

[1]陈博,彭代智,左海斌,等.渗透冲击联合超声和去污剂处理对新生牛真皮组织学和生物活性成分的影响[J].第三军医大学学报,2012,34(09):797-800.

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渗透冲击联合超声和去污剂处理对新生牛真皮组织学和生物活性成分

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Title: Effect of osmotic shock combined with sonication and detergent treatment on histology and bioactive components of newborn bovine dermis

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摘要: 目的 采用渗透冲击联合超声和去污剂处理新生牛真皮, 观察处理前后新生牛真皮基质的组织学和生物活性成分的变化。 方法 从8头健康雄性新生牛皮肤获取网状层真皮组织。采用反复渗透压改变、超声震荡和去污剂联合法对新生牛网状层真皮进行处理, HE染色、DAPI染色和核酸电泳检测细胞成分残留情况; 扫描电镜(SEM)观察胶原束结构和细胞成分; PicoGreen 法、DMMB 法和BCA法对脱细胞前后真皮基质的DNA、sGAG和蛋白质含量进行定量分析; ELISA 法测定脱细胞前后真皮基质内TGF- β 1、EGF、bFGF和KGF含量。 结果 形态学观察可见脱细胞处理后的真皮三维结构完整, 胶原纤维束排列较疏松, 细胞清除较彻底, 未见明显细胞及细胞碎片残留。与脱细胞处理前比较, 脱细胞处理后的DNA含量[(2516.1 \pm 324.2) ng/mg vs (249.5 \pm 53.8)ng/mg, $P=0.000$]、sGAG含量[(5.92 \pm 0.50) μ g/mg vs (2.48 \pm 0.24) μ g/mg, $P=0.000$]、TGF- β 1含量 [(478.8 \pm 196.1) pg/g vs (180.3 \pm 111.0) pg/g, $P=0.009$]和EGF含量[(10.52 \pm 2.78) pg/g vs 未能检测到EGF含量]均明显减少; 而bFGF含量[(788.6 \pm 333.8) pg/g vs (364.8 \pm 294.8) pg/g, $P=0.424$]下降了53.7%, KGF含量[(0.033 \pm 0.000) pg/g vs (0.033 \pm 0.000) pg/g, $P=0.433$]变化不显著。 结论 反复渗透冲击联合超声和去污剂处理对新生牛真皮基质明显地清除了细胞及细胞碎片, 较好地保留了真皮细胞外基质的三维结构和主要生物活性成分。

Abstract: Objective To determine the effect of osmotic shock combined with sonication and detergent treatment on the histology and bioactive components of newborn bovine dermis. Methods The reticular dermis was prepared from neonatal male bovine skin ($n=8$), and sequentially treated with osmotic shock, sonication and Triton X-100. HE staining, DAPI staining and nucleic acid electrophoresis were used to evaluate cellular debris. The collagen bundle structure and cellular components were observed by scanning electron microscopy (SEM). PicoGreen, DMMB and BCA were applied to assay the contents of DNA, sulfate polysaccharide (sGAG) and protein in the dermis before and after decellularization. The contents of TGF- β 1, EGF, bFGF and KGF in the dermis were measured by ELISA. Results Morphological observation displayed that the decellularized dermis showed the integral three-dimensional structure, interspersed collagen bundles, and satisfying removal of cells, and no obvious cell or cellular debris were seen. Compared with natural dermis, the treated sample showed significant decrease in the contents of DNA (2 516.1 \pm 324.2 vs 249.5 \pm 53.8 ng/mg, $P=0.000$), sGAG (5.92 \pm 0.50 vs 2.48 \pm 0.24 μ g/mg, $P=0.000$), TGF- β 1 (478.8 \pm 196.1 vs 180.3 \pm 111.0 pg/g, $P=0.009$) and EGF (10.52 \pm 2.78 pg/g vs undetectable). The content of bFGF (788.6 \pm 333.8 pg/g vs 364.8 \pm 294.8 pg/g, $P=0.424$) was reduced by 53.7%. No significant change was found in the content of KGF (0.033 \pm 0.000 vs 0.033 \pm 0.000 pg/g, $P=0.433$). Conclusion Osmotic shock combined with sonication and detergent treatment effectively achieves acellular extracellular matrix with integral histological structure and partially preserved major bioactive components of the newborn bovine dermis.

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