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METHODOLOGY

Fibroblasts of skin fragments as a tool for the investigation of genetic diseases: technical recommendations

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Abstract

Skin biopsies are frequently indicated for investigation and/or confirmation of genetic disorders. Although relatively simple and noninvasive, these procedures require care in order to increase probability of success and to avoid patient discomfort and unnecessary repeated analyses and associated laboratory fees. The present report highlights the importance of skin biopsies in genetic disorder diagnosis and presents general rules for collecting, storing, transporting and processing samples. We recommend its reading to professionals intending to use this important and sometimes fundamental diagnostic tool.

INTRODUCTION

Tissue culture was introduced at the beginning of the century (Harrison, 1907; Carrel, 1912) as a method for studying behavior of animal cells free of possibly increased systemic variations due to stress experienced during either experiments or even normal homeostasis. As the name indicates, this technique was elaborated with disaggregated tissue fragments. The term "cell culture" refers to disperse cell cultures obtained from either original tissue or a cell line by enzymatic, mechanical or chemical aggregation (Freshney, 1983).

Among the various uses of a skin biopsy, the most frequent probably is the use in investigating or confirming clinical diagnoses (Smith, 1992).

Material obtained from the skin biopsy can be utilized in several investigative procedures, e.g., ultrastructural examinations, immunofluorescence, histochemical and enzymatic immunohistochemical studies, microbiological studies, karyotype, autoradiography, and in research methods, e.g., *in situ* hybridization. In the specific area of inborn errors of metabolism, cell culture from skin biopsies obtained by elliptical surgery (Harrison, 1980) has been developed to a greater extent in order to obtain material for enzymatic measurements possibly leading to definitive diagnoses. While these procedures may differ, the specimen collection, packing, transport and processing techniques are mostly similar.

Based on the authors' experience of about ten years with collection, processing and analysis of skin fragments for enzymatic studies, the objective of the present study is to describe the ideal procedural conditions for successful diagnosis of genetic and non-genetic diseases.

COLLECTION OF THE SKIN FRAGMENT

Material for biopsy should be collected using sterile scissors and forceps; no punch is necessary. Although any skin fragment can be cultured, samples from the anterior surface of the forearm are mostly frequently used. The procedure should be performed in absolutely aseptic conditions. The forearm area from which the sample will be taken is first washed with water, then cleaned with 70% ethanol. A 2% lidocaine (1 or 2 ml is sufficient) injection is made close to the collection site. Using sterilized forceps, a fold is made in the anesthetized skin and a 2- to 3-mm fragment removed with sterile scissors (Figure 1). Some bleeding is positive, indicating that the fragment was not too superficial to be adequate. After collection, site should be covered with sterile gauze fixed with adhesive tape.

The collected skin fragment should be immediately transferred to a sterile flask containing HAM-F10 cell culture medium plus 20% fetal calf serum or a similar product. In laboratories equipped for cytogenetics, the same culture medium used for karyotypes can be employed. If the culture medium does not contain penicillin and transport to the laboratory exceeds 48 h, 400 µg/ml 400 U penicillin should be added to the medium. If culture medium is not available, the fragment should be placed in a flask containing sterile physiological saline, with penicillin added when possible.

The flask containing the skin fragment should be thoroughly sealed to avoid contamination and can then be stored or transported for a few days at room temperature before using.

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SKIN PREPARATION PROCESS

Primary culture

After collection, skin should be placed in a sterile polyethylene cell culture flask of the Falcon T25 type. Handling should preferably be carried out under a laminar flow hood using sterile materials and reagents, with a Bunsen burner ignited throughout the procedure. Using a surgical knife with a number 10 blade, skin is cut into several pieces (explants) of approximately 1 mm in diameter on Petri dishes containing 1 ml HAM-F10 culture medium plus 20% fetal calf serum. Explants are transferred to the cell culture flask with a needle (Figure 2), following which 3 ml of HAM-F10 culture medium containing 20% fetal calf serum is added. The flask is sealed and placed in a 37°C oven to grow the fibroblasts.

Culture medium is changed every three days, old medium being discarded and 3 ml fresh medium added to culture flask (Verma and Babu, 1995).

Secondary culture

When cells from the explants present adequate growth (growth halos start to approach one another), fibroblasts are detached from the bottom of the flask with a 0.25% trypsin-EDTA solution and transferred to two new sterile flasks to which 3-ml HAM-F10 culture medium containing 10% fetal calf serum is then added (Verma and Babu, 1995).

This procedure continues for several weeks until enough confluent flasks are obtained for collecting or freezing in cell bank. We define a confluent flask as one containing grown fibroblasts that have covered the bottom almost completely. When material is to be sent to another laboratory, flask should show 80% confluence so that some space will remain for growth during transportation.

Fibroblasts are collected without asepsis on an ice bath. Medium is removed from the culture flask, the cells are submitted to three washings with PBS buffer, washed with 0.02% PBS, and removed from the flask. After centrifugation, a fibroblast precipitate is obtained and washed twice with 0.9% NaCl before being ready for analysis.

Even after being collected for analysis, these fibroblasts must be kept in culture until analysis completion. Alternatively, if the laboratory has a cell bank (liquid nitrogen tank), they can be frozen. In this case, culture medium is discarded and fibroblasts are washed with 0.25% trypsin-EDTA solution. After detachment from the flask, culture medium is added for freezing, i.e., HAM-F10 medium containing 10% fetal calf serum and 10% dimethylsulfoxide. Cells are then transferred to plastic ampoules and frozen at -70°C for 1 h and a half, then transferred to liquid nitrogen.

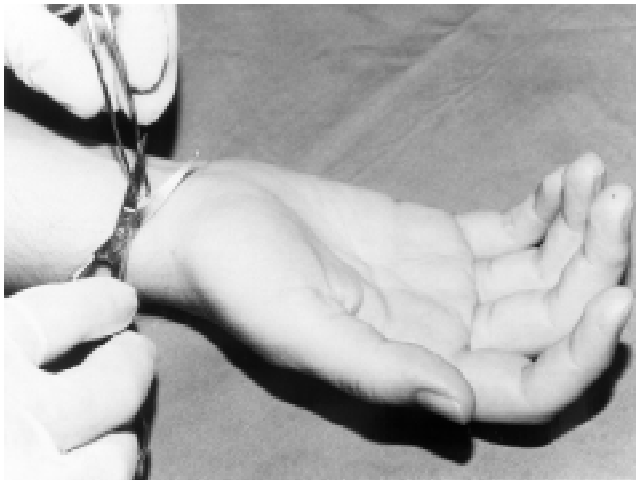

Figure 1 - Procedure for collection of a skin sample from the anterior forearm.

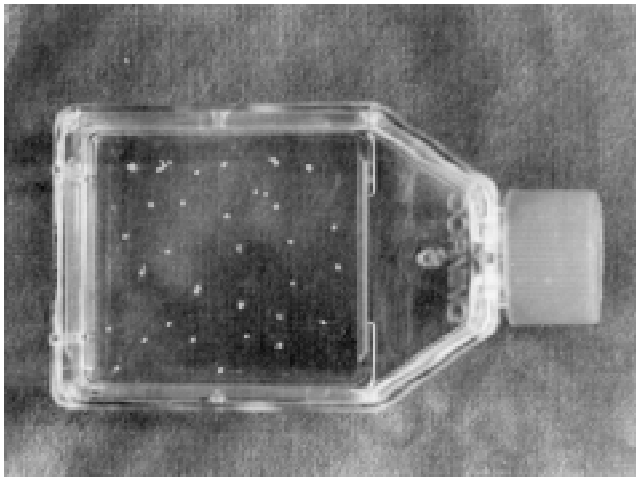

Figure 2 - Cell culture flask containing explants after skin fragmentation.

Shipping the material

If cultured fibroblasts must be sent to a distant laboratory, the packing steps below should be followed.

Skin fragments

After collection, skin fragment should be placed in a small sterile flask containing cell culture medium (HAM-F10 or a similar medium containing 20% fetal calf serum), karyotype medium or sterile physiological saline. The flask should always be completed with culture medium so that skin fragments remain covered with medium during transport, regardless of flask position. The flask should be closed and tightly sealed, preferably with plastic film, and identified.

Material should be sent express at room temperature

in a styrofoam or cardboard box. Time between collection and arrival at destination should not exceed 72 h.

Cultured fibroblasts

Flasks containing cultured fibroblasts (preferentially at 80% confluence) can also be transported. Again, the flask should be completed with culture medium containing 10% fetal calf serum and fully sealed with plastic film to prevent leakage. The container must be completely full so that the cells will always be covered by medium no matter what is the flask position during transport. The flask should be transported in a rigid box to prevent damage or breakage. Again, during transportation the flask should be kept at ambient temperature.

Possible problems

Upon reception, skin fragments should be examined to see if it presents some dermis in addition to epidermis. Samples consisting only of epidermis do not grow, nor do those accompanied by fatty tissue which may prevent fibroblast growth, and therefore should be removed during explant preparation.

Sample contamination can result from collection and/or preliminary processing contamination, frequently by difficult-to-detect mycoplasma, and for the presence of which all biopsies should be tested (McGarrity *et al.*, 1983); if results are positive, material should be disinfected with mycoplasma removal agent.

When sample is sent, the flask often reaches its destination with little remaining medium, even after having been properly sealed, since flask may rupture due to inadequate packing or depressurized air transport. In either case, for lack of sufficient contact with the medium, cells either die or become contaminated.

Final comments

Although relatively simple and of low invasiveness, skin biopsies cause patient discomfort and involve high laboratory costs, so that all possible measures should be taken to assure their success and avoid the need for further collection. These measures include absolute asepsis throughout the procedure, correct packing of collected material, especially when transportation is necessary, and adequate primary culture preparation. Following the norms presented in this paper should contribute to more efficient use of this important diagnostic tool.

RESUMO

Biópsias de pele são freqüentemente indicadas para a investigação e/ou confirmação de um distúrbio genético. Embora relativamente simples e não invasivo, este procedimento deve ser executado com cuidado de modo a aumentar as chances de sucesso, evitando o desconforto para o paciente e os custos para o laboratório gerados por uma eventual necessidade de repetição da análise. Este trabalho destaca a importância da biópsia de pele para o diagnóstico de doenças genéticas e descreve as normas gerais para coleta, acondicionamento, transporte e processamento da amostra. Sua leitura é recomendável para profissionais que pretendem utilizar esta importante, e às vezes fundamental, ferramenta diagnóstica.

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