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Determination of viability of cryopreserved cartilage grafts

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Abstract Although transplantation of preserved cartilage has assumed a role of great importance in reconstructive surgery, there are many divergent and contradictory opinions with regard to the outcome of cryopreserved cartilage. This study was formulated to assess the functional state of chondrocytes after cryopreservation. Freeze injury and survival were studied using the trypan blue dye exclusion test, functional assay for cell adhesion and transmission electron microscopy. The methods applied clearly proved that a greater part of the cartilage cells was irreversibly damaged by cryopreservation. Findings demonstrated that cryopreserved cartilage remained non-viable and was not able to originate new cartilage. Thus, such cartilage will be subject to resorption processes and not practical for reconstruction of parts of the skeleton subject to mechanical stress. The feasibility of cryopreservation techniques for providing vital cartilage substitutes needs further evaluation.

Key words Cartilage grafting · Cryopreservation · Cell biology · Ultrastructure

Introduction

At present, cartilage grafting is commonly performed to reconstruct skeletal defects produced in various body sites, including those of the head and neck [11]. The use of autologous cartilage is the ideal method. However, for technical reasons, chemically preserved homologous cartilage has also proved to be a popular tissue [13]. The preservation of banked homologous cartilage with chemical methods continues to present a clinical challenge, especially because the storage methods available have the disadvantage of diminishing or eliminating cell viability. Unfortunately, these devitalized cartilage grafts induce a combination of enveloping fibrosis and resorption after transplantation [1].

The preservation of a wide variety of tissues by freezing and storage at low temperatures with liquid nitrogen for subsequent use in surgical procedures has been extensively practiced in recent years. The greatest amount of transplant success after freezing and thawing procedures has been achieved with epithelial tissue [3, 25]. However, despite the early encouraging results with frozen cartilage allotransplantation in humans and experimental animals, late complications due to degenerative changes are common [11, 13]. An important factor in the behavior of these cartilage grafts is the preservation of chondrocyte viability during storage and maintenance of the cell's ability to function following storage.

To our knowledge, there have been few studies examining the function of cells after cryopreservation of intact cartilage grafts and results have been conflicting [6, 12]. The present study was set up to examine the functional state of cartilage cells after cryopreservation. Additionally, the ultrastructural changes that occur in frozen and thawed cartilage were also investigated.

Materials and methods

Sampling of material

During reconstructive surgery of the nose excised septal cartilage tissue was obtained from ten adult healthy patients. The sexes and ages of the patients were not considered to be relevant to the present study.

Preparation of specimens

Sterile conditions were kept by continuing work in laminar air flow (CEAG, Dortmund, Germany) and precautions were taken to

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