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Freeze-dryind as na alternative method for human scleral preservation

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ABSTRACT

Background: Human scleral homograft and autograft techniques are commonly employed today to manage ocular diseases that compromise the tectonic stability of the eye, and to patch-in ocular surgery. Although freeze-drying method has been largely used for preserving human tissue, attention to scleral preservation has been neglected in the sense that only scarce literature on freeze-drying specifically applied to scleral tissue is available. This study aims at comparing freeze-dryed and 95% ethanol scleral samples during 18/45/90/174 days period preservation.

Methods: ninety-six samples of formalin-fixed and paraffin-embedded human sclera were studied. Half of them were submitted to freeze-drying process and the other half preserved in 95% ethanol. Automated immunostaining was carried out in the Ventana BenchMarkR LT platform by using the pathways COL-1 and FIB antibodies. Histological analysis was also performed for Hematoxilin-Eosin to evaluate collagen fiber structure (collagen fiber parallelism), and Mason-Trichrome to evaluate its integrity. Samples were classified according to the degree of collagen fibers parallelism (0-2), intensity of Mason staining (0-2), and the expression of both Collagen 1 (COL-1) and Fibronectin (NCL-FIB) monoclonal antibodies (0-3).

Results: Friedman and Wilcoxon tests were applied to compare preservation methods, freeze-drying and 95% ethanol, through statistical analysis of the mean scores of each scleral sample studied. Inherent to the methods used, p-values bellow 0.05 were considered to have statistical significance. Significant results were found in four situations: (i) Friedman's test applied to two sample groups of freeze-dried scleras showed higher level of collagen fiber integrity in the group re-hydrated after 174 day preservation as compared to the 90/day preservation group; (ii) Wilcoxon test showed better collagen fiber integrity in the freeze-dried sclera group after 18 and 174 days/preservation as compared to the ethanol sclera group with the same exposition period; (iii) when submitted to the Friedman test, freeze-dried group disclosed higher immunohistochemical expression for COL-1 antibody in the sclera samples re-hydrated after 45, 90 and 174 preservation period as compared to the ones re-hydrated after 18 days; (iv) freeze-dried samples re-hydrated after 174 preservation period showed lesser immunohistochemical expression for FIB antibody as compared to ethanol preserved samples by Wilcoxon test.

Discussion: Both methods, Freeze-drying and 95% Ethanol, are considered effective for scleral preservation. Histopathological and immunohistochemical analysis proved freeze-drying preservation to be an effective or even superior method for scleral graft preservation than 95% Ethanol, providing the former easier tissue manipulation, longer shelf life and the capability of storage at room temperature.

Key words: freeze-drying, scleral preservation, automated immunohistochemistry