

Impact of differently processed amniotic membrane grafts on the outcome of corneal ulcers in rabbit models.

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Introduction. Thanks to its regenerative properties, the human amniotic membrane (AM) is extensively used in ophthalmology to treat a wide range of eye surface conditions. Despite the vast amount of studies justifying its ophthalmic use, there is lacking scientific data on the effectiveness of differently processed AM in the treatment of corneal ulcers. In this paper, we aim to compare the effectiveness of cryopreserved, lyophilized and decellularized amniotic membrane transplantation (AMT) in the management of corneal ulcers in a rabbit model.

Material and methods. Corneal ulcers were surgically induced in the left eyes of 28 rabbits. Four groups, each containing 7 specimens, were formed: group A (control) – rabbits treated conservatively, with no AMT; group B – rabbits operated with cryopreserved AM; group C – rabbits operated with decellularized AM; and group D – rabbits operated with lyophilized AM. The rabbits were clinically observed for a 3-month period, with the assessment of objective signs, the evolution of the corneal lesion and/or complications. After the follow-up period, the rabbits were euthanized; the left corneas were excised, fixed in 10% formaldehyde and then embedded in paraffin, cut into thin sections, stained with hematoxylin-eosin, and studied by light microscopy.

Results. The cornea regenerated the slowest in the control group (28 days), the fastest in group B (15 days), followed by group D (18 days) and then group C (21 days). The highest rate of infectious complications was found in rabbits from group A (57%, n=4), and the lowest – in the group operated with cryopreserved AM (14%, n=1). Corneal neovascularization and opacification were most intense in the control group. In the groups operated with AM, corneal transparency was relatively uniformly recovered, with a slightly poorer outcome in the group C. In conservatively treated corneas (A), histological examination revealed a thickened and deformed epithelium, patchy connective tissue and epithelial cell depletion; in corneas treated with cryopreserved AM (B), fibroblast proliferation and solitary lymphocytic infiltrate below Bowman's membrane were revealed; in corneas treated with decellularized (C) and lyophilized (D) AM, polymorphic and atrophied epitheliocytes were found.

Conclusion. While there were significant differences between the control group and the eyes treated with AMT, the clinical signs did not differ significantly between groups operated with cryopreserved, decellularized and lyophilized AM. Still, the cryopreserved AM showed the best results in terms of post-operative complications, regenerative capacities and restoration of corneal transparency.

Keywords: amniotic membrane transplantation, corneal ulcer, rabbit model.

Bibliography

1. PROCOPCIUC V., CUȘNIR V., NACU V. Amniotic membrane transplantation in the management of corneal ulcers refractory to conventional treatment. In: Sănătate publică, economie și management în medicină. 2022, nr. 1(92), pp. 58-59. ISSN 1729-8687.
2. DALLAL M., NIKKHAHI F., IMENI S., et al. Amniotic Membrane Transplantation for Persistent Epithelial Defects and Ulceration due to Pseudomonas Keratitis in a Rabbit Model. *J Ophthalmic Vis Res.* 2021;16(4):552-557. Published 2021 Oct 25. doi:10.18502/jovr.v16i4.9744
3. IGNATOV O., MELNIC A., PROCOPCIUC V., MIHALUȚA V., PASCAL O., NACU V. Could human amniotic membrane be a source for acupoint thread embedding therapy? In: *Moldovan Medical Journal.* 2021, nr. 6(64), pp. 41-48. ISSN 2537-6373.
4. COCIUG A., MACAGONOVA O., CUȘNIR V., CUȘNIR V., NACU V. Evaluation of the endothelial cell regenerative properties of the cornea in the culture media. results and prospects. In: *IFMBE Proceedings.* Edition nr. 4, 18-21 of September 2019, Chișinău. Switzerland: Springer Nature Switzerland AG, 2020, pp. 601-605. ISBN 978-303031865-9.