

COMPARISON OF CYTOKINE EXPRESSION AND ULTRASTRUCTURE CHANGES IN
CRYOPRESERVED AND DEHYDRATED HUMAN AMNIOTIC MEMBRANELee SR¹, Lee MK¹, So JW¹, Kim TG¹, Kang JH², Chung YG^{3*}¹Department of R&D, Korea Public Tissue Bank, Seoul, Republic of Korea²Department of Ophthalmology, Kyung Hee University Hospital at KangDong, Seoul, Republic of Korea³Department of Orthopaedic Surgery, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

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SUMMARY

Introduction: Amniotic membrane(AM) has been used to treat corneal injuries in ophthalmic area due to its structural features and secreting ability for various cytokines. Cryopreserved AM(cAM) has been used for a long time. Recently, dehydrated AM(dAM) has been developed and its use for chronic wounds such as diabetic foot ulcers.has been increased due to its convenience and efficacy. Processing of AM may destroy the structure and degrade cytokines. Much cytokines are known to be present in the chorion attached to the amniotic membrane and can be usaful. We compared the ultrastructure and cytokine secretion of cAM and dAM. **Materials:** Upon study agreement, AMs(n=5) were obtained from placenta after elective cesarean section. AM was separates from the chorion and disinfected by the antibiotic solution. cAM was cryopreserved in cryoprotectant of 50% glycerol in RPMI 1640 medium. dAM was dried for 30 to 120 minutes in 30~50oC incubator and sterilized with 25 KGy electron beam irradiation. Fresh AM, cAM, dAM, fresh AM+chorion, dAM+chorion were compared. H&E staining and TEM was used to evaluate structure. ELISA kit was used to quantify cytokines (EGF, VEGF, HGF, FGF-2, TGF-a, PDGF-AA, PDGF-BB, PIGF, G-CSF, IL-4, IL-10, TIMP-1, TIMP-2, TIMP-3, TIMP-4). **Results:** The total amount of cytokines was preserved 77.1% in dAM and 78.1% in cAM compared to fresh AM. The epidermal growth factor (EGF), which is important cytokine for epithelial regeneration and corneal regeneration, was more preserved in dAM. In addition, PDGF, PIGF, and TIMP2 were more abundant in dAM, and TGF-a, HGF, and TIMP3 were more in cAM. dAM thickness was thinner, but epithelial cell and basement membrane damage were not observed in both groups. TEM results confirmed the preservation of basement membrane. In fresh AM+chorion, there was 10.9 times more amount of cytokines than fresh AM. Dehydrated AM+chorion has 3.2 times more cytokines than dAM has. **Discussion & conclusion:** Growth factors such as EGF were more preserved in dAM and other cytokines differed in two groups. It is difficult to predict how this difference will affect clinical outcome. Histologic test results can predict similar clinical effects of dAM and cAM. Because the chorion has more cytokines than AM, the simultaneous transplantation of chorion with AM can be expected to have the better clinical effects.