

# Experimental model for fibroblast culture<sup>1</sup>

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Keira SM, Ferreira LM, Gragnani A, Duarte IS, Santos IAN. Experimental model for fibroblast culture. Acta Cir Bras [serial online] 2004 Vol 19 Special Edition. Available on URL: <http://www.scielo.br/acb>.

**ABSTRACT** - The use of cell culture methods in Plastic Surgery opened a new horizon in the research of cellular mechanisms of proliferation and biosynthesis functions. Several types of cells have been investigated in the cutaneous compartment. Keratinocytes and fibroblasts have been studied aiming the possibility of developing biomaterial for skin substitution. The present study describes the standardization for the development of fibroblast primary culture, its utilization in experiments and its storage.

**KEY WORDS** - Cell culture. Fibroblasts.

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## Introduction

Skin, with a 2m<sup>2</sup> surface, has an important role in the defense of human body against microorganisms from the environment and also in the maintenance of hydric homeostasis<sup>1</sup>.

When skin integrity is broken by wound, the organism begins immediately the healing process using a sequence of biochemical and biophysical events in order to reestablish the skin integrity in the wound site<sup>2</sup>.

Cell culture has been used as an important tool to investigate the healing process. This method permits the study of the biological behavior of skin cells, considering either the cellular proliferation kinetics or the biosynthesis of several components of extra-cellular matrix.

## Proposition

The present study intends to show an experimental model of fibroblast culture with the standardization of the initial process of the cutaneous material and the maintenance of the cell culture.

## Method description

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### *Initial processing of the skin specimens*

Skin material for primary culture of fibroblasts is obtained from surgical specimens that are disposed. These specimens are used once the patients have read and signed a Consent Term.

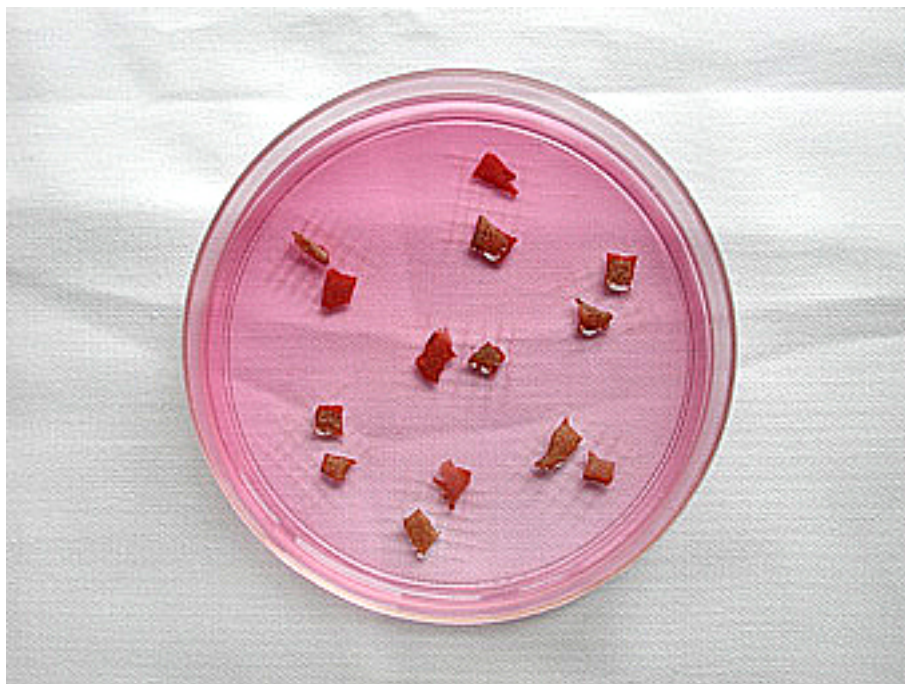
These specimens of skin are initially processed within the surgical ward, when epidermal and dermal portions of the skin is dissected with scalpels and scissors, disposing the subjacent subcutaneous tissue. The obtained samples are maintained in 50 ml conic tubes with 40 ml of Dulbecco's modified Eagle's medium (DMEM) (*Gibco, California, USA*), stored at 4°C and processed within four hours in laminar flow.

Initially, the samples are put in 50 ml conic tubes and exhaustively rinsed (eight times) with 40 ml of phosphate buffered saline (PBS) (*Sigma Chemical Co., Saint Louis, USA*) under vigorous agitation. The conic tube and the PBS are changed at each repetition.

### *Primary culture of fibroblasts*

Fibroblast harvesting is done by explant, isolating the dermis from the epidermis with scalpels and scissors. Then dermis specimens are fragmented in 5,0 mm<sup>2</sup> pieces. These fragments are laid onto the surface of 100 mm<sup>2</sup> Petri dishes, in square areas marked by perpendicular lines made with scalpel blades.

The Petri dishes are maintained semi-opened into the laminar flow for 40 minutes in order do adhere the dermis specimens in the culture surface. It is important to avoid desiccating the dermis specimens by instilling physiologic solution over them in order to maintain cellular viability (FIGURE 1).



**FIGURE 1** - Petri dish with skin fragments in its surface into culture medium after fixation in laminar flow.

Once the fragments are fixed in the Petri dish surface, 10 ml of DMEM with 20% fetal bovine serum<sup>3</sup> (*Sigma Chemical Co., Saint Louis, USA*), penicillin (100 UI/ml) and streptomycin (100 µg/ml), at 37°C, was poured in the dish.

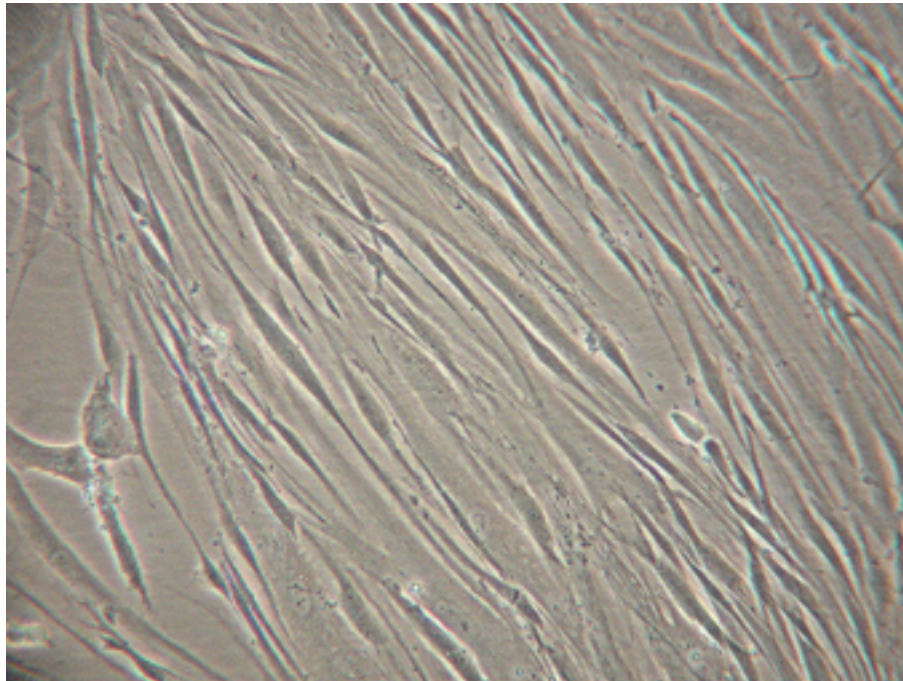
Then, the culture dish is maintained in a humidified incubator at 37°C, under 5% CO<sub>2</sub> in air.

The culture medium is changed every two days, for this rate enables the maintenance of ideal conditions of pH between 7,6 and 7,8 without non-physiologic upheavals<sup>4,5</sup>. This pH stability aimed a balance between cellular proliferation and cellular biosynthesis activity of the fibroblasts<sup>6</sup>.

Fibroblast satisfactory proliferation is observed in approximately seven days<sup>7</sup>.

#### *Subculture of fibroblasts*

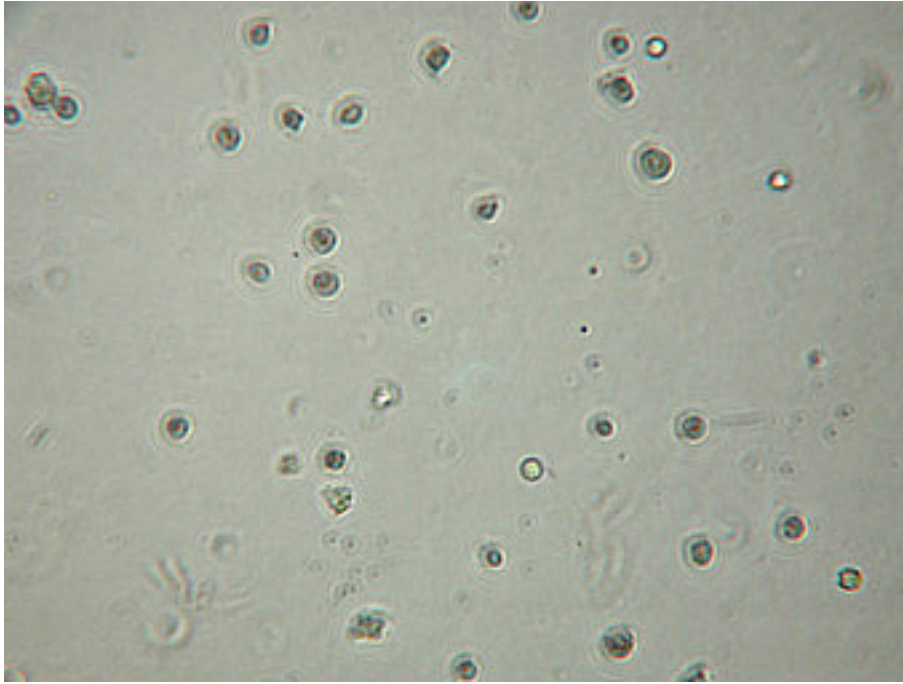
Subculture (passage) of fibroblasts was done with 80% cellular confluence in the Petri dish. (FIGURE2).



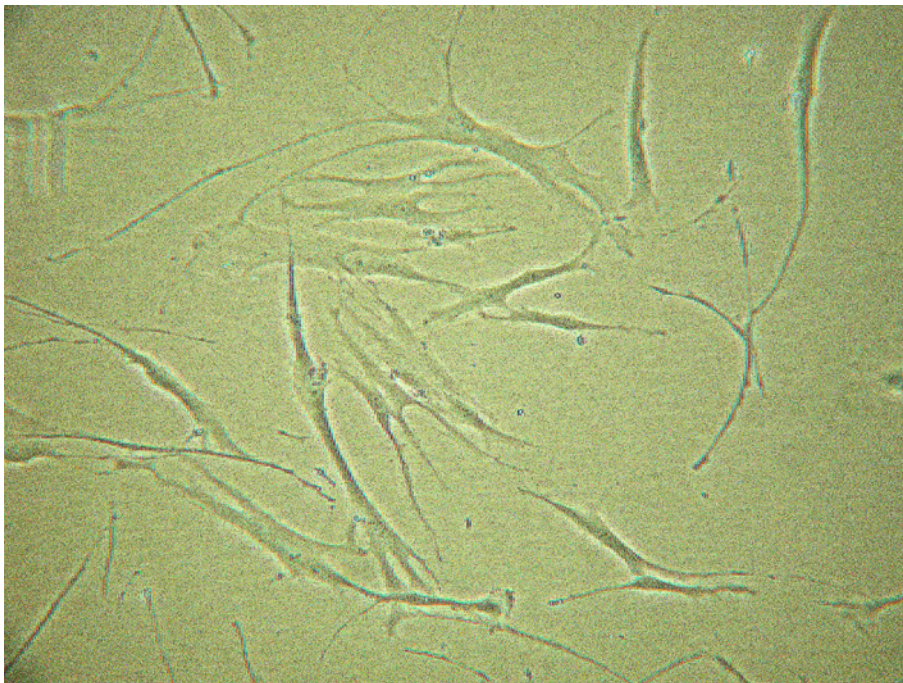
**FIGURE 2** - Human fibroblasts in Petri dish with fusiform morphology. (100x magnification)

After aspirating the culture medium, the original skin fragments are removed with pincers. Fibroblasts grown in the Petri dish are rinsed with PBS twice and then, maintained in a humidified incubator with 10 ml of PBS for 20 minutes.

Afterwards, PBS is aspirated and 1 ml of trypsin 0,25% with EDTA 0,02% (*Sigma Chemical Co., Saint Louis, USA*) is instilled in the Petri dish and maintained in humidified incubator for two minutes. Fibroblasts are observed detached from the dish surface with spherical morphology (FIGURE 3), opposite to the fusiform morphology usually observed in fibroblasts (FIGURE 2) or with cytoplasmic prolongation of fibroblasts found scattered in the culture surface (FIGURE 4).



**FIGURE 3** - Human fibroblasts in suspension detached from the surface of a Petri dish with spherical morphology. (100x magnification)



**FIGURE 4** - Human fibroblasts in Petri dish with cytoplasmic prolongations (100x magnification).

For neutralization of the enzymatic action of trypsin, 5,0 ml of culture medium supplemented with 20% fetal bovine serum is instilled in the Petri dish. The cellular suspension is centrifuged ( $100 \times g$ ) for six minutes. The supernatant (culture medium with neutralized trypsin) is disposed and the cellular pellet is suspended once more in 10 ml of culture medium

for fibroblasts. This suspension may be stored in nitrogen or used for experiment if cultivated in culture flasks (25, 75 or 175 cm<sup>2</sup>).

#### *Fibroblast storage in nitrogen*

In the third passage, fibroblasts were frozen for final storage in nitrogen.

After suspending the detached fibroblasts in 5,0 ml of culture medium, cells are counted in a Neubauer chamber. Then this solution is once more centrifuged and suspended again in 5,0 ml of storage solution (DMEM with 20% bovine fetal serum and 10% dimethylsulfoxide)<sup>5</sup> at 4°C. These cells are transferred to five cryotubes containing 5,0 x 10<sup>6</sup> to 10,0 x 10<sup>6</sup> cells in a volume of 1,0 mL.

These cryotubes are immediately transferred to a freezer at -20°C, where they are maintained for 30 minutes. Afterwards, the cryotubes are placed in a freezer at -80°C for 24 hours. Finally, they are stored in liquid nitrogen at -196°C, where they can be maintained indefinitely.

#### *Fibroblast experimental culture*

In experimental culture, fibroblasts are used between the fourth and seventh passage in order to avoid the influence of plasmatic factors<sup>9</sup> and senescent changes in the cellular morphology.

A standard amount of fibroblasts is cultivated (1,0 x 10<sup>5</sup> cells) in 75 cm<sup>2</sup> flasks and maintained at 37°C, under 5% CO<sub>2</sub> in air, changing the culture medium every 2 days.

The culture medium consists of 10 g/L DMEM, 3,7 g/L sodium bicarbonate (*Merck, Darmstadt, Germany*), 2,4 g/L HEPES buffer (*Sigma-Aldrich Chemical Co., Saint Louis, USA*) and 10% bovine fetal serum in distilled water filtered through 0,22 µm cellulosic membrane. Additional components may be used depending on the aim of experience.

## **Perspectives**

The standardization of conducts in processing the skin specimens, establishment of primary culture of fibroblasts and in the proceeding of maintenance showed satisfactory in experiments with fibroblast culture in order to investigate cellular proliferation kinetics and biosynthesis functions<sup>7,8</sup>.

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**RESUMO** - A utilização de métodos de cultura de células em Cirurgia Plástica abriu um novo horizonte na pesquisa dos mecanismos celulares de proliferação e biossíntese. Vários tipos de células têm sido investigados no compartimento cutâneo. Os queratinócitos e os fibroblastos têm sido estudados objetivando-se o desenvolvimento de biomateriais para substituição de pele. O presente estudo descreve uma padronização metodológica para o estabelecimento de uma cultura primária de fibroblastos, sua utilização em experimentos e seu armazenamento.

**DESCRITORES** - Cultura de células. Fibroblastos.

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Conflito de interesse: nenhum  
Fonte de financiamento: nenhum

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