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METHODOLOGY:

A simple and fast procedure to grow bat fibroblasts from lung biopsies for cytogenetic studies

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ABSTRACT

A fast, simple, and inexpensive procedure to establish fibroblast culture from bat lungs is presented. Explants plated following mechanical disaggregation provide good quality preparations for cytogenetics studies in about one week. Cultures established with this procedure may also be used for other biological studies.

INTRODUCTION

Comparative cytogenetics has been restricted by the use of *in situ* procedures, using for example bone marrow, or lymphatic organs. In these tissues the metaphase chromosomes usually are condensed, making chromosome band pattern analysis difficult (Baker and Haiduk, 1985).

Living cells can be exposed to several agents that facilitate chromosome analysis for taxonomic and evolutive studies. Cells in culture can also be used for biological investigations, such as DNA extraction and sequencing, cellular metabolism analysis, detection of surface proteins, and others.

Cell cultures for bats are generally obtained from fibroblasts, derived from ear or tail skin, or from the lung.

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MATERIAL AND METHOD

Biopsy obtention and primary cell culture

The lungs are taken from freshly killed specimens under sterile conditions and placed in a tube containing Hanks Balanced Saline Solution Ca and Mg free (HBSS) supplemented with penicillin (250 U/ml), quemicetin (250 μg/ml), gentamicin (0.04 mg/ml), and amphotericin B (1 μg/ml). The material is incubated at 4°C a maximum of 24 hours, for disinfection. Then, under a hood, the biopsy is transferred to a sterile culture dish containing medium (Ham-F10 or MEM-Eagle) or HBSS, and is minced with scissors into small fragments (mechanical disaggregation).

Using sterile Pasteur pipettes, the fragments are transferred into polystyrene T-25 culture flasks. The fragments should be evenly distributed over the inferior surface of the flask, which was previously wetted with 2 ml of growth medium [Ham F-10 supplemented by penicillin (125 U/ml), quemicetin (125 μg/ml), glutamine (40 mM), fetal bovine serum (20%)]. The flasks are incubated at 37°C for 48 hours to allow the attachment of the fragments to the flask. It is important to correct for pH variations since bat cells grow best at pH 6.5-7.0.

Lung has fibroblast-rich tissue and mechanical disaggregation proved to be sufficient to expose the fibroblasts and induce their proliferation. Usually within 48 to 72 hours after plating, the fibroblasts begin migration from the fragments. At this time, 3 ml of the growth medium should be added to the flask. The growth medium can be changed every three days. When there is thick growth around the fragments or when colonies start to merge, the culture is ready for cytological preparation or for subculturing. This is observable usually within one week.

Subculture

Subcultures are established by trypsinization. The growth medium from the culture flask is removed and cells are washed in 5 ml of HBSS at 37°C for releasing of the serum. HBSS is then removed and about 1 ml of trypsin/EDTA solution (0.05 g of trypsin + 0.53 mM EDTA in 100 ml of HBSS) also at 37°C is added to the flask for two minutes. The trypsin/EDTA is carefully removed and 5 ml of fresh growth medium is added. The flask is then vigorously agitated to detach the cells. The cell suspension is split into two flasks and 2.5 ml of the growth medium is added to each flask to complete 5 ml of medium. The free cells usually attach to the flask surface within a few hours, establishing the secondary cell cultures.

Lung-derived fibroblast growth rate in bat cells is high and the cells are suitable for chromosomal analysis in the earlier passages (about one week) or till they exhibit senescence signs, which are generally present after 45 days. Cells from actively growing cultures should be frozen for future studies.

DISCUSSION

Many methods are available for growing animal biopsies. However, most of them require digestion by proteolytic enzymes such as trypsin and collagenase, after mechanical disaggregation. Trypsinization of fragments before cultivation has proven to be useful for some tissues, but in collagenous fiber-rich tissues it generally fails to dissociate the cells. Besides, overtrypsinization plus agitation may cause cellular damage. Procedures using collagenase generally result in excellent cell growth rate, but the methods are not simple and collagenase is rather expensive. Lung has a fibroblast-rich tissue and mechanical disaggregation proved to be sufficient to expose the fibroblasts and induce their proliferation.

Lung fibroblasts can be easily and rapidly obtained. The establishment of fibroblast cultures from skin, however, is much slower than that from lung. When obtained from skin-derived cultures, fibroblasts start growing after five to seven days, and usually epithelial cells grow first (Figure 1), which sometimes delays fibroblast growth (Morielle-Versute, 1992).

One basic requirement to succeed in cell culturing is to avoid microbiological contamination. Failures in aseptic techniques and incorrect use of antibiotics and fungicides are the principal cause of contamination in cell cultures. Microbial contamination is much more likely in skin than in lung-derived cultures, due especially to the presence in the skin of hairs that shelter fungi.

The procedure described provides cytogenetic preparations of adequate quality for banding procedures, as illustrated in Figure 2.

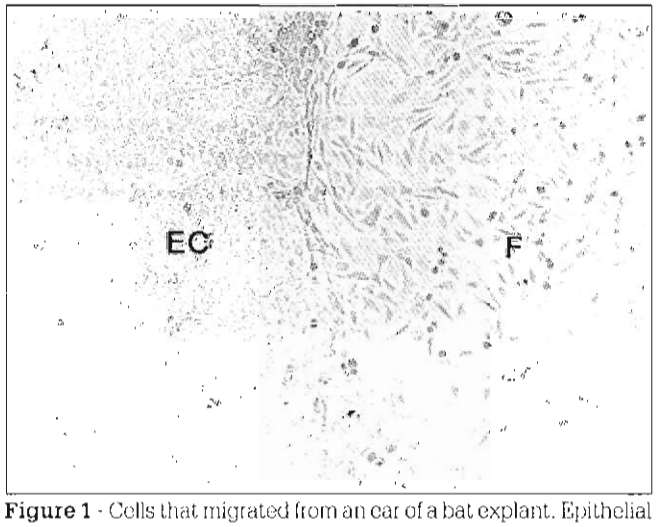

Figure 1 - Cells that migrated from an ear of a bat explant. Epithelial cells (EC) and fibroblasts (F).

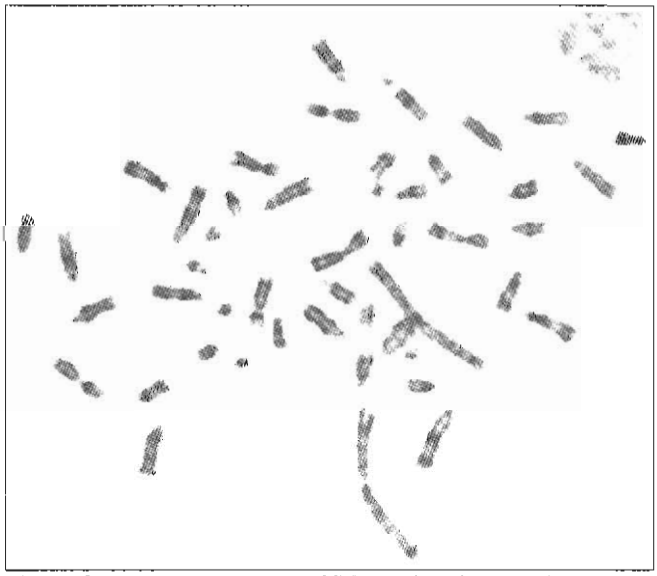

Figure 2 - Metaphase cell with GTG-banding from a culture of the bat species *Molossus molossus*.

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RESUMO

Apresenta-se um procedimento rápido e de baixo custo para a obtenção de cultura de fibroblastos de pulmão de morcegos. Fragmentos obtidos por desagregação mecânica e colocados em cultivo, propiciam, em cerca de uma semana, a obtenção de preparações de boa qualidade para os estudos citogenéticos e os de outras naturezas.

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