Cell Biology
2020; 8(1): 8-11
http://www.sciencepublishinggroup.com/j/cb
doi: 10.11648/j.cb.20200801.12
ISSN: 2330-0175 (Print); ISSN: 2330-0183 (Online)

Report

Culture of Human Gingival Fibroblasts: An Experimental Model

Raoul Bationo¹,*, Ablassé Rouamba², Abdoulaziz Diarra³, Monique Lydie Beugré-Kouassi⁴, Fabienne Jordana⁵, Jean-Bertin Beugré⁴

¹CHU de Bogodogo, Ouagadougou, Burkina Faso, Université Félix Houphouët-Boigny, Abidjan, Côte d’Ivoire
²Université Joseph Ki-Zerbo/Laboratoire de Biochimie et de Chimie Appliquées, Ouagadougou, Burkina Faso
³CHU de Tengandogo, Université Joseph Ki-Zerbo/UFR SDS, Ouagadougou, Burkina Faso
⁴Département d’Orthopédie Dentofaciale/UFR d’Odonto-stomatologie, Université Félix Houphouët-Boigny, Abidjan, Côte d’Ivoire
⁵CHU de Nantes/Service d’Odontologie, Université de Nantes/UFR d’Odontologie, Nantes, France

Email address: *Corresponding author
raobat10@yahoo.fr (R. Bationo)

To cite this article:
Raoul Bationo, Ablassé Rouamba, Abdoulaziz Diarra, Monique Lydie Beugré-Kouassi, Fabienne Jordana, Jean-Bertin Beugré. Culture of Human Gingival Fibroblasts: An Experimental Model. Cell Biology. Vol. 8, No. 1, 2020, pp. 8-11. doi: 10.11648/j.cb.20200801.12

Received: May 5, 2020; Accepted: June 11, 2020; Published: June 20, 2020

Abstract: Cell culture is an important tool in medical, odontological and biological research laboratories, supporting cell therapies and tissue bioengineering strategies. It is used as a means for in vitro testing of the biocompatibility of resin polymers used in dentistry. Dulbecco's modified Eagle's medium (DMEM) or RPMI supplemented with fetal bovine serum. Several cell types are being studied including gingival fibroblasts. Gingival fibroblasts are the main cells of gingival connective tissue. These cells play an active and important role in almost all coating fabric processes, and its involvement in various pathophysiological conditions, including, healing, repair, aging, psoriasis, cancer among others, is only beginning to be understood. DMEM is the most widely used fibroblastic culture medium. This model describes a method for obtaining and cultivating human gingival fibroblasts, by explants derived from surgical discards. Fibroblasts were isolated mechanically and cultured in RPMI 1640 culture medium supplemented with fetal bovine serum 10%, Penicillin (10000 U/ml)/Streptomycin (10 mg/ml) 1% and L-Glutamine (200 mM) 1%. The culture medium is replaced every two days. Cells forming a fairly dense network were observed after a period of 4 days of culture. Human gingival fibroblasts can be cultured by direct explant technique with RPMI 1640 culture medium supplemented with fetal bovine serum and antibiotics.

Keywords: Cell Culture, Gingival Fibroblast, RPMI 1640 Culture Medium

1. Introduction

Gingival fibroblasts are the main cells of gingival connective tissue [1]. They are involved in the repair of gingival connective tissue and in the mediation of epithelial cell morphogenesis, also aiding in the restoration of oral epithelium [2, 3].

Fibroblast is ubiquitous mesenchymal cell involved in many vital functions including embryogenesis and development, homeostasis and tissue repair [4-6]. Fibroblasts play an active and important role in almost all coating fabric processes, and its involvement in various pathophysiological conditions, including, healing, repair, aging, psoriasis, cancer among others, is only beginning to be understood [7].

Cell culture is an important tool in medical, odontological and biological research laboratories, supporting cell therapies and tissue bioengineering strategies. It is used as a means for in vitro testing of the biocompatibility of resin polymers used in dentistry. Dulbecco's modified Eagle's medium (DMEM) is the most used culture medium for cell culture.

The present study intends to describe a method for obtaining and cultivating human gingival fibroblasts, by explants derived from surgical discards, for in vitro research. This model was
inspired by that of Keira et al. [8]. It will allow the assessment of the biocompatibility of resin-based materials.

2. Method Description

2.1. Gingival Tissue Removal

Surgical gum waste from dental extraction in a patient who has given his consent and has no periodontal pathology are cut into small pieces with surgical blade.

Gum specimens are placed in a tube containing RPMI 1640 culture medium (Sigma-Aldrich, St Louis, USA). The sample is stored at room temperature and then transported to the laboratory within 4 hours.

2.2. Fibroblasts Primary Culture

The explants are rinsed with phosphate buffered saline (PBS) (Medicago AB Sweden) and then laid onto the connective side in a 100 mm² Petri dish. The dish is kept semi-open under laminar flow for 30 minutes so that explants can adhere to the culture surface. Culture medium is instilled on the explants to prevent them from drying out and to maintain cell viability.

10 ml of RPMI 1640 culture medium supplemented with fetal bovine serum 10% (Sigma-Aldrich, St Louis, USA), Penicillin (10000 U/ml)/Streptomycin (10 mg/ml) 1% (Sigma-Aldrich, St Louis, USA) and L-Glutamine (200 mM) 1% (Sigma-Aldrich, St Louis, USA) is poured in the dish then it is stored in humidified incubator at 37 °C under 5% CO₂ in air. (Figure 1).

The culture medium is replaced every two days up to confluence.

2.3. Fibroblasts Subculture

After aspiration of the culture medium, the Petri dish is rinsed with 5 ml of PBS for 30 seconds in order to remove dead cells. PBS is aspirated and then 10 ml of culture medium is poured to the dish.

Cells are observed under an inverted microscope (Figures 2 and 3).

When cells have reached the confluence stage, explants are removed from the dish and culture medium is aspirated. Cells are rinsed twice at PBS and then kept in humidified incubator for 20 minutes with 10 ml of PBS.

PBS is aspirated and 1.5 ml of trypsin + EDTA (0.25% + 0.02% in PBS 1X) is instilled in the dish for 3 minutes at 37°C to detach and separate the cells. Mechanical separation is also carried by multiple pipetting. 8.5 ml of culture medium is then added to the dish to deactivate the trypsin. The whole is transferred into Falcon 15 tubes and centrifuged for 5 minutes to 0.2 rpm (Figure 4).

The supernatant is aspirated without going to the bottom. 10 ml of culture medium is added to each tube to resuspend the cells. This suspension can be used in the context of biocompatibility tests.
fibroblasts isolated by tissue explants and by enzyme digestion methods. They concluded that enzyme-digestion showed much more mesenchymal stem cell-like properties with significantly higher self-renewal capacity and adipogenic and osteogenic differentiation ability compared with tissue-explant.

4. Conclusion

The direct explant technique of human gingival fibroblasts culture is a viable model for obtaining gingival cells suitable for laboratory tests.

Human gingival fibroblasts can be cultured in RPMI 1640 culture medium supplemented with fetal bovine serum, Penicillin/Streptomycin and L-Glutamine.

Acknowledgements

Manipulations were carried out to the Plateforme de culture cellulaire of Laboratoire de Biochimie et de Chimie Appliquées (LABIOCA) of Université Joseph Ki-Zerbo.

References

[1] Schroeder, H. E. and Listgarten, M. A. (1997). The gingival tissues: the architecture of periodontal protection. Periodontol 2000 13: 91-120.
[2] Mussig, E., Steinberg, T., Schulz, S., Spatz, J. P., Ulmer, J., Grabe, N., Kohl, A., Komposch, G. and Tomakidi, P. (2008). Connective-tissue fibroblasts established on micropatterned interfaces are pivotal for epithelial-tissue morphogenesis. Adv Funct Mater 18 (19): 2919-2929.
[3] Locke, M., Hyland, P. L., Irwin, C. R. and Mackenzie, I. C. (2008). Modulation of gingival epithelial phenotypes by interactions with regionally defined populations of fibroblasts. J. Periodontal. Res 43 (3): 279-89.
[4] Carrel, A. and Ebeling, A. H. (1921). Age and Multiplication of Fibroblasts. J Exp Med 34 (6): 599-623.
[5] Carrel, A. and Ebeling, A. H. (1921). The Multiplication of Fibroblasts in Vitro. J Exp Med 34 (4): 317-337.
[6] Carrel, A. and Ebeling, A. H. (1926). The Fundamental Properties of the Fibroblast and the Macrophage: I. The Fibroblast. J Exp Med 44 (2): 261-284.
[7] Sriram, G., Bigliardi, P. L. and Bigliardi-Qi, M. (2015). Fibroblast heterogeneity and its implications for engineering organotypic skin models in vitro. Eur J Cell Biol 94 (11): 483-512.
[8] Keira, S. M., Ferreira, L. M., Gragnani, A., Duarte, I. S. and Santos, I. A. N. (2004). Experimental model for fibroblast culture. Acta Cir Bras 19: 11-16.
[9] Reid, C. B. A., Cloos, J., Snow, G. B. and Braakhuis, B. J. M. (1997). A simple and reliable technique for culturing of human oral keratinocytes and fibroblasts. Acta Otolaryngol 117 (4): 628-633.
[10] Bernice, M. M. (1994). Tissue Culture techniques. An Introduction, Brown-Brumfield, Inc. Ann. Arbor, MI, U.S.A, pp. 116-124.
[11] Kedjarune, U., Pongprerachok, S., Arpornmeaklong, P. and Ungkusonmongkhon, K. (2001). Culturing primary human gingival epithelial cells: comparison of two isolation techniques. Journal of Carnio-Maxillofacial Surgery 29 (4): 224-231.

[12] Klingbeil, M. F. G., Herson, M. R., Cristo, E. B., Pinto, Jr. D. S., Yoshito, D. and Mathor, M. B. (2009). Comparison of two cellular harvesting methods for primary human oral culture of keratinocytes. Cell Tissue Bank 10 (3): 197-204.

[13] Lauer, G., Otten, J. E., Von Specht, B. U. and Schilli, W. (1991). Cultured gingival epithelium. A possible suitable material for pre-prosthetic surgery. Journal of Carniomaxillofacial Surgery 19 (1): 21-26.

[14] Flaxman, B. A., Lutzner, M. A. and Van Scott, E. J. (1967). Cell maturation and tissues organization in epithelial outgrowths from skin and buccal mucosa in vitro. The Journal of Investigative Dermatology 67: 8-14.

[15] Daniels, J. T., Kearney, J. N. and Ingham, E. (1996). Human keratinocyte isolation and cell culture: a survey of current practices in the UK. Burns 22: 35-39.

[16] Abreu, F. A. M., Reis, I. D. G., Silva, G. A. B. and Jorge, E. C. (2019). Collection and culture of human connective tissue cells from gingival explant technique for oral tissue bioengineering. Int J Morphol 37 (4): 1229-1233.

[17] Tsugeno, Y., Sato, F., Muragaki, Y. and Kato, Y. (2014). Cell culture of human gingival fibroblasts, oral cancer cells and mesothelioma cells with serum-free media, STK1 and STK2. Biomedical Reports 2: 644-648.

[18] Saczko, J., Dominiak, M., Kulbacka, J., Chwilowska, A. and Krawczykowska, H. (2008). A simple and established method of tissue culture of human gingival fibroblasts for gingival augmentation. Folia Histochem Cytobiol 46 (1): 117-119.

[19] Pini Prato, G. P., Rotundo, R., Magnani, C. and Soranzo, C. (2000). Tissue engineering technology for gingival augmentation procedures: A case report. Int J Periodont Restorative Dent 20: 553-559.

[20] Xu, Q., Ji, Q., Yang, J., Xu, X., Yu, X., Wang, Z. and Deng, J. (2016). Comparison of the characteristics of human gingival fibroblasts isolated by tissue explants and by enzyme digestion methods. Int J Clin Exp Med 9 (10): 19757-19763.