# Experimental model for establishment of hypoxia in 75 $cm^2$ culture flasks[1]

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**ABSTRACT -** In Plastic Surgery, cell culture represents the perspective of studying cellular mechanisms that guide the healing process of several tissues. Some steps of the healing process depend on physical factors as the tissular partial pressure of $O_2$. In cell culture, it is possible to submit cells to hypoxic enviroment. The present study reports an alternative method at low cost for the establishment of hypoxic environment in cell culture flasks.

**KEY WORDS –** Cells, cultured. Hypoxia.

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## Introduction

*In vitro* experimental models in Plastic Surgery has been useful to the study of biochemical and biophysical aspects of cellular dynamics from different tissues manipulated in this specialty.

In skin cell culture, it is possible to standardize factors that may have influence cutaneous cells. This fact enables the study of several physical factors (temperature, characteristics of culture medium and partial pressure of oxygen[1]) or chemical factors (drugs[2], hormones, citokynes[1,3,4,5] and vitamins).

In previous literature, the establishment of hypoxic environment in culture flasks was mentioned by IANNONNE *et al.*[6], in a study concerning free radical biosynthesis in the metabolism of organic hydro-peroxides by normal human keratinocytes.

## Proposition

Hypoxic environment may be established by using incubators with simultaneous flow of air, $CO_2$ and $N_2$. Nevertheless, this method has in its expensive cost its main disadvantage for it is necessary to settle an incubator for this exclusive purpose.

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The present study proposes an alternative method for the establishment of hypoxic environment in 75 $cm^2$ culture flasks, with its reproducibility determined by standardization of culture medium volume, composition of hypoxic gas mixture and gas flow rate.

**Method description**

The 75 $cm^2$ culture flasks with 7,0 ml of culture medium were closed with silicon stoppers (30x24x32mm) and sealed with a double layer cellulose film (Parafilm M®). Two needles (40x8 mm) were introduced in the flasks through the stopper. One of them was connected in a infusion system with the hypoxic gas mixture. The second needle was used as an output way to the gas flow (FIGURES 1, 2).

**FIGURE 1 -** Hypoxia system with gas mixture of 95% $N_2$ and 5% $CO_2$ consisting of gas torpedo, conductors of the gas mixture to the culture flask, inside a laminar flow system.


**FIGURE 2 -** Hypoxia system with the gas mixture of 95% $N_2$ and 5% $CO_2$ connected in the culture flask, onto a vortex shaker.

The hypoxic gas flow was maintained for 30 minutes with a constant flow of 100 ml/min and under constant agitation in a vortex shaker. All these procedures were done inside a laminar flow.

Both needles were taken away together at the end of the hypoxia session and the culture flasks were maintained sealed at 37 °C for more 30 minutes or 24 hours.

Partial pressure of oxygen in the culture medium was estabilished by gasometry in a pHmeter and gas analyser (Radiometer Copenhagën®, model ABL 330) (TABLE 1). With this hypoxia establishment procedure it was possible to lower the partial pressure of $O_2$ to an average of 30 mmHg.

**TABLE 1 -** Partial pressure of $O_2$ (mmHg) of culture medium (7,0 ml) collected from 75% culture flasks in air, in incubator (5% $CO_2$ in air), 30 minutes after hypoxic infusion and 24 hours after hypoxic infusion and averages.

| SAMPLE | *AIR* | *INCUBATION* | *POST-INFUSION* | *POST-24h* |
|---|---|---|---|---|
| **1** | 192,9 | 151,3 | 32,1 | 30,3 |
| **2** | 190,8 | 148,5 | 24,6 | 28,7 |
| **3** | 181,2 | 151,6 | 26,7 | 26,5 |
| **4** | 192,6 | 146,8 | 30,6 | 29,2 |
| **5** | 192,1 | 148,5 | 27,1 | 29,7 |
| **6** | 184,3 | 152,6 | 28,3 | 32,0 |
| **7** | 187,4 | 152,9 | 28,3 | 24,1 |
| **8** | 184,3 | 152,6 | 27,7 | 24,1 |
| **9** | 175,5 | 152,5 | 26,4 | 35,8 |
| **10** | 178,5 | 153,1 | 21,8 | 25,0 |
| **11** | 180,8 | 153,3 | 28,4 | 24,9 |
| **12** | 179,3 | 151,7 | 26,8 | 24,0 |
| **13** | 182,2 | 151,9 | 28,2 | 30,3 |
| **14** | 182,4 | 151,6 | 28,0 | 35,1 |
| **15** | 183,6 | 152,8 | 28,1 | 30,9 |
| **16** | 183,5 | 153,1 | 27,8 | 29,9 |
| **17** | 181,8 | 151,9 | 22,5 | 28,4 |
| **18** | 180,6 | 151,5 | 21,8 | 25,6 |
| **19** | 180,5 | 151,5 | 25,2 | 26,6 |
| **20** | 179,4 | 152,9 | 30,3 | 29,4 |
| **Average** | 183,7 | 151,6 | 27,0 | 28,5 |

Analyses of significant differences between data sets were performed by the use of Friedman's test and Multiple Comparison test. Values of $p < 0,05$ were considered statistical significant.

It was observed that there is statistical significant difference ($\chi^2_{calc} = 54,240$ - $p = 0,000$) among the groups maintained in air, in incubation under 5% $CO_2$ in air and after infusion of hypoxic gas mixture and maintenance of hypoxia for 30 minutes. These results show that the present method was efficient in establishing a hypoxic environment into the culture flask.

On the other, there was not statistical significant difference (TABLE 2) between the group submitted to hypoxic infusion and stabilized for 30 minutes and the group maintained sealed for 24 hours with the hypoxic environment after the infusion. This result showed that the present experimental method is effective in maintaining the hypoxic environment into the culture flask for so long as 24 hours after the hypoxic infusion procedure (FIGURE 3).

**TABLE 2 -** Multiple Comparison test results between groups of 75 $cm^2$ culture flasks with culture medium (7,0 ml) maintained in air, in incubator (5% $CO_2$ in air), 30 minutes after hypoxic infusion and 24 hours after hypoxic infusion. msd - minimal significant difference, [ * ] - significant, n.s. - non-significant.

| msd = 20,980 | AIR | INCUBATOR | post-INFUSION | post-24h |
|---|---|---|---|---|
| **AIR** | ----- | 20,000 n.s. | 52,000 * | 48,000 * |
| **INCUBATOR** | ----- | ----- | 32,000 * | 28,000 * |
| **post-INFUSION** | ----- | ----- | ----- | 4,000 n.s. |
| **post-24h** | ----- | ----- | ----- | ----- |

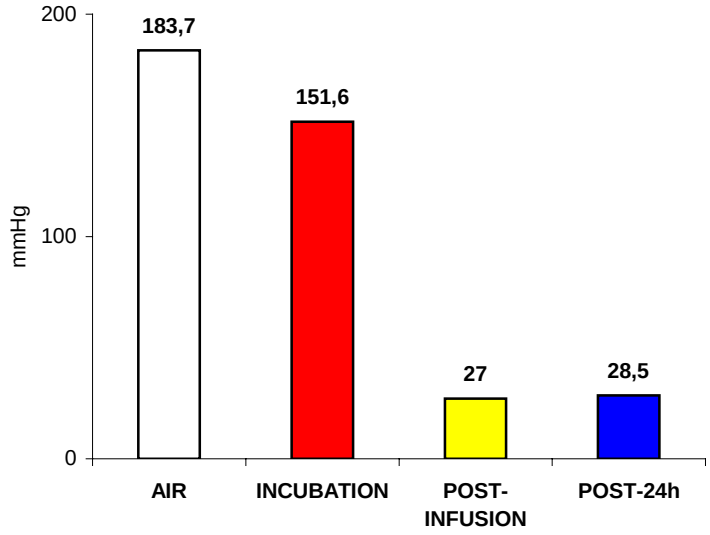


**FIGURE 3 -** Averages of partial pressure of $O_2$ (mmHg) of culture medium collected from 75$cm^2$ culture flasks maintained in air, in incubator (5% $CO_2$ in air), 30 minutes after hypoxic infusion and 24 hours after hypoxic infusion.

**Perspectives**

The present experimental model showed efficient to establish a hypoxic environment in 75 $cm^2$ culture flasks maintained as a sealed system with the hypoxia apparatus. The theoretical basis that justifies the reproduction of this model is the standardization of culture medium volume and superficial area, duration of the hypoxic infusion and the hypoxic gas mixture gradient between the gas and liquid phase in the culture flask. So the theoretical substratum of Fick Law (theory of gas diffusion in the interface between two distinct phases) was applied to the cell culture system[7].

This experimental model consists an alternative for the establishment of hypoxic environment in 75 $cm^2$ culture flasks at low cost.

**References**


1. Falanga V, Qian SW, Danielpour D, Katz MH, Roberts AB, Sporn MB - Hypoxia upregulates the synthesis of TGF-beta 1 by human dermal fibroblasts. J Invest Dermatol. 1991;97:634-7.
2. Castor CW, Muirden KD - Collagen formation in monolayer cultures of human fibroblasts. The effects of hydrocortisone. Lab Invest. 1964;13:560-74.
3. Haisa M, Okochi H, Grotendorst GR - Elevated levels of PDGF alpha receptors in keloid fibroblasts contribute to an enhanced response to PDGF. J Invest Dermatol. 1994;103:560-3.
4. Bettinger DA, Yager DR, Diegelmann RF, Cohen IK - The effect of TGF-beta on keloid fibroblast proliferation and collagen synthesis. Plast Reconstr Surg. 1996;98(5):827-33.
5. Steinbrech DS, Mehrara BJ, Chau D, Rowe NM, Chin G, Lee T - Hypoxia upregulates VEGF production in keloid fibroblasts. Ann Plast Surg. 1999;42(5):514-9.
6. Iannonne A, Marconi A, Zambruno G, Giannetti A, Vanninni V, Tomasi A - Free radical production during metabolism of organic hydroperoxides by normal human keratinocytes. J Invest Dermatol. 1993;101(1):59-63.
7. McLimans WF, Crouse EJ, Tunnah KV, Moore GE - Kinetics and gas diffusion in mammalian cell cultures systems. I: Exper Biotech Bioeng. 1968;10:725-63.


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**RESUMO -** A cultura de células, na Cirurgia Plástica, representa uma perspective para o estudo dos mecanismos celulares que norteiam o processo cicatricial de diversos tecidos. Algumas etapas do processo de cicatrização dependem de fatores físicos como a pressão parcial de $O_2$. Em uma cultura de células, é possível submeter células a um ambiente hipóxico. O presente estudo relata um método alternativo de baixo custo para o estabelecimento de um ambiente hipóxico em frascos de cultura de células.



**DESCRITORES** - Cultura de células. Hipóxia.


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