tissues while the process of natural repair in the affected area is promoted. The number of people in Brazil that need a skin transplant has constantly increased over the last 10 years. There are many dermal substitutes available nowadays, and they are, in the vast majority of cases, acellular. Considering the high price of dermal substitutes available on the market, new options must be sought to lower the cost. Decellularized skin presents a great potential as a skin substitute. It is necessary to have a good and effective protocol for decellularization of skin and to test its biocompatibility. Objective: The aim of this study was to develop a protocol for decellularization of rat skin. In this study, a dermal substitute by decellularized skin rat was researched and human keratinocytes were cultivated on this decellularized matrix and their viability was analyzed. Materials and Methods: Discarded rat skin (CEUA 32510) was used to start the decellularization process using different incubations with hypertonic solutions for cell lysis,using detergents (Triton X-100) and digestive enzymes (Trypsin) under continuous agitation. The entire protocol extended for a period of 12 days. The genomic DNA was extracted and quantified using NanoDrop 2000 and compared with a control skin. In order to confirm the protocol efficacy, histological analyses were done. The samples were sectioned on cryostat and stained with DAPI, verifying the presence of cell nucleus with haematoxylin and eosin (HE). Results: Three different protocols were tested during the padronization procedure using cycles of freezing and defrosting, washes with water, incubation in different NaCl concentrations and treatment with Trypsin and 1% Triton X-100 for 3 days. The DNA quantification analyses showed that control skin presents a much higher amount of DNA (111.8±7.02 mg gDNA/mg tissue) compared with decellularized samples that exhibit a low concentration of DNA (3.026±1.06 mg gDNA/mg tissue). The histological sections stained with DAPI presented normal nuclear distribution in the skin in non-decellularized samples, but cell nuclei were not detected on decellularized samples. The HE staining of the decellularized samples exhibited a conserved matrix structure, with the dermis extracellular matrix maintained. Conclusion and perspectives: In this study, it was possible to establish an efficient protocol of the rat skin that can serve as a matrix for developing a skin substitute. Future studies will be under