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ORIGINAL ARTICLE

Cytopathological and chemico-physical analyses of smears of mucosa surrounding oral piercing

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OBJECTIVE: The aim of this comparative study was to analyze cytopathologically and chemico-physically the mucosa surrounding oral piercing to correlate results with adverse tissue signs.

MATERIALS AND METHODS: The tongue superficial mucosa of 15 young subjects (control group) and the superficial mucosa surrounding oral piercing of 15 young subjects (test group, TG) were smeared on slides, Papanicolaou stained and analyzed under the optical microscope. Some smears were prepared for (back-scattered) scanning electron microscope (SEM) and X-ray microanalysis to study piercing fragments.

RESULTS: Smears of TG displayed a variable extent of bacterial cytolysis of epithelial cells, fungi, hyperkeratosis, parakeratosis, granulocyte infiltration, calcium formations and bacterial flora; the four last statistically significant ($P < 0.05$). Foreign bodies surrounded by keratinocytes were detected under both light and SEM. X-ray microanalyses highlighted piercing alloy aggression, ion release and an inverse gradient of ion concentration inside keratinocytes.

CONCLUSIONS: The pathological findings in smears correlated with adverse effects of oral piercing. Ion release may be related to direct toxic effects and belated reactions because of metal sensitization. A strict regulation of piercing is warranted.

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Keywords: piercing; smears; cytopathology; scanning electron microscope; X-ray microanalysis

Introduction

Body piercing has become a common form of body adornment in young people. The extensive use of oral piercing is probably because of the common notion that piercing has minimal risks when properly performed, notwithstanding the contrasting conclusions of several works, in particular for oral sites (Price and Lewis, 1997; De Moor *et al*, 2000; Akhondi and Rahimi, 2002; Campbell *et al*, 2002; Shacham *et al*, 2003; Dubose and Pratt, 2004; Kieser *et al*, 2005; Leichter and Monteith, 2006; Lopez-Jornet *et al*, 2006; Slutzkey and Levin, 2008).

Almost all studies on piercing examine its psychosocial, epidemiological or clinical aspects (Price and Lewis, 1997; De Moor *et al*, 2000; Akhondi and Rahimi, 2002; Campbell *et al*, 2002; Shacham *et al*, 2003; Armstrong *et al*, 2004; Dubose and Pratt, 2004; Kieser *et al*, 2005; Leichter and Monteith, 2006; Lopez-Jornet *et al*, 2006; Garcia-Pola *et al*, 2008; Slutzkey and Levin, 2008). They highlight several direct risks and adverse outcomes of oral piercing. These can affect the oral mucosa, gums and teeth (Price and Lewis, 1997; De Moor *et al*, 2000; Campbell *et al*, 2002; Shacham *et al*, 2003; Kieser *et al*, 2005; Leichter and Monteith, 2006; Lopez-Jornet *et al*, 2006; Garcia-Pola *et al*, 2008; Slutzkey and Levin, 2008), or cause allergy (Kerosuo *et al*, 1996), endocarditis (Akhondi and Rahimi, 2002; Dubose and Pratt, 2004; Kloppenburg and Maessen, 2007), thrombophlebitis (Nicolas *et al*, 2007) and other infective diseases (Garcia-Pola *et al*, 2008), or even death (Cremonese, 2003). Histopathological studies on tissues surrounding oral piercing are not, to our knowledge, present in the literature, as a biopsy cannot usually be performed for ethical reasons. Histopathological analyses are justified when piercing produces great tissue damage (Ng *et al*, 1997; Horle and Kuba, 2002; Garcia-Pola *et al*, 2008) needing surgical debridement. However, it is possible to analyze tissues surrounding the piercing site and to obviate ethical or interpersonal problems with the technique of tissue surface brushing. This method is regularly used in dermatology, gynaecology and gastroenterology, and it allows the harvest of superficial cells of the skin, cervix of uterus and gastric mucosa respectively. Brushing is scarcely used in oral pathology, even though it enables cytopathological diagnosis and can be correctly applied also in subjects with piercing, as it does not require jewellery removal or tissue excision.

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Aim of this comparative work was to investigate cytopathologically the smears obtained from brushing without bleeding of tissues surrounding the piercing, to analyze the adjacent oral mucosa and correlating results with adverse tissue signs of oral piercing if possible. This study applied cytopathological methods, scanning electron microscope (SEM) and X-ray microanalysis to detect and characterize also possible piercing fragments in smears.

Materials and methods

Young, informed and consenting male and female subjects, over 18 years old, recruited, examined and treated in private dental office, with tongue metal piercing (test group – TG), were asked to complete a questionnaire which collected data on age, gender, type and time of piercing, smoking habits, alcohol use, drug assumption or addiction, oral discomfort and any complication arising from the piercing. The patients were asked to perform the normal regular oral hygiene two hours before the visit and not to rinse with any medications during this period. Each subject underwent an accurate oral inspection aimed at evaluating dental and oral hygiene, and oral mucosa status. Subjects with diseases of the oral cavity, except for those arising from the presence of the piercing, were excluded from the study. Subjects with prosthetic reconstructions, metallic restorations, oral implants or recent orthodontic therapy were also excluded from the study. In addition to that, informed and consenting subjects of the same age and gender of each TG subject were randomly recruited to constitute the control group (CG) of this comparative study; these subjects were chosen with same characteristics of TG subjects. After removal of the piercing post in TG and a one-min rinse with saline, the tongue mucosa surrounding the piercing body of the TG subjects was brushed, in an area 1–5 mm far away from the piercing hole, as well as the one located in the corresponding sites of CG subjects. In TG subjects, both dorsum and inferior surfaces were brushed (not bleeding). Superficial brushing was performed by means of a plastic spatula (Ramspatula – plastic Ayre's spatula, RI.MOS s.r.l., Mirandola MO, Italy), the same type used to produce gentle and atraumatic samples of cervical smears. Brushing was performed using a different spatula in every site. According to the informed consent given by the patient, which did not include the piercing donation, piercing was repositioned in the original oral site. The removed superficial epithelial cells were smeared on polished glass slides and immediately fixed with Fissy (RI.MOS s.r.l., Mirandola MO, Italy) until dry. Smeared slides were stained with the Papanicolaou trichrome method, analyzed and photographed under transmitted light microscopy (Axiophot; Carl Zeiss AG, Oberkochen, Germany).

Some slides (one for each brushing) were rehydrated and desiccated in a critical point dryer (CPD030; Bal-Tec AG, Balzers, FL, USA) at 40°C and 7.4 MPa pressure, using CO_2 as and intermediate agent. Specimens were carbon coated (CED020; Bal-Tec), examined by SEM using the back-scattered electron detector (ESEM Quanta-200; FEI Company, Heindhoven, NL, USA) and analyzed with an X-ray microprobe (INCA 350; Oxford Instruments, Oxfordshire, UK), energy dispersive spectroscopy system, at 25 kV. Semiquantitative analysis was subsequently run after appropriate ZAF (Z, atomic number; A, absorption; F, secondary fluorescence) correction (Sanchez-Quevedo *et al*, 1989), using proprietary software (INCA; Oxford Instruments).

The Fisher's exact test was applied to investigate the effect of piercing on cytopathological findings since the expected frequency was always < 5 (Glantz, 2003). Data were stratified by smoke habit to assess confounding effect of smoking. The statistical significance level was set at 0.05.

Results

Thirty non-remunerated subjects, 15 (CG, three males and 12 females, ranging in age from 20 to 29 years, mean ± s.d. = 22.87 ± 2.45) and 15 (TG, three males and 12 females, ranging in age from 20 to 29 years (mean ± s.d. = 22.94 ± 2.74), voluntarily participated in this comparative study (Table 1). All subjects did not present any clinical sign of infection or allergy to metals. All subjects were habitual drinkers and more than 2/3 (CG = 73.3%, TG = 80%) smokers (Table 1). Tongue piercing had been present in the oral cavity of TG subjects for time (mean ± s.d. = 3.42 ± 1.84) ranging from 6 months to 7 years (Table 1). All subjects showed a normal hygiene level without clinical signs of parodontal inflammation (periodontal probing depth < 3 mm; bleeding on probing = 0). All subjects referred that their piercing was made of 'surgical steel', but they had no certification to prove it.

The mucosa smears of CG subjects displayed a normal morphology with isolated and mature keratinocytes showing different sized nuclei besides nests of eosinophil keratin lamellae (in all dorsum brushes). Only 10% of smokers displayed weak bacterial infection without serious neutrophil infiltration (Table 2). On the contrary, smears of the mucosa surrounding the piercing body of TG subjects showed several alterations:

1. More than half of smears of the mucosa surrounding the piercing body (66.7% of subjects) showed a copious and diffuse bacterial flora (Table 2). Bacterial flora inside oral epithelium surrounding piercing was more frequent in smokers (75%) than non-

Table 1 Gender, age, habits and piercing dwell time of subjects

	Control group	*Test group*
Males (*n*)	3	3
Females (*n*)	12	12
Mean age (years)	22.87	22.94
s.d. (years)	2.45	2.74
Habitual drinkers (%)	100	100
Smokers (%)	73.3	80
Mean dwell time (years)	0	3.42
s.d. (years)	0	1.84

Table 2 Cytopathological findings in subject smears

	Control group		*Test group*	
	S	*NS*	*S*	*NS*
Bacterial flora	1 (9.1)[a]	0 (0)	9 (75)	1 (33.3)
Fungi	0 (0)	0 (0)	2 (16.7)	1 (33.3)
Widespread granulocytosis	0 (0)	0 (0)	4 (33.3)	1 (33.3)
Cytolysis	0 (0)	0 (0)	3 (25)	1 (33.3)
Calcium formations	0 (0)	0 (0)	4 (33.3)	2 (66.7)
Basophilic keratin lamellae	0 (0)	0 (0)	2 (16.6)	0 (0)
Parakeratosis	0 (0)	0 (0)	8 (66.6)	0 (0)
Foreign bodies	0 (0)	0 (0)	2 (40)[b]	1 (100)[b]

Values are expressed as *n* (%).
S, smokers; NS, non-smokers.
[a]Weak.
[b]Piercing with more than 3-year-dwell time.

smokers (33.3%). Intercellullar spaces were amply infected with bacteria and intracytoplasmic concretions, i.e. intracellular bacteria, were occasionally recorded (Figure 1a). Intracytoplasmic bacteria were often Gram-positive (82%).

2. Fungi (Table 2) were found in 20% of subjects, two smokers and one non-smoker. Hyphae and spores were located intercellularly (Figure 1b) and associated with bacterial infection and cytolysis.
3. Neutrophil infiltration (Table 2) was abundant in 33.3% of subjects, four smokers and one non-smoker. Neutrophils often formed large aggregates overlapping keratinocytes (Figure 1c).
4. Cytolysis of epithelial cells (Table 2) was observed in about 26.6% of subjects, three smokers and one non-smoker. In some smears, the process was so advanced to produce shadow cells and nuclear dust (Figure 1d).
5. Calcium formations (Table 2) were observed in a great number of cases: 40% of subjects, four smokers and two non-smokers, displayed basophilic bodies among keratinocytes.
6. Anuclear cells (Figure 1e), i.e. basophilic keratin lamellae (Table 2) without nuclei (hyperkeratosis), were found in 16.7% of TG smokers, all having installation of oral piercing longer than 3 years.
7. Variable degrees of parakeratosis were found in almost all smears. The widest expression of parakeratosis (Table 2) was found in about 50% of piercing, affecting the tongue dorsum (53.8%), particularly in smokers (66.6%). Keratinocytes were found to form aggregates, showed larger nucleus and extended cytoplasm (Figure 1f).

In addition to the reported cytopathological findings, foreign bodies (Table 2) were found in smears of mucosa surrounding piercings present for more than 3 years (Figure 2). Fifty percent of piercings (three cases, two smokers and one non-smoker) with more than 3 years of dwell time (six cases in all, five smokers and one non-smoker) produced foreign bodies. These bodies had a green-blue hue and size ranging from 5 to 70 μm in stained slides. They were partially (Figure 2a), particularly if bigger, or completely, when smaller (Figure 2b), surrounded by keratinocytes that appeared in close contact with their surface.

The Fisher's test (Table 3) showed a statistical significance of bacterial flora ($P < 0.002$), widespread granulocytosis ($P < 0.04$), calcium formations ($P < 0.02$) and parakeratosis ($P < 0.002$) comparisons between test and CG. The Fisher's test (Table 3) also showed a statistical significance of bacterial flora ($P < 0.003$) and parakeratosis ($P < 0.001$) comparisons between smokers of test and CG.

Scanning electron microscope observations, performed using the back-scattered electron detector, were performed to confirm the cytological findings and allow a better discrimination of organic and inorganic materials, because of the higher atomic weight of the metallic body components (Figure 2c). The SEM analyses revealed large metallic bodies, greater than 10 μm, but also of small particles with size smaller than 1–2 μm in many keratinocyte aggregates (Figure 2c). The smaller metallic bodies appeared highly fragmented and displayed irregular edges.

X-ray microanalyses, performed on fields formed by aggregates of keratinocytes enveloping metal bodies >5 μm (Figure 3a), confirmed the back-scattered appearance. The putative metal bodies (Figure 3b) proved to be an alloy of chromium/iron/nickel (Cr/Fe/Ni) containing small amounts of silicon (Si),

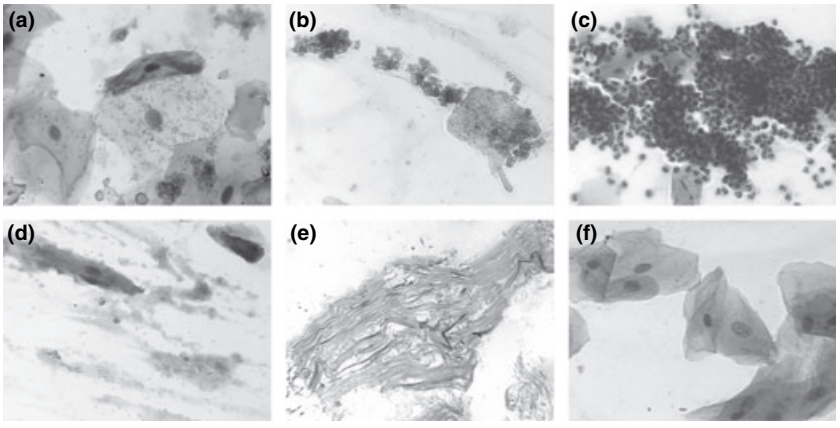

Figure 1 Smears of the mucosa surrounding piercing jewellery, stained with Papanicolaou technique: (**a**) keratinocytes showing intracytoplasmic bacteria settlement; (**b**) extracellular bacteria settlement and fungal spores; (**c**) epithelial cells infiltrate by several granulocytes; (**d**), partially (dark red) and totally (blue-grey) lysed keratinocytes; (**e**) conspicuous basophilic keratin lamellae formation of a smear; (**f**) parakeratosis of the superficial epithelial cells of tongue dorsum. Field width: $a = 175$ μm; $b = 400$ μm; $c = 275$ μm; $d = 175$ μm; $e = 400$ μm; $a = 175$ μm

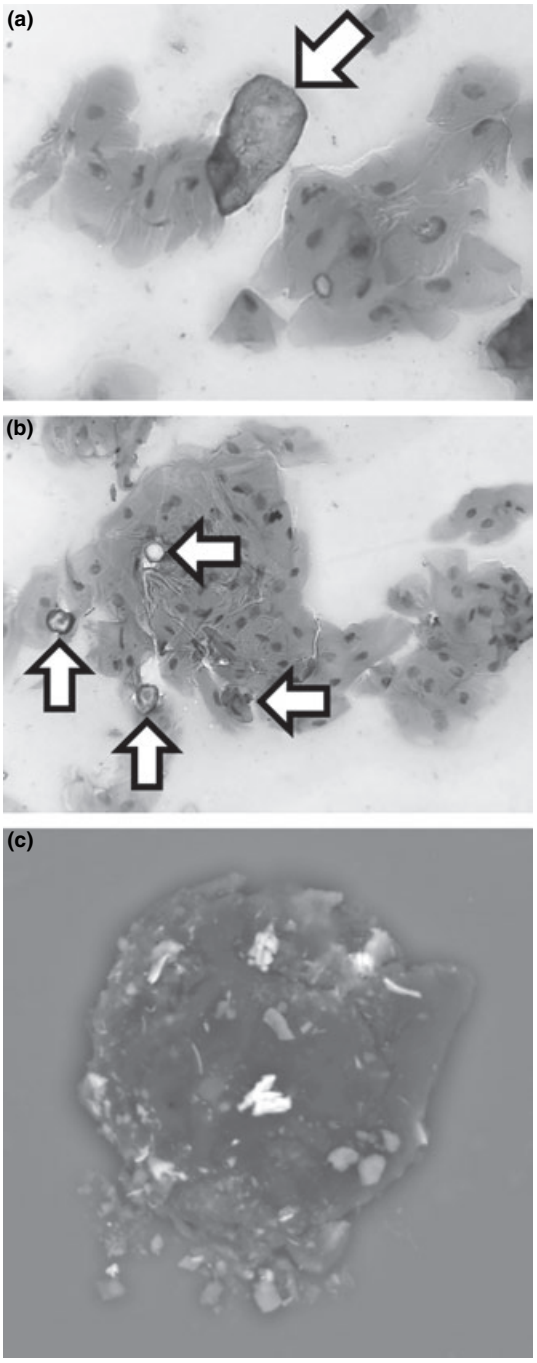

Figure 2 Smears of the mucosa surrounding piercing jewellery observed under optical microscope (**a**, **b**) or SEM (c): (**a**) keratinocyte nests adhering to a large foreign body (arrow); (**b**) keratinocyte nests engulfing several small foreign bodies (arrows); (**c**) large keratinocyte nest engulfing several small to minute metallic (white) fragments. Field width: $a = 260$ μm; $b = 400$ μm; $c = 68$ μm

and coated with carbon (C) (Figure 3c). Semi-quantitative analyses (without standard) of these metal fragments revealed a content of 0.9% Si, 9.8% Ni, 18.8% Cr and 70.5% Fe, identical in all samples of smears containing metal bodies, an alloy falling within the surgical steel group, i.e. a fragment of the jewellery. A similar composition (i.e. 316L AISI stainless steel; American Iron and Steel Institute, Washington, DC, USA) was found in not implanted piercings used as control. X-ray analyses of keratinocytes enveloping the piercing fragment (Figure 3b) showed metallic ions (elements of the alloy) in addition to usual ions inside the keratinocyte cytoplasm (graphs 1, 2 and 4). X-ray analyses revealed the presence of carbon (C), oxygen (O), sulfur (S) and potassium (K) as per normal content but also Cr, Fe and Ni as pathological mineral content of the keratinocyte cytoplasm. In each graph, X-ray analyses also revealed the presence of sodium (Na), magnesium (Mg), aluminum (Al), silicon (Si) and calcium (Ca) because of unavoidable analysis of the glass slide beneath the keratinocytes. The metallic ions were found in larger amounts at 1 μm (graph 1) from the piercing fragment surface and decreased with distance (graphs 2 and 4). The Fe/C ratio (Figure 4), calculated after semi-quantitative analyses, was very high at 1 μm, rather high at 2 μm and low at 4 μm from the piercing site, i.e. a decreasing concentration gradient was observed in the cytoplasm of keratinocytes facing the piercing fragment surface.

Table 3 Statistics of cytopathological findings

Control group	Test group		
	A	S	NS
BF			
A	+		
S		+	
ns			
F			
A			
S			
ns			
WG			
A	+		
S			
ns			
C			
A			
S			
ns			
CF			
A	+		
S			
ns			
BKL			
A			
S			
ns			
P			
A	+		
S		+	
ns			
FB			
A			
S			
ns			

BF, bacterial flora; F, fungi; WG, widespread granulocytosis; C, cytolysis; CF, calcium formations; BKL, basophilic keratin lamellae; P, parakeratosis; FB, foreign bodies; A, all (smokers + non-smokers); S, smokers; NS, non-smokers.
$+ P < 0.05$ (Fisher's exact test).

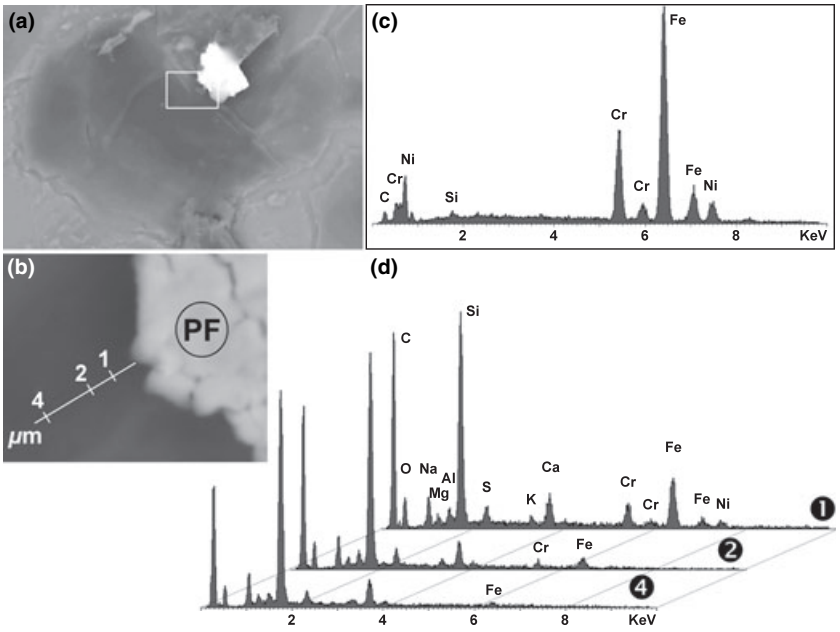

Figure 3 Scanning electron microscope images and X-ray microanalyses of a piercing fragment surrounded by keratinocytes: (**a**) keratinocyte nests surrounding a large foreign body; (**b**) boxed in portion of image **a** at higher magnification; (**c**) graph showing the X-ray microanalysis performed into the (PF) site indicated in B; (**d**) graphs showing the X-ray microanalyses performed inside the keratinocyte cytoplasm at a distance of 1, 2 and 4 μm from the piercing fragment surface (as indicated in **b**) respectively. Field width: $a = 82$ μm; $b = 10$ μm

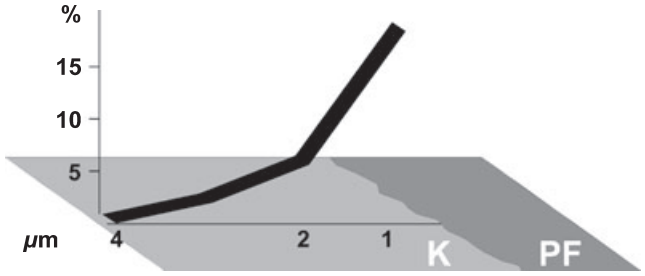

Figure 4 Graph showing the pattern of the Fe/C ratio (Wt/Wt), expressed as percentage, inside the keratinocyte (K) cytoplasm at a distance of 1, 2 and 4 μm from the piercing fragment surface (PF)

Discussion

Piercing has become a popular and fashionable trend by means of which young people, mainly teenagers, challenge or antagonize adult perception and rules of society. Piercing practice is so widespread that it involves about 45% of US college students (Armstrong *et al*, 2004), nevertheless our sample had a limited size mainly for ethical, type of piercing and follow-up constraints. Even if piercing is particularly widespread among teenagers, only people over 18 years old can legally consent to an informed study. Oral piercing, and in particular tongue piercing, concerns only 1/8 of young people with piercing in the USA (Armstrong *et al*, 2004). On the other hand, our interest was in cytological findings only detectable with long follow-up, in the age when a teenager becomes a legal-adult and frequently ceases the practice of piercing.

Our TG sample was consistent with the literature (Armstrong *et al*, 2004; Kieser *et al*, 2005) for gender, rate, smoke and alcohol use and site of oral piercing (Kieser *et al*, 2005). Overlapping results were found for piercing adverse effects in the oral cavity (Price and Lewis, 1997; De Moor *et al*, 2000; Campbell *et al*, 2002; Shacham *et al*, 2003; Kieser *et al*, 2005; Leichter and Monteith, 2006; Lopez-Jornet *et al*, 2006; Garcia-Pola *et al*, 2008; Slutzkey and Levin, 2008).

Cytopathological analyses of smears highlighted both cytological and microbiological disorders, in particular, if compared with CG findings. Keratinocytes were affected by calcifications, parakeratosis and hyperkeratosis that could transform them into basophilic keratin lamellae. All these data are mainly collected from piercing located inside the oral mucosa, even though a possible action of ions released by the piercing device cannot be excluded (Grìmsdóttir *et al*, 1994; Masel, 2005). These epithelial hyperplasic reactions were not merely a pathological state, but seem to be in an initial phase of a process that could lead to hyperkeratosis and acanthosis. Smears showed fungi and bacteria in a remarkable number of subjects. Their presence induced granulocyte infiltration, but also caused several keratinocyte cytolysis. In addition to the lack of oral hygiene, these findings may warrant inflammation evidence as a major recurrence in oral piercing risk (Price and Lewis, 1997; Shacham *et al*, 2003; Armstrong *et al*, 2004; Kieser *et al*, 2005; Leichter and Monteith, 2006; Garcia-Pola *et al*, 2008). The pathogenic propagation could be so severe as to cause damage to internal organs (Akhondi and Rahimi, 2002; Dubose and Pratt, 2004; Kloppenburg and Maessen, 2007; Garcia-Pola *et al*, 2008) or even patient death (Cremonese, 2003).

Notwithstanding the partial result of statistical comparisons, probably because of the small size of the sample, four cytopathological findings of TG resulted statistically significant, thus corroborating the belief of the negative piercing effect on the oral mucosa. Two comparisons between smokers of TG and CG resulted statistically significant, and so the smoke would not seem to have a significant effect on cytopathological findings. As our findings relate to a rather limited

number of subjects, further studies will be necessary to confirm our results.

Scanning electron microscope and X-ray microanalyses highlighted a possible concurrence of keratinocytes in piercing device corrosion and disruption. The dispersal of piercing fragments surrounded by keratinocytes and the high content of elements of piercing alloy found in the cytoplasm, near the keratinocyte membrane facing the fragment, provide evidence that points away from a direct activity of keratinocytes on metal dissolution. The released ions could only propagate through the cytoplasm where a decreasing gradient was formed by drainage.

Particularly in exposed piercing parts, the main cause of piercing alloy (of low-quality) corrosion (Grìmsdóttir *et al*, 1994; Kerosuo *et al*, 1996; Hwang *et al*, 2001) was undoubtedly oral fluids, but this initial metal aggression probably could belatedly stimulate keratinocytes to continue the corrosion. The cytotoxicity of ions initially released from piercing devices could damage keratinocytes (Rogero *et al*, 2000; Faccioni *et al*, 2003) that attempt to eliminate the source of danger instead amplifying the effect. The ions drained by keratinocyte cytoplasm could then propagate inside the oral cavity, but also into the interstitial spaces, reaching the corion and vessels (i.e. systemic diffusion). This is probably the reason for ion sensitization of a subject. Even if some authors found Ni allergy to orthodontic devices (Grìmsdóttir *et al*, 1994; Kerosuo *et al*, 1996; Kalimo *et al*, 2004), others (Setcos *et al*, 2006) disclaim Ni hypersensitivity, but affirm that a prior sensitization (for instance caused by piercing) could trigger a subsequent allergy. This is supported by a reported correlation between the number of piercing and the incidence of metal allergy in men (Garner, 2004).

In conclusion, our results and the continual scientific reports on piercing risks and adverse effects in chorus delineate piercing practices as a paramedical activity. Materials should be accurately selected and chemically not corrodible. Piercing should be executed with maximum safety and hygiene. On the contrary, piercing is in demand amongst teenagers that usually have limited financial resources and consequently favour low-cost piercing jewellery, and also because of ignorance. These low-quality devices are usually made of surgical steel (AISI 316L), containing 10–14% Ni that must not be used inside or in contact with the human body (European Parliament and Council Directive 94/27/EC). Regulation of piercing is mandatory (Civatte and Bazex, 2007): piercing should be performed by licensed qualified personnel in a clean, safe environment, and not by unlicensed practitioners in an unsuitable environment, as it often happens. The various aspects of oral pathology recorded (bacteria and fungi settlement, keratinocyte morphological alteration, ion release and diffusion) suggest the need for a new restrictive regulation of piercing practice (at least in EU) and clear information to people (young and old) about the risks and adverse effects of piercing (Levin and Zadik, 2008) with more emphasis on material corrosion and chronic inflammation.

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Author contributions

Saturnino Marco Lupi, Ruggero Rodriguez y Baena and Silvana Rizzo contributed to the research design. SM Lupi contributed to the acquisition of specimens. Annibale Renzo Botticelli contributed to the cytopathological analysis. Davide Zaffe contributed to the SEM and X-ray microanalysis. S Lupi and D Zaffe contributed in drafting the paper. R Rodriguez y Baena, S Rizzo and AR Botticelli worked on the critical revision of the paper.

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