



New method for detection of mutagenicity in oral mucosa through the micronucleus test

Dionatas Ulises de Oliveira Meneguetti^{1*}, Francisco da Silva², Rosa Bosso², Renato Zan³, and Leandro Ramos⁴

Correspondence: dionatas@icbusp.org

¹ Department of biology, Faculty of Education and the Environment, Ariquemes, Rondônia, Brazil.

² Department of biology, Centro Universitário Luterano de Ji-paraná, Rondônia, Brasil.

³ Department of chemistry, Faculty of Education and the Environment, Ariquemes, Rondônia, Brazil.

⁴ Department of Health, Faculty of Education and the Environment, Ariquemes, Rondônia, Brazil.

Abstract

Background: The micronucleus assay using a tissue rapidly dividing, such as human oral mucosa, allows for assessment of damage of genetic material, without the need for a stage of ex vivo cell replication.

Methods: The present study describes a new protocol for the detection of MN in oral mucosa, replacing methanol and giemsa by triarilmetano, xanthene and thiazine.

Results: The blades were well visible with ungrouped cells, having a good view of the cell boundaries.

Conclusions: Good results were obtained with the substitution of methanol and giemsa by triarilmetano, thiazine and xanthene, because of its efficiency in bluish nucleic acid, getting a good visualization of cell nuclei and micronucleus, and this methodology it is indicated for mutagenicity analysis of the oral mucosa.

Keywords: Mutagenicity, oral mucosa and micronucleus

Background

The action of carcinogens can induce chromosomal instabilities such as deletions, translocations, gain or loss of entire chromosomes, contributing to the development of malignant cellular processes [1].

These chromosomal disorders give rise to micronuclei, which are globular structures resulting from chromosomal fragments or entire chromosomes that lag behind at anaphase during nuclear division, which allows us to detect the action of clastogenic and aneugenic agents [2,3].

Among the bioassays used to evaluate the impact of environmental factors, genetic and lifestyle on genomic stability in humans, the micronucleus assay (MN-assay) has detached by its ease of use and a low cost associated the precision of viewing a larger number of cells [4].

For evaluation of genetic damage have been developed several techniques among them, the micronucleus test in human oral mucosa has detached by using metaphase cells. Methods like this that uses oral mucosal tissue with large numbers of metaphase it is of fundamental relevant for the reliability of statistical analysis [5].

The use of oral epithelial cells as bioindicator of genotoxic damage resulting from exposure to environmental carcinogen by inhalation and ingestion, added to the digestive tract damage can be explained by the ability of cells metabolize

these compounds become the first physical barrier for these organs, resulting in increased incidence of cancers in human epithelial cells [6].

The micronucleus assay using a tissue rapidly dividing, such as human oral mucosa, allows for assessment of damage of genetic material, without the need for a stage of ex vivo cell replication. In order to delineate and obeter data from different centers and populations, a collaborative program global Human MicroNucleus Project (HUMNXL) addressed a series of technical mechanisms and epidemiological items regarding the micronucleus test in cells of human oral mucosa providing reference values frequency, thereby creating the basis for a detailed validation of the same [7].

The number of publications that refer to this test has increased greatly in the last decade and can be explained by several factors, including its relative technical simplicity and variety of toxicological endpoints which may be evaluated [6].

The utilization of tests that demonstrate the action of mutagenic agents allows the understanding of the interaction between the chemical Agents, physical and biological that is of fundamental importance for understanding and controlling the factors that predispose the occurrence of degenerative diseases [2]. However, the establishment of an efficient protocol and more economically viable it is of the great importance to the use of MN-test cells for human oral mucosa. In this sense,

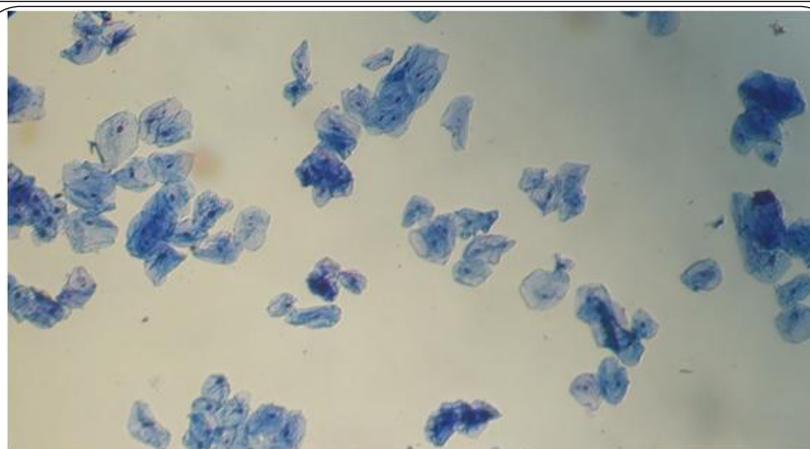


Figure 1. Oral mucosa cells (ocular 10x, objective 10x).

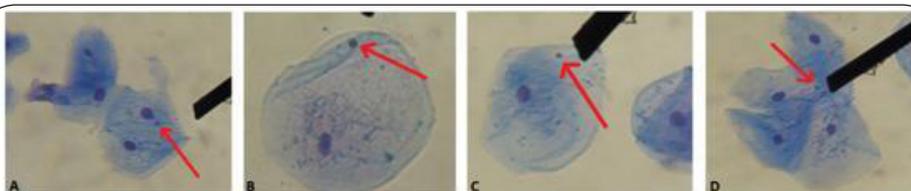


Figure 2. Micronucleus in oral mucosa cells (ocular:10x, objective:10x).

the development of new tests using new equipment and reagents, expands the possibilities of improving this method including speed, accessibility and efficiency us results.

The present study aimed describes a new protocol for the detection of MN in oral mucosa, replacing methanol and giemsa by triarilmetano, xanthene and thiazine.

Methods

Collection of samples

Perform mouth wash with distilled water (three replicates) to remove traces of saliva and surface mucous. Then perform exfoliation within the two cheeks to maximize the cell sample and remove any trace unknown, which may be caused by sampling of only one cheek.

The exfoliation should use a disposable brush similar to that used for Pap smear, performing 10 rotations of the brush against the inner wall of the cheeks starting in the center and gradually increasing the circumference, producing a spiral effect to increase the sampling of a larger area and prevent erosion continuous of a single region [8].

Storage and Transport of Samples

After exfoliation, the brushhead must be placed in a container (test tube) with 04ml of buffer containing: 0.01 M Tris hydrochloride (Tris HCl), 0.1 M acid ethylenediaminetetraacetic (EDTA) and 0.02M Sodium Chloride (NaCl) with pH 6.8, turning in the way that the cells are released and displaced on the inner edge of the container. The containers must then be sealed tight to prevent leakage of cells (during transport to the laboratory).

Preparation of Blades

In the laboratory the tubes should be homogenized by vortex, and removed the brush from the test tube, centrifuged the 1,000 rpm for 10 minutes, and then discarded the supernatant and added 04 ml of buffer solution for further washing the cells, promoting removing of bacteria. This procedure must be repeated twice more time, completing three washes.

Then added to 2ml of Triarilmetano 0.1% and 2ml of xanthenes 0.1% (fixative solution), The solution was again homogenized in a vortex and centrifuged at 1000 rpm for 10 minutes, the supernatant was discarded leaving only the white pellet plus three times the amount of pellet of solution 50% Triarilmetano to 0.1% and 50% xanthenes 0,1%, and again homogenized. In sequence the homogenization of the resulting product will be removed and dripped 3 drops in blades preheated to 37 °C with the aid of a Pasteur pipette and placed to dry at room temperature for 30 minutes.

After drying, the blades has to be stained with triarilmetano 0.1%, xanthenes 0,1% and thiazine 0,1%, and the blades dipped 10 times in each container with the immersion duration of 1 second, using the sequence described above . Thereafter, the blades should be washed in distilled water to remove excess dye and dried at room temperature for 30 minutes, for cell counting later.

Results and Discussion

The blades were well visible with ungrouped cells, having a good view of the cell boundaries (Figure 1).

There was also a better staining of nuclei and micronuclei, as can be seen in (Figure 2, A, B, C and D), being equivalent to the blades using

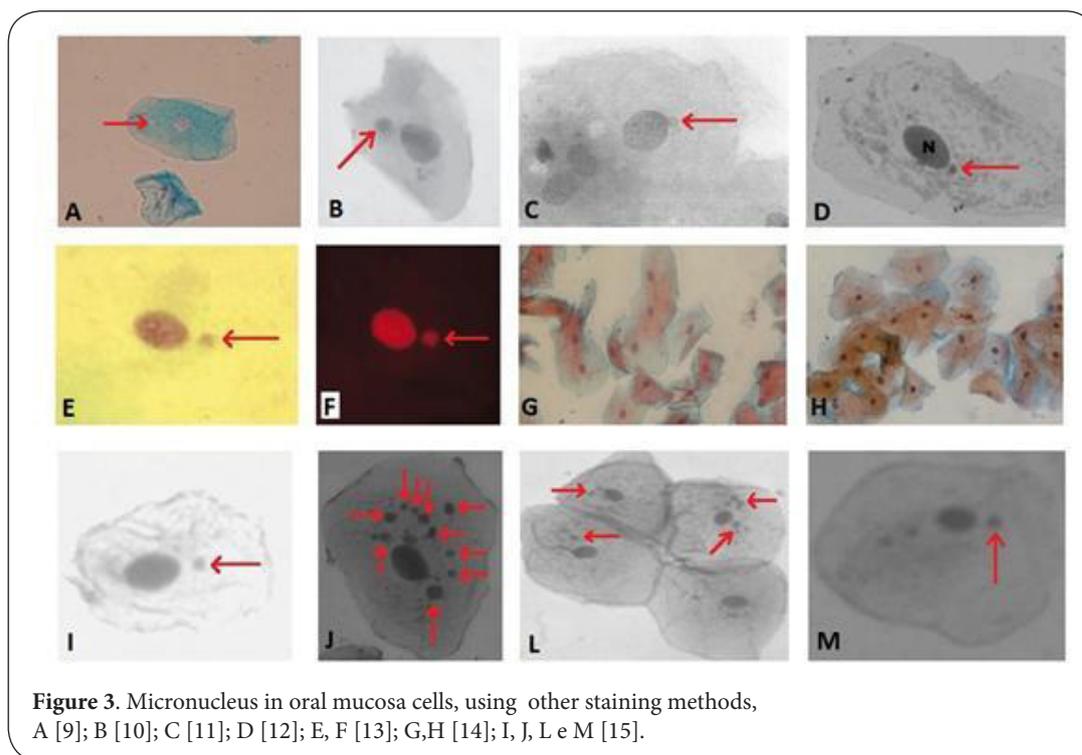


Figure 3. Micronucleus in oral mucosa cells, using other staining methods, A [9]; B [10]; C [11]; D [12]; E, F [13]; G,H [14]; I, J, L e M [15].

other staining methods (**Figure 3A, B, C, D, E, F, G, H, I, J, L and M**).

It is important to highlight that in (**Figure 2**) was not used any software to improve the quality of images, can get an even better resolution.

The reagents, triarilmetano, thiazine and xanthenes, already have been use e successfully in the preparation of blades for micronucleus *Allium cepa*, seen their efficiency in the blush nucleic acid, obtaining a good visualization of cells, mitotic index, micronucleus, anaphase bridges and telophase [12-15]. This success in staining was also observed in the preparation of these blades micronucleus oral mucosa, seeing that using the same pattern of staining nucleic acids. Another advantage is the speed for the preparation of the blades, since the reagents used is ready in kits for rapid staining, and the low cost thereof.

Conclusion

Good results were obtained with the substitution of methanol and giemsa by triarilmetano, thiazine and xanthene, because of its efficiency in blush nucleic acid, getting a good visualization of cell nuclei and micronucleus, and this methodology it is indicated for mutagenicity analysis of the oral mucosa.

Competing Interests

The Authors declare that they have no competing interests.

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