PROTOCOL OPTIMIZATION FOR OBTAINING LYOPHILIZED HUMAN AMNIOTIC MEMBRANE EXTRACT

Shramko A., Umbayev B., Olzh ayev F., Idrissova D., Masoud A., Sadyrbekov D, Askarova Sh.

National Laboratory Astana, Nazarbayev University (Astana, Kazakhstan)
alla.shramko@nu.edu.kz

Introduction: The amniotic membrane (AM) is the inner amniotic fluid–filled membrane which forms a cavity around the embryo. AM has long been used in the treatment of venous ulcers and burns. Freshly extracted AM has been shown to possess the highest effect, thus making the extraction method costly and severely limiting its possible usage. To counter this problem, several extraction methods have been developed aimed at preserving the growth factors present in AM and maintaining the AM in a sterile condition. The use of lyophilized AM extract could help preserve its active properties which is associated with the presence of a variety of cytokines necessary for wound-healing. However, there are no standard methods for obtaining amniotic-membrane extracts to date. We chose two available methods in which the resulting extract had the highest regenerative properties. Comparative analyses of these two methods were not carried out. Hence, in order to determine which method gave AM lyophilisates with the highest cytokine composition, we determined the cytokine content of the obtained lyophilisates with reference to that of the native membrane.

Materials and methods: Fifty (50) biological samples were collected aseptically at the National Research Centre for Maternity and Childhood from healthy women who tested negative for HIV, hepatitis B and C, syphilis. The samples were processed using the following methods: method 1 involved ultrasound processing; method two involved treatment of AM with liquid nitrogen and a subsequent heat treatment. FGF, EGF, PDGF-BB, TNF-alpha and TGF-beta-1 contents in the lyophilized AM extracts were measured using the ELISA technique.

Results: Lyophilized AM extracts were obtained. Method №1 produced AM extracts with higher protein contents. AM extracts were lyophilized to approximately 98% dryness. The water content in the lyophilized AM extracts was less than 0.5%. It was observed that by combing these two methods, extracts with decreased EGF, PDGF-BB and TGF-beta-1 concentrations were obtained compared to method №1 only. FGF and TNF-alpha levels were approximately equal in both methods.

Conclusion: Methods for solubilizing and lyophilizing amniotic membrane samples were developed.