PRIMARY FIXATION OF VERVET MONKEY ORAL EPITHELIUM FOR SEM AND TEM

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There is a fivefold difference in the osmolarity of primary fixatives used in previous published electron microscope studies of the oral mucosa[1]. This study was therefore undertaken to determine an optimal primary fixation procedure (by either perfusion or immersion) and fixative osmolarity for vervet monkey (Cercopithecus pygerythrus) oral epithelium for SEM and TEM prior to a histometric study of this tissue.

Eight anaesthetised vervet monkeys of 3.5-5.0kg body mass with clinically healthy oral mucosa were used immediately after nephrectomy for poliomyelitis vaccine production. In five monkeys 250-500ml of 0.9% sodium chloride at 35°C plus heparin at 1000IU/kg body mass was retrogradely perfused through the head via the dorsal aorta at 136mm Hg pressure[2]. Immediately after the saline prewash a separate monkey was perfused with one litre of the listed fixatives (Solns. 1, 8, 9, 10, 11) at the same temperature and pressure. Thereafter the heads were dissected free and immersed in the same fixative for 24 hours at 4°C. After 24 hours blocks of mucosa were dissected from the buccal mucosa, alveolar mucosa, tongue, attached gingiva and hard palate and further processed for TEM. The remaining three monkeys provided specimens of buccal mucosa, alveolar mucosa and hard palate for TEM study and attached gingiva and alveolar mucosa for SEM examination. Blocks of tissue were fixed by immersion in one of each of the following Solns. 1-11 for the TEM study and Solns. 1-12 for the SEM study: Karnovsky's fixative[3] at the following strengths:

1. 100% - 2010mOsm. 2. 90% - 1400mOsm. 3. 80% - 920mOsm.
4. 70% - 830mOsm. 5. 60% - 740 mOsm. 6. 50% - 580mOsm.
7. 40% - 450mOsm. 8. 30% - 320mOsm. 9. A fixative used by Landay and Schroeder[4] ie. 2.5% glutaraldehyde and 2% paraformaldehyde in a 0.02M sodium cacodylate buffer - 900mOsm. 10. A standard 2.5% glutaraldehyde fixative in a 0.1M sodium cacodylate buffer - 470mOsm. 11. A standard 2.5% glutaraldehyde fixative in a 0.1M phosphate buffer - 550mOsm. 12. 10% buffered formol saline - 440mOsm.

After fixation all tissue was briefly rinsed in 0.185M sodium cacodylate buffer of 350mOsm at pH 7.4 for ten minutes and postfixed for two hours in a 2% aqueous solution of osmium tetroxide all at 4°C. The blocks were then rinsed for ten minutes in the same buffer. Thereafter the SEM and TEM specimens were processed according to standard procedure, viewed and assessed as to quality of fixation.

Good quality fixation could not be uniformly nor consistently obtained with the perfusion technique. Areas of good fixation were found adjacent to poor. Curling of the dissected tissue proved a drawback in the immersion fixation TEM and SEM studies, which was overcome by immersing the entire head in fixative and dissecting out the required tissue once it was fixed. TEM examination revealed that tissue immersion fixed in Solns. 1 and 2 produced severe plasmolysis, whereas Solns. 4, 5, 6, 7, 8, 10, 11 resulted in swollen cells with eroded nuclei. Solns. 3, 9 produced well fixed oral epithelium and 9 produced consistently good results over a number of TEM studies. Morphologically no difference was apparent between the SEM specimens fixed in the different primary fixatives. The epithelial cells of the attached gingiva showed the characteristic pitting while the cells of the alveolar mucosa were typically microplicated. In all specimens, cells with relatively smooth surfaces were found. This is a normal feature of oral epithelium where pressure, abrasion and aging of the exposed cell surface is responsible for this loss of surface detail. However point counting methods revealed significant differences at $p<0.001$ in the surface density of the microvilli present on the epithelial cell undersurface of the attached gingiva and the microplications of the alveolar mucosa epithelial cell undersurface fixed in the highest (1) and lowest (8) osmolar solutions. It was also found that when cells were stripped to expose the cell undersurfaces for point counting measurement, the higher osmolarity fixed alveolar mucosa epithelial cells would separate intercellularly, whereas alveolar mucosa epithelial cells fixed in (8) tended to separate intracytoplasmically.

This investigation has shown that oral epithelium is best fixed by immersion and at about 900mOsm for TEM study. Osmolarity does not seem to influence the overall SEM appearance of the oral epithelium, but detailed investigation does reveal changes in morphology attributable to osmolarity. If cell stripping techniques are employed to examine the deeper cell layers of this tissue in the SEM, primary fixative solutions of a higher osmolarity should be used. Further, this study emphasises the difficulties encountered when one attempts to determine "optimal" fixation methods for SEM processing and raises rhetoric questions about the nature of artefacts.

References

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