

Analysis of the histopathological artifacts in punch biopsies of the normal oral mucosa

Fabio Camacho Alonso ¹, Pía López Jornet ¹, María José Jiménez Torres ², Albina Orduña Domingo ²

(1) Department of Oral Medicine, Faculty of Medicine and Odontology, University of Murcia, Spain

(2) Service of Pathology. Alicante University General Hospital, Spain

Correspondence:

Dr. Fabio Camacho-Alonso

Dentistry Clinic University

Hospital Morales Meseguer (Second Floor)

C/ Marqués de los Vélez s/n

30008 – Murcia (Spain)

E-mail: fcamacho@um.es

Received: 07/02/2008

Accepted: 08/06/2008

Indexed in:

-Index Medicus / MEDLINE / PubMed
-EMBASE, Excerpta Medica
-SCOPUS
-Indice Médico Español
-IBECS

Camacho-Alonso F, López-Jornet P, Jiménez-Torres MJ, Orduña-Domingo A. Analysis of the histopathological artefacts in punch biopsies of the normal oral mucosa. Med Oral Patol Oral Cir Bucal. 2008 Oct;13(10):E636-9.

© Medicina Oral S. L. C.I.F. B 96689336 - ISSN 1698-6946

<http://www.medicinaoral.com/medoralfree01/v13i10/medoralv13i10p636.pdf>

Abstract

Objectives: To study the most frequent artifacts of punch biopsies of the healthy oral mucosa, distinguishing between those attributable to the surgical technique and those resulting from sample processing in the laboratory. **Study design:** A total of 186 samples of oral mucosa (dorsal lingual mucosa) were obtained from 186 adult albino rats by the experienced oral surgeons, evaluating the presence of artifacts attributable to the surgical technique (crush, splits, fragmentation, pseudocysts and haemorrhage) and those attributable to sample processing in the laboratory (orientation). **Results:** The distribution of artifacts attributable to the surgical procedure was as follows: crush 16.67%, splits 0%, fragmentation 0%, pseudocysts 1.08% and hemorrhage 0%. The artifacts corresponding to sample processing in the laboratory (orientation) represented 37.63%. **Conclusions:** The present study shows use of the punch for obtaining biopsies of the healthy oral mucosa to produce few artifacts. In addition, good or poor orientation of the samples is not attributable to the surgical technique but to processing of the samples in the laboratory.

Key words: Oral biopsy, artifacts, punch, oral mucosa.

Introduction

The biopsy of oral mucosa lesions constitutes minor surgery, defined as the obtainment of tissue from a live organism for microscopic study in order to establish a diagnosis. The biopsy specimen can be affected by numerous types of artifacts (1-3). Some, such as freezing or fulguration of the sample, may be caused by the surgical instruments used: cryosurgery, electrocautery and the CO2 laser (2,4,5).

In relation to use of the cold scalpel in oral biopsy, some authors (6,7) suggest that the punch biopsy reduces the number of artifacts compared with the conventional scalpel. In effect, such authors found punch biopsy to reduce the incidence of crush, splits, fragmentation, pseudocysts and haemorrhage. One of these investigators (6) concluded that it also reduces the incidence of poor sample orientation. However, no study to date has examined which of

these artifacts are attributable to the surgical technique (punch biopsy) and which are a consequence of sample processing in the laboratory. Finally, some of these studies (6,8) evaluate the presence of artifacts such as splits, fragmentation and/or haemorrhage in biopsies obtained from patients with underlying inflammatory processes that can facilitate the generation of artifacts.

The aims of this study were to investigate which artifacts appear most often in punch biopsies of the healthy oral mucosa, and to determine which are attributable to the surgical technique and which are a consequence of sample processing in the laboratory.

Materials and Methods

The animals used in this study were obtained from the Animalary Medicine Faculty of the University of Murcia (Spain), and the experiment was approved on December

10, 2003, by the Bioethics Committee of the same university.

- Animals

One hundred and eighty-six adult male Sprague-Dawley rats, with a mean weight of 243.41 g (range 141-382 g) were used. Housing and care for the animals was in accordance with the Advice of the European Communities (9).

- Surgical procedure

The animals were anesthetized with a mixture of ketamine and xylazine (50%) administered as an intramuscular injection (0.1 mL/100 g). All biopsies were made on the midline in the middle third of the dorsal surface of the tongue. An 8-mm diameter circular scalpel or biopsy punch (Stiefel Laboratories, Madrid, Spain) was used to obtain the oral mucosa biopsy, since this diameter punch is the maximum available for the punch biopsy of oral mucosa and therefore with which we can bring about the maximum number of artifacts. We helped ourselves of tweezers of dissection and surgical scissors with which we cut the sample from its base. All punch biopsies were carried out by the same experienced oral surgeons. Finally, the edges of the wound were brought together mesially and distally with two simple 4/0 polypropylene sutures (Propilorc, Murcia, Spain).

- Histopathological study

The 186 specimens were immediately introduced in a wide-mouthed container and fixed in abundant 10% formalin-buffered saline (without being sectioned after its initial fixation). The specimens were finally embedded in paraffin. Samples were cut into 4- μ m sections and stained with hematoxylin and eosin, in the cases in which it appeared some artefact the blocks were exhausted to assure to us that the artefact was present in full series of sample sections. All samples were studied by the same experienced pathologist.

The presence of artifacts associated with the surgical technique was determined based on the criteria established by Moule et al. (6) and used by Seoane et al. (7,8): crush (0, none; 1, superficial; 2, base), splits (0, none; 1, superficial; 2, deep), fragmentation (0, none; 1, superficial; 2, deep), pseudocyst (0, none; 1, superficial; 2, deep) (the true presence of a pseudocyst in a given specimen was defined by the persistent presence of the pseudocyst in the entire serial sections sequence of the specimen, lesions not present in the entire series of sections of the sample were regarded as false pseudocysts, and were excluded), and haemorrhage (0, none; 1, edge; 2, specimen). Finally, to determine the presence of artifacts attributable to sample processing in the laboratory, use was made of the criteria defined by Moule et al. (6): orientation (1, poor; 2, moderate; 3, oblique; 4, good).

- Statistical analysis

The data were processed using the SPSS version 12.0 (SPSS Inc, Chicago, IL, USA) statistical package for Microsoft Windows. A descriptive study was made of each variable.

Results

The artifacts identified on histopathological examination are listed in Table 1. As regards those attributable to the surgical technique, no sample showed splits, fragmentation or haemorrhage. Thirty-one cases (16.67%) showed crush (Figure 1). Lastly, in two cases (1.08%) (Figure 2) we observed the presence of true pseudocysts. In this context, a first histopathological evaluation identified 23 pseudocysts, though 21 of them were not present in the full series of sample sections, and were thus excluded. Regarding the presence of artifacts attributable to sample processing in the laboratory, poor sample orientation was recorded in 70 cases (37.63%).

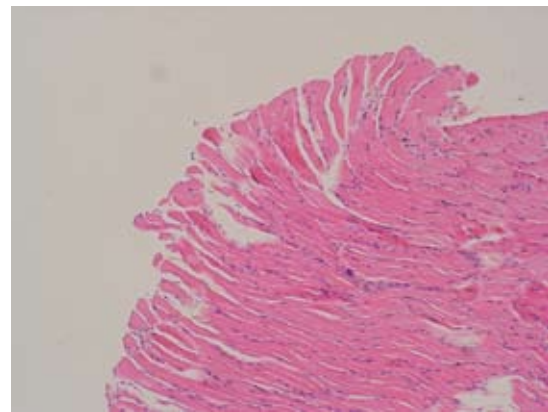


Fig. 1. Histopathological section of an oral mucosa biopsy showing a crush artifact (superficial) - punch biopsy (Hematoxylin and eosin stain; original magnification $\times 100$).

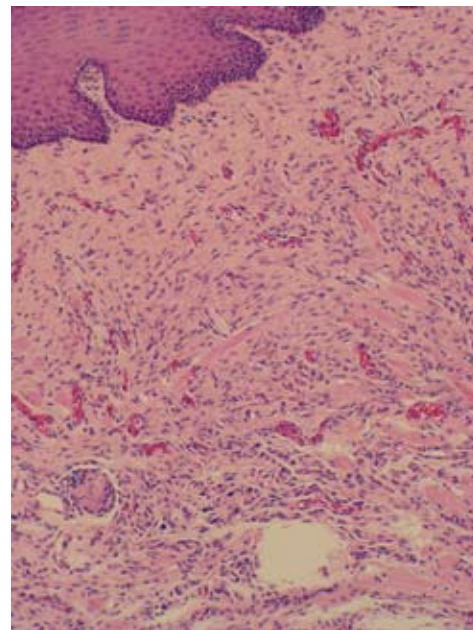


Fig. 2. Histopathological section of an oral mucosa biopsy showing a deep pseudocyst - punch biopsy (Hematoxylin and eosin stain; original magnification $\times 100$).

Table 1. Frequency and location of the artifacts found in specimens obtained using the punch biopsy.

Artifacts found on histological examination	Punch (n = 186) n (%)	Presence of the artifact (n = 186) n (%)
Artifacts attributable to the surgical technique:		
Crush		31 (16.67)
None	155 (83.33)	
Superficial	10 (5.38)	
Base	21 (11.29)	
Splits		0 (0)
None	186 (100)	
Superficial	0 (0)	
Deep	0 (0)	
Fragmentation		0 (0)
None	186 (100)	
Superficial	0 (0)	
Deep	0 (0)	
Pseudocyst		2 (1.08)
None	184 (98.92)	
Superficial	1 (0.54)	
Deep	1 (0.54)	
Haemorrhage		0 (0)
None	186 (100)	
Edge	0 (0)	
Specimen	0 (0)	
Artifacts attributable to sample processing in the laboratory:		
Orientation		70 (37.63)
Good	116 (62.37)	
Oblique	0 (0)	
Moderate	0 (0)	
Poor	70 (37.63)	

Discussion

Several tissue alterations during oral biopsy procedures have been described (10,11). Intralesional injection of anesthetic solution should also be avoided, as it can cause bleeding with extravasation and separation of connective tissue bands, with vacuolization (2). Some artifacts such as freezing or fulguration of the sample can be caused by the surgical instrumentation used: cryosurgery, electrocautery and the CO₂ laser (2,4,5).

In relation to use of the cold scalpel in oral biopsy, some authors (6,7) suggest that the punch biopsy reduces the number of artifacts compared with the conventional scalpel. Regarding the presence of artifacts secondary to the surgical technique employed, such as splits, fragmentation or haemorrhage, Moule et al. (6) reported a total 20.83% (splits), 16.67% (fragmentation) and 66.67% (haemorrhage) in punch biopsies. In our series there were no splits, fragmentation or haemorrhage. However, the fact that Moule et al. (6) carried out their study in biopsies obtained

from patients with underlying inflammatory processes could explain the appearance of such artifacts. In this sense, Seoane et al. (8) also reported a high incidence of splits (12.15%), fragmentation (4.24%) and haemorrhage (14.12%) in biopsies obtained with the conventional technique using the cold scalpel, in 354 patients with inflammatory disorders, benign tumors, precancerous lesions and malignancies of the oral cavity. We therefore consider that the absence of such artifacts in our series may be explained by the nature of the samples studied (healthy oral mucosa), since a study conducted in 2002 by Seoane et al. (7) in healthy oral mucosa of the pig (dorsal lingual mucosa) likewise reported no splits.

In our series, 31 cases (16.67%) presented crush. Similar frequencies of this type of artefact have been reported by other authors such as Moule et al. (6) (41.67% in punch biopsies), Seoane et al. (7) (7.5% in punch biopsies) and Seoane et al. (8) (18.64% in biopsies obtained with the cold scalpel). The presence of this artefact in biopsies obtained

with a punch or conventional scalpel could be explained by the need to use dissection forceps to exert traction upon the sample before final sectioning at the base, and to then transport it to the wide-mouthed container.

Regarding the presence of pseudocysts, in two cases (1.08%) we observed the presence of true pseudocysts. In relation to this artefact, Seoane et al. (7) found no true pseudocysts in 40 punch biopsies of healthy oral mucosa. The presence of pseudocysts in our series can likewise be explained by the use of toothed dissection forceps, since the incorrect utilization of traumatic forceps produces the formation of pseudomicrocysts, apparently lined with surface epithelium twisted inwards by the teeth of the instrument (2,3,12). Compression of the specimen results in the loss of cytological detail. In biopsies taken from epithelial neoplasms, these alterations may be sufficient to compromise the diagnosis (2,12). Toothed forceps when applied too forcefully leave holes, sometimes resembling mucosal pits or an epidermoid cyst in histological terms. Finally, regarding the presence of artifacts attributable to sample processing in the laboratory, sample orientation was poor in 37.63% of the cases. In this context, Moule et al. (6) reported 16.67% of samples with a poor orientation in punch biopsies. Poor orientation of small specimens due to curling while in fixative has been noted as a problem. A proposed solution is to place the specimen epithelial surface down on a piece of card prior to immersion in fixative (12). In any case, the presence or absence of this artefact, which in earlier studies (6) has been used to compare different surgical techniques, cannot be attributed to the use of a given technique - since its appearance is exclusively dependent upon sample processing in the laboratory.

In conclusion, the present study shows use of the punch for obtaining biopsies of the healthy oral mucosa to produce few artifacts.

References

- Bernstein ML. Biopsy technique: the pathological considerations. *J Am Dent Assoc.* 1978 Mar;96(3):438-43.
- Margarone JE, Natiella JR, Vaughan CD. Artifacts in oral biopsy specimens. *J Oral Maxillofac Surg.* 1985 Mar;43(3):163-72.
- Ficarra G, McClintock B, Hansen LS. Artifacts created during oral biopsy procedures. *J Craniomaxillofac Surg.* 1987 Feb;15(1):34-7.
- Pogrel MA, Yen CK, Hansen LS. A comparison of carbon dioxide laser, liquid nitrogen cryosurgery, and scalpel wounds in healing. *Oral Surg Oral Med Oral Pathol.* 1990 Mar;69(3):269-73.
- Eversole LR. Laser artifacts and diagnostic biopsy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1997 Jun;83(6):639-40.
- Moule I, Parsons PA, Irvine GH. Avoiding artifacts in oral biopsies: the punch biopsy versus the incisional biopsy. *Br J Oral Maxillofac Surg.* 1995 Aug;33(4):244-7.
- Seoane J, Varela-Centelles P, Ramirez JR, Romero MA, De La Cruz A. Artifacts produced by suture traction during incisional biopsy of oral lesions. *Clin Otolaryngol Allied Sci.* 2002 Dec;27(6):549-53.
- Seoane J, Varela-Centelles PI, Ramirez JR, Cameselle-Teijeiro J, Romero MA. Artifacts in oral incisional biopsies in general dental practice: a pathology audit. *Oral Dis.* 2004 Mar;10(2):113-7.
- Directive 86/609/CEE, of 24 of November by that the experimentation animal protection settles down. Brussels (1986).
- Sinha UK, Gallagher LA. Effects of steel scalpel, ultrasonic scalpel,

CO2 laser, and monopolar and bipolar electrosurgery on wound healing in guinea pig oral mucosa. *Laryngoscope.* 2003 Feb;113(2):228-36.

11. Camacho-Alonso F, López-Jornet P, Bermejo-Fenoll A. Effects of scalpel (with and without tissue adhesive) and cryosurgery on wound healing in rat tongues. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2005 Sep;100(3):e58-63.

12. Lynch DP, Morris LF. The oral mucosal punch biopsy: indications and technique. *J Am Dent Assoc.* 1990 Jul;121(1):145-9.