BioCompatibility of Acellular Dermal Matrix Graft Evaluated in Culture of Murine Macrophages

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INTRODUCTION

Several techniques have been developed to solve gingival aesthetic problems. The techniques can be grouped as pediculated grafts and autogenous grafts (epithelial free grafts and connective tissue graft)\(^\text{16,22}\). Aiming at reducing the problems with these treatments, new techniques and materials have been developed\(^\text{9,18-22,24,26,31}\).

A recent material developed for soft tissue grafting was the acellular dermal matrix (AlloDerm®, Lifecore Biomedical, Oral Restorative Division, Chaska,MN)\(^\text{14,25,30,32}\). This allograft material has advantages to reduce surgical complications, decrease donor site discomfort and improve esthetic tissue. This graft is obtained from an allograft donor skin and produced by a carefully controlled process that removes the epidermis and dermis cells without altering the extracellular matrix structure, reducing the possible immune response and the transmission of diseases.

The need for biocompatible materials implies the necessity of in vitro toxicity tests, animal experimentation usage tests and clinical studies in humans\(^\text{33}\). The biological system used in in vitro cytotoxicity testing of some materials...
are cells in culture. Thus, culture of macrophage cells can be used to evaluate the biocompatibility of materials. These cells participate in many aspects of host defense, inflammation and immunity, partly through their ability to undergo adaptive responses to the conditions or stimuli that prevail at sites to which they have been attracted2.

Among the numerous secretory products of macrophages there are two groups of inorganic compounds with a high degree of chemical reactivity: the ROI (including super oxide, hydrogen peroxide, and in some populations of mononuclear phagocytes, the products of myeloperoxidase), and the RNI including nitrite (NO₂⁻) and highly related reactive oxides such as nitric oxide and nitrogen dioxide1. Macrophage-derived RNI are of interest for at least three reasons: their production is under strict immunological control17,28, they are synthesized by enzymes novel to mammalian biochemistry, which have not been yet well characterized13, and they appear to play an important role in some of the carcinogenic13,15, antitumor12,29 and immunological control27,28, they are synthesized by enzymes novel to mammalian biochemistry, which have not been yet well characterized13, and they appear to play an important role in some of the carcinogenic13,15, antitumor12,29 and antitumor12,29 and antimicrobial1 actions of the activated macrophage.

Although AlloDerm® is tested before being released for sale; we assume that it is important to evaluate the biocompatibility in macrophages culture.

**MATERIAL AND METHODS**

This study was approved by the Institutional Committee on Animal Research, School of Pharmaceutical Science.

**Animals**

The Animals Laboratory of the School of Pharmaceutical Science, UNESP (State University of São Paulo) Araraquara, SP, BRAZIL supplied six-week old male Swiss mice weighing 18 to 25 g.

**Acellular dermal matrix samples**

Samples of acellular dermal matrix (ADM), commercially known as AlloDerm®, measuring 4x4 mm, from different batches were obtained at the Department of Periodontology of Araraquara Dental School UNESP-Araraquara, São Paulo, Brazil.

**Cell macrophage**

Mice were injected i.p. 3 to 4 days before harvesting with 3 ml of thioglycollate broth. Macrophages were obtained after killing the mice with chloroform, and the peritoneum was exposed using sterile scissors. Saline solution (0.85% NaCl) was introduced into the peritoneum and after digital massage, the suspension was removed by aspiration. This suspension of peritoneal cells and saline was placed in a Neubauer chamber and counted in order to obtain the ideal concentration for each test. The adherent was placed in a Neubauer chamber and counted in order to obtain the ideal concentration for each test. The adherent aspirates were removed from conditioned medium and incubated with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylthylenediamine dihydrochloride, 0.25% H₂PO₄) at room temperature for 10 minutes. The absorbance at 550 nm was determined in a micro plate reader. The tests were made in quadruplicate and the results were expressed in micromoles of NO / 5 x 10⁶ peritoneal cells, from a standard curve established in each test, constituted of known molar concentrations of NO in RPMI – 1640 medium.

**H₂O₂ release**

The method depends on the determination of hydrogen peroxide (H₂O₂) release in the culture of peritoneal macrophages from Swiss mice. Suspensions of peritoneal cells were performed using a concentration of 2 x 10⁶ cells/ml in a solution of phenol red, containing 140 mM NaCl, 10 mM potassium phosphate, pH 7.0; 5.5 mM dextrose; 0.56 mM phenol red and type II horseradish peroxidase 0.01 mg/ml (Sigma). Aliquots of 0.1 m were transferred to culture plates, flat bottomed containing 96 wells (Corning). The acellular dermal matrix or 50 mL of Zymosan solution (5 mg/ml, Sigma) were added to each well. The samples were incubated for one hour at 37°C in a 5% CO₂ atmosphere. After the period of incubation, the reaction was interrupted by addition of 10 mL of NaOH 4N. Experiments were done in quadruplicate and the absorbance was determined in an ELISA automatic photometer, with a 620 nm filter. The results were expressed in nanomols of H₂O₂ released per 10⁶ peritoneal cells, from a standard curve established in each test, constituted of known molar concentrations of H₂O₂ in buffered phenol red.

**Statistical Analysis**

Comparisons between groups were performed using Student’s T test. P values <0.05 were considered statistically significant.

**RESULTS**

Figures 1 and 2 illustrate the release of nitric oxide and hydrogen peroxide when macrophages were exposed to...
acellular dermal matrix, respectively. Statistical analysis showed no significant difference ($p \leq 0.05$) when compared with negative control. However, the level of release of these mediators in positive control was statistically different ($p \geq 0.05$).

**DISCUSSION**

This study evaluated the cell inflammatory response of in vitro macrophages in contact with the acellular dermal matrix measuring the release of nitric oxide and hydrogen peroxide. The positive control using zymosan-SIGMA to H$_2$O$_2$ release and LPS-SIGMA to NO release showed a high potential of macrophages activation and liberation of H$_2$O$_2$ and NO, respectively. The acellular dermal matrix was not able to stimulate macrophage liberation of hydrogen peroxide (H$_2$O$_2$) and nitric oxide (NO).

In respect to these results we pointed out that Liversey, et al. (1994) evaluating immunohistochimically the material AlloDerm® did not find any antigen that could develop an immune reaction.

Cellular events were evaluated in some human studies with ADM grafts for root coverage$^{4,10,11,33}$. In 1998, Harris$^{10}$, using acellular dermal matrix graft in treatment of gingival recessions in patients, obtained complete root coverage on two of three defects. In histological analysis, the AlloDerm® had incorporated and became part of the gingival tissue in the area. The same author$^{11}$ in 2001 reported a comparative clinical study of root coverage obtained with ADM versus connective tissue. Biopsy of the grafted area revealed elastin fibers. The author stated that the presence of these fibers implied that the ADM was being incorporated into the host tissue.

In a recent study Cummings, et al.$^{4}$ (2005) histologically evaluated the acellular dermal matrix graft. The findings of the study showed new fibroblast, vascular elements, and collagen were present throughout the ADM, with retention of the transplanted elastin fibers.

All of these results can be directly related with the laboratory processing of human skin obtained in tissue banks with the removal of all cells without altering the connective tissue structure, composed of type I collagen fibers$^{14}$. In this respect, the AlloDerm® matrix is rigorously controlled by the FDA according to the guidelines of the American Association of Tissue Banks. Apart from the selection of possible donors, serologic and microbiologic exams are also performed to screen for diseases such as AIDS, syphilis, hepatitis, etc. The patented process of preparation and lyophilization removes all cells, preserving the collagen structure of the connective tissue. In the manufactured controls, histological and immunohistochemistry tests are carried out to check the complete removal of all cellular components.

Within the limits of this study the present results show that there was no release of hydrogen peroxide (H$_2$O$_2$) and nitric oxide (NO), suggesting that the acellular dermal matrix did not activate the cell inflammatory response, although new studies should be accomplished with Alloderm® use.

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