Scanning electron microscopic analysis of the efficacy of acid etching on cat enamel

Claudio Gallottini[1], Giancarlo Barraco[2], Stefano Pagano[2], Carlo De Carolis[2], Alessandro Dolci[3], Manila Chieruzzi[4], Stefano Eramo[2]

[1]School of Veterinary Medical Sciences, University of Camerino, Via Fidanza 15, Matelica (MC) 62024, Italy
[2]Department of Restorative Dentistry, Faculty of Medicine and Surgery, University of Perugia, S. Andrea delle Fratte, Perugia 06156, Italy
[3]Department of Clinical Science and Translational Medicine, Faculty of Medicine and Surgery, University of Rome 'Tor Vergata', Via Montpellier 1, Rome 00133, Italy
[4]Civil and Environmental Engineering Department, UdR INSTM University of Perugia, Strada di Pentima 4, Terni 05100, Italy
E-mail: manila.chieruzzi@unipg.it

Published in Micro & Nano Letters; Received on 12th December 2013; Revised on 18th February 2014; Accepted on 12th March 2014

The effect of etching on cat enamel made with 40% orthophosphoric acid for different times was evaluated. Twenty-one cat teeth were selected and randomly divided into three groups of seven teeth each. They were subjected to etching on a circular area of the coronal enamel (diameter = 2 mm) for 30 s (group A), 45 s (group B) and 60 s (group C). The samples obtained were observed by a scanning electron microscope focusing on the border area between etched and unetched enamel, to highlight the differences. The micrographs were subjected to blind assessment of three experienced operators. The groups were statistically assessed with the Wilcoxon test. At 30, 45 and 60 s, the acid attack results only in the formation of an irregular enamel surface and without uncovering and attack of the prismatic organisation. Prismatic areas with preferential interprismatic action could be detected in few samples etched for 60 s. Analysis with ImageJ was also used to quantify the efficacy of acid etching in the conditions used for human enamel, by an evaluation of grey levels. In cat enamel the etching times considered are not as effective as in human enamel for the purpose of adhesion and the presence of a thick prismless layer could explain this result.

1. Introduction: Always, and more frequently in the domestic cat, dental restorations are made after fractures [1–3] using composite and adhesive systems originally designed for man. The adhesion usually occurs on the dentine and enamel, rarely on the cement, but there is a lack of micromorphological specific works in the literature about the relationship between these hard tissues of cat tooth and the adhesive/composite clinically used systems. Optical microscopy and scanning electron microscopy (SEM) have widely described both the growth [4–8] and the structure and composition of mature cat enamel. About the latter, in particular, the surface of the cat enamel tends to appear smooth, with notable deficiency of perikymata and prisms exposed in the surface [9, 10]; this smooth surface would be formed 'as a result of slowed incremental growth of enamel prisms by ameloblasts, which is associated with a change in the shape of the Tomes process, resulting in a lack of distinction between pitfloor and interpit enamel' [9, 11]. However, small areas of lesion with exposure of prismatic 'cobbled' enamel were noted. They are probably related to wear and trauma that occurred during the lifetime [9, 11, 12]. The thickness of the enamel varies, in the cat, from 0.1 to 1 mm [13] and it is composed by three layers [10]:

- a surface prismless layer (Fig. 1, 5000 ×), that shows a thickness ranging from 5 to 20 μm [9] and appears to cover all the surfaces of the tooth with the partial exception of the cervical areas [10];

- an intermediate layer with parallel prisms only at some sites; and

- a deep layer, with parallel prisms and prominent bands of Hunter-Schreger, in which the diameter of the individual prisms seems to vary significantly and the course suddenly changes with respect to that of the surrounding prisms [10].

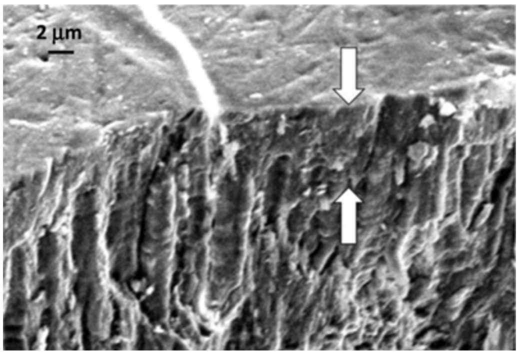

Figure 1 *SEM micrograph (5000 ×) of the surface of cat enamel*
Arrows indicate thickness of the prismless layer

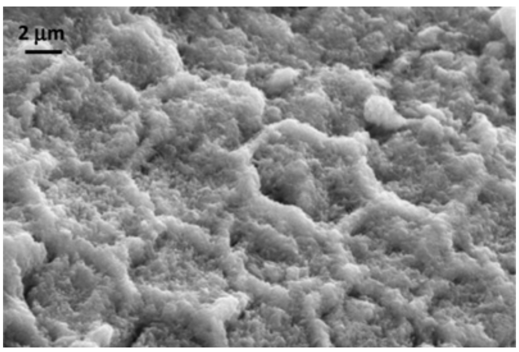

Figure 2 *SEM micrograph (7000 ×) of the fractured cat enamel with prisms visible in cross-section*

However, the average diameter of the prisms in the cross-section is 5 μm [10, 14, 15], as in humans (Fig. 2, 7000 ×). The composition of cat enamel is, of course, based on calcium and phosphorus as in all mammals (man included), but it seems that high concentrations of

doi: 10.1049/mnl.2013.0729

fluorides, sodium and magnesium (< 5% of the total elemental composition) in addition to the combined presence of sulphur, potassium and iron combined (< 0.1%) are also present [16]. Furthermore, the surface layer of the enamel seems to show the lowest concentration of calcium [8] and there are differences in composition between males and females [16].

Hence, it is evident that cat enamel is different from human enamel [10]. Nevertheless, in the veterinary literature, the same etching times and concentrations of orthophosphoric acid used for the enamel of human teeth are recommended.

The aim of this work was to assess by SEM the effect of etching on cat enamel made with 40% orthophosphoric acid for 30, 45 and 60 s (usually used for man). The hypothesis is that cat enamel reacts to these etching conditions in a different way with respect to that of human permanent teeth.

2. Materials and methods: Twenty-one teeth from domestic cats, that had died from various causes, including twelve canines and nine premolars were used. The teeth were extracted by closed extraction, avoiding any damage to the enamel. Once extracted, the dental roots were cleaned and the organic residues were removed by manual scaling and then immersed for 12 h in a 5% aqueous solution of sodium hypochlorite. The samples were then thoroughly washed in distilled water and air-dried for 30 s. The enamel of each extracted tooth was checked by a stereomicroscope (Officine Galileo, Milan, Italy) at a magnification of 40× and there was no evidence of any hypoplasias, resorptions, abrasions, infractions and fractures. The samples were then randomly divided into three groups of seven each. They were subjected to etching with orthophosphoric acid gel (Acid Etch 40% – H Schein Inc., NY, USA) using a disposable syringe, on a circular area of the coronal enamel with a diameter of 2 mm. At the end of the etching each sample was sprayed with an abundant mix of air and water and then air-dried for 30 s by an air jet. The three groups differ for the etching time: in group A each of the seven samples was subjected to etching for 30 s, in group B the samples were treated as those of group A but with an etching time of 45 s, in group C an etching time of 60 s was used [17].

All the samples obtained were then coated with a 300 Å carbon layer by means of a sputter coater KQ150R (DentonVacuum, NY, USA). The samples were then subjected to a microscopic evaluation by a LEO 1525 field-emission gun SEM with a constant magnification of 1000× focusing, in particular, for each sample on three areas: the area of unetched enamel, the area between the part of the enamel subjected to etching and the part not exposed to etching and the area of the etched enamel. To evaluate some particular characteristics of the observed surfaces, micrographs with magnifications ranging from 50 to 7000× were also taken. Twenty-one micrographs at 1000× magnification focusing on the border areas between the etched and unetched enamel were obtained.

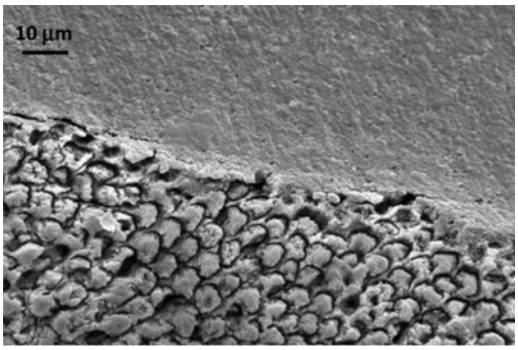

Figure 3 *SEM micrograph (1000×) of human enamel: border area between etched enamel (lower part) and unetched enamel (upper part) after an etching time of 45 s*

The 21 micrographs were then subjected to blind assessment by three experienced operators (two dentists and a vet) who evaluated the validity of the treatment for adhesive purposes, assigning the following scores: 3 (effective), 2 (discrete), 1 (poor) and 0 (uncertain or null). Fig. 3 (1000×) shows the border area between the unetched enamel and enamel subjected to etching with orthophosphoric acid for 45 s in human deciduous teeth. In this case, the etching was evaluated as 'effective' and the Figure was used for comparison.

The micrographs were also analysed using image analyser software (ImageJ, v1.47, U.S. National Institute of Health, Bethesda, Maryland, USA). TIFF images were normalised to have the same mean grey-level intensities. Grey levels ranging from 0 for black to 255 for white were taken to assess the presence of valleys after etching. In particular, grey levels were measured for each SEM image on the etched and unetched areas for the three groups of cat enamel and for human enamel (considered as a reference). A region of interest (ROI) was selected for each image and for each ROI a profile was obtained.

The *x*-axis of this profile represented the horizontal distance along the rectangle, whereas the *y*-axis represented the vertically average pixel intensity (grey-scale values). A ratio *R* of grey amplitudes between the etched and unetched zones for cat enamel was also calculated (the human enamel was also taken as a reference).

3. Results and discussion: 63 micrographs at 1000× magnification and 12 micrographs at magnifications ranging from 50 to 7000× were obtained on 21 samples. Among the 63 pictures, 21 were related to the border area between the etched and unetched enamel. Fig. 4 presents SEM micrographs at 1000× of the border areas between the etched and unetched enamel of the samples etched for 30 s (group A). In Fig. 4*a*, there is clear evidence of the etched zone (on the right) and the unetched enamel (on the left) for sample A1. Moreover, it was possible to note a poor action of the acid and a partially missing prismatic zone. The SEM image of sample A2 shows a poor action of the acid (upper right) with no evidence of prisms (Fig. 4*b*), whereas sample A3 in Fig. 4*c* shows traces of prismatic areas (etching on the lower part). A poor action of the acid in sample A4 (on the left) was also noted but in this case a little zone with 7–10 prisms could be detected (Fig. 4*d*). For sample A5 (etching on the left), SEM analysis revealed a lamellar-like structure of the enamel (Fig. 4*e*), whereas for sample A6 it was not possible to easily distinguish the etched zone from the unetched one (Fig. 4*f*). The last sample of group A (Fig. 4*g*) showed a prismatic zone on the upper part where the etching attack was conducted.

Fig. 5 shows SEM micrographs of cat teeth subjected to acid etching for 45 s (group B). Even in this case the micrographs were taken at 1000× magnification. It is possible to note in Fig. 5*a* for sample B1 (where the etched enamel is on the left and the unetched on the right), the etching was irregular with no prismatic evidence. SEM micrographs of samples B2, B3, B4, B5, B6 and B7 are also reported in Figs. 5*b*–*g*. Sample B2 showed the etched zone in the lower part. In this case a patchily uncovering of the prismatic area was evident, whereas in sample B3 (etching on the upper part) it was scarcely evident (Figs. 5*b* and *c*, respectively). In sample B4 (etching on the left) a superficial prismatic uncovering occurred. The uncovering resulted diffusion and a loss of inorganic substance resulted in the interprismatic areas. Fig. 5*e* shows sample B5 with the etching zone on the right where the etching treatment was not effective. The analysis of sample B6 with etching visible on the upper part, revealed that part of the prisms on the enamel surface were left uncovered (Fig. 5*f*). Moreover, slight evidence of an interprismatic attack was also detected in this sample. Similar observations could be done on the last sample etched for

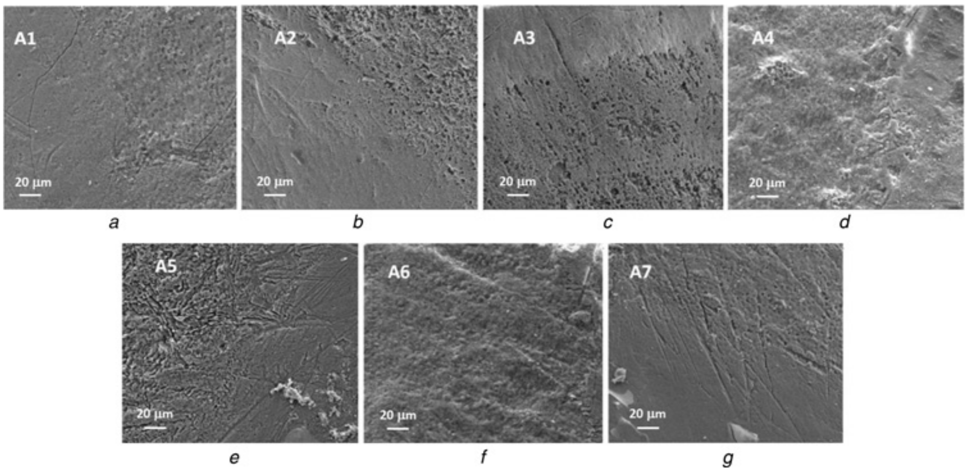

Figure 4 *SEM micrographs (1000 ×) of group A samples etched for 30 s showing the border area between etched and unetched enamel. The etched areas are visible*
a On the right for sample A1
b On the upper right part for sample A2
c On the lower part for sample A3
d On the left for sample A4
e On the left for sample A5
f On the entire area for sample A6
g On the upper part for sample A7

45 s (Fig. 5*g*), whose etching is visible on the bottom area. In this case, the attack was more superficial than that in B6. The border areas between etched enamel (right) and unetched enamel (left) of the samples C1–C7 (etching for 60 s) are reported in Fig. 6 (SEM, 1000 ×). Even in the case of the first sample of this group (Fig. 6*a*) the etching was irregular and no prismatic uncovering was evident. From this SEM image the presence of linear notches could be detected, probably because of traumas experienced by the tooth during the lifetime. In sample C2 (etching in the upper left part visible in Fig. 6*b*) a superficial prismatic uncovering occurred. The uncovering caused diffusion and with an interprismatic loss of substance (similar to sample B4). In Fig. 6*c*, showing sample C3 with etching in the upper right part, it was difficult to distinguish the etched zone with respect to the unetched one. A chaotic etching of the enamel surface was shown for sample C4 (etching in the lower right zone, Fig. 6*d*).

Analysis of sample C5 (etching on the right) revealed the presence of prisms that were also slightly attacked in the interprismatic zones.

In Figs. 6*f* and *g*, related to the samples C6 and C7 (etching on the bottom left and on the left, respectively), the acid attack seemed important if compared with the untreated surface but was not able to show the prismatic pattern.

In the present Letter which is focused on SEM analysis of cat enamel surfaces, it was observed that 40% orthophosphoric acid did not have the ability to create (in the range of 30–60 s) an etched surface with a morphology that could encourage the use of etch-and-rise adhesive systems. To quantify this attitude, the

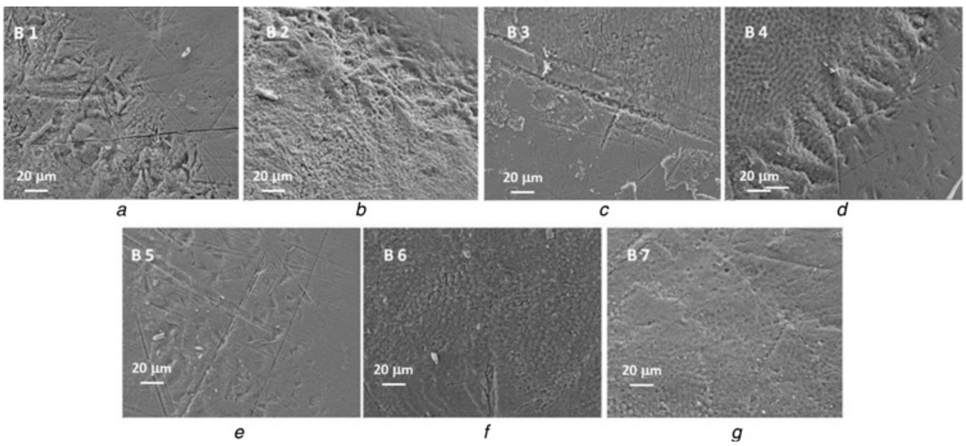

Figure 5 *SEM micrographs (1000x) of group B samples etched for 45 s showing the border area between etched and unetched enamel. The etched areas are visible*
a On the left for sample B1
b On the lower part for sample B2
c On the upper part for sample B3
d On the left for sample B4
e On the right for sample B5
f On the upper part for sample B6
g On the lower part for sample B7

doi: 10.1049/mnl.2013.0729

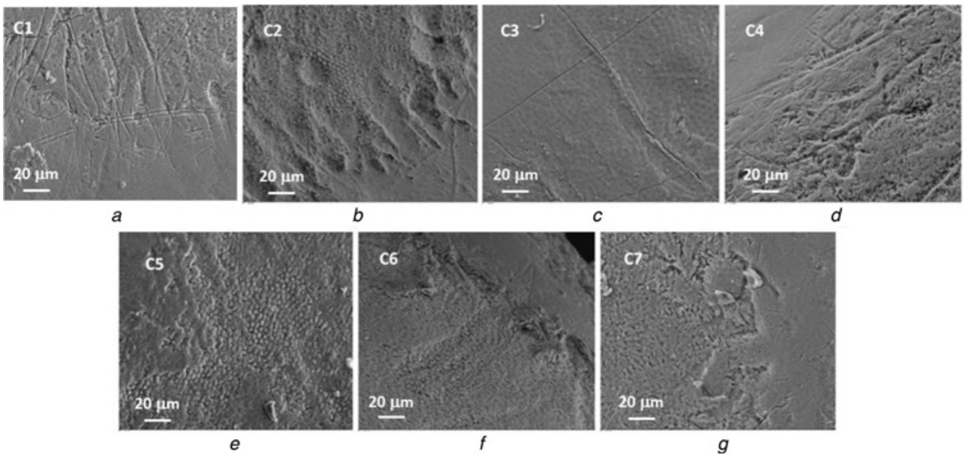

Figure 6 *SEM micrographs (1000 ×) of group C samples showing the border areas between etched and unetched enamel. The etched areas are visible*
a On the right for sample C1
b On the upper left part for sample C2
c On the upper right part for sample C3
d On the lower right part for sample C4
e On the right for sample C5
f On the bottom left for sample C6
g On the left for sample C7

images were subjected to blind assessment by three experienced operators. The assessments were then related to the three groups of seven samples and the means were obtained. They resulted in 0.43 for group A (etching for 30 s), 0.43 for group B (etching for 45 s) and 0.67 for group C (etching for 60 s). The average assessment for the three groups was then located between uncertain or null and poor even if group C shows a better performance with respect to the other two.

The results obtained were also subjected to statistical analysis using the Wilcoxon test. No significant differences ($P > 0.05$) between the three groups were found, which demonstrated the high reliability of the examiners. This means that the weak effect of etching on the cat enamel surface for all the samples analysed was not different after 30, 45 or 60 s of etching with 40% orthophosphoric acid.

This work demonstrated that the effect of etching times usually used for human enamel is weak when applied on cat enamel. The reason can probably be ascribed to the presence of a thick prismless layer [9, 10] having a lower calcium concentration [8], that is the element on which the chemical attack of orthophosphoric acid preferentially occurs [18]. A similar situation can be found in human deciduous teeth, where the presence of a substantial prismless layer [19–21] led to hypothesize that different etching times should be used to overcome the barrier formed by the prismless enamel (ineffective solutions) and, subsequently, the addition of mechanical pre-treatment of the enamel surface could be useful to provide in evidence of the prismatic component [22, 23]. More recently, the human dental clinic overcame this problem by proposing several enamel preparation techniques. Different cavity-preparation designs currently exist. Among these, the preparation of a bevel or a chamfer on the cavity margin were the more widely used to enhance retention of the restoration. In particular, a bevel is obtained at the edge of the enamel surrounding the cavity, whereas in the case of a chamfer the surface obtained is not plane (as in the bevel) but it is concave [24]. The use of bevel and chamfer preparation in the enamel margin of the cavity [25–27] leads to an increase of the adhesive surface exposing a more favourable adhesion substrate. The adhesion area is increased with a bevel or a chamfer by more than 50% with respect to other techniques. Moreover, in this way the enamel prisms can be discovered [26].

Some studies have shown that the use of chamfered techniques provided greater restoration fracture resistance and higher retention of tooth structures [28, 29]. Other studies have shown that the use of bevels resulted in improved fracture resistance of composite restorations [30], improved restoration retention [31] and avoided microleakage [32].

In this study, ImageJ was not used to estimate the exact percentage of area represented by valleys produced by etching. Nevertheless, it was useful to obtain an indication of the degree of efficacy of acid etching on cat enamel as peak values of grey intensity. Fig. 7 shows an example of the analysis made on SEM images with ImageJ. In this Figure, sample C4 is reported because it gave a more representative profile. The rectangles in Fig. 7*a* represented the ROIs considered for this image and Figs. 7*b* and *c* show the grey value against distance profile (in microns) obtained for unetched and etched enamel, respectively. As can be seen, the amplitude of peaks was more evident in the etched zone; it represents the part of the enamel attacked by the acid and therefore eroded.

In the case of sample C4 the profile showed grey values ranging from about 182 to 172 representing the unetched region (Fig. 7*b*) that appeared also as almost homogeneous. On the right, the profile showed a change in peak amplitude representing a more inhomogeneous zone with peaks ranging from 140 to 107 (Fig. 7*c*). This part represented the etched region. The peaks revealed the presence of valleys because of etching. Moreover, the peaks related to the etched zone appeared wider with respect to the untreated enamel. This also could be explained with the presence of eroded enamel. The ratio R between the etched peak amplitude and the unetched one was evaluated as 3.3. The same approach was conducted for each image and the values obtained are reported in Table 1. In this Table, the ratio of grey amplitudes between etched and unetched zones for cat enamel are reported (the human enamel was used as a reference). Cat enamel samples revealed different profiles with respect to the human enamel when subjected to the same acid etching treatment. As it is possible to detect, the differences in grey-level peak amplitudes related to the two zones analysed (etched and unetched) was more evident for cat enamel subjected to etching for 60 s (group C). In this case, in fact, R was 2.8 with respect to 2.0 and 2.5 for groups A and B, respectively. This analysis showed that increasing the etching time led to a corresponding increase of efficacy of etching on cat enamel. At the same time, the treatment resulted was not as effective as in human enamel. The efficacy of the treatment on cat enamel was in fact 44, 30 and

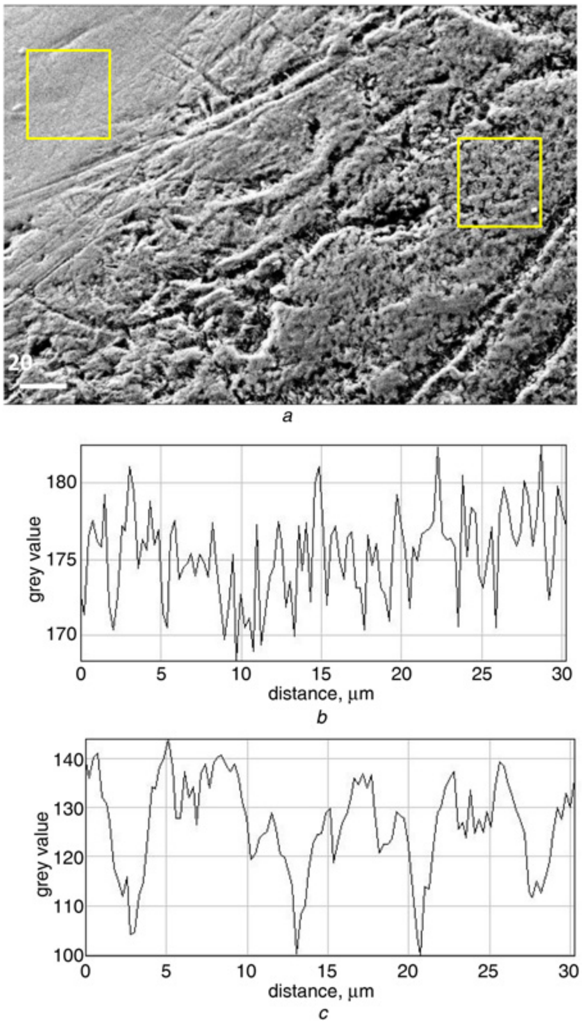

Figure 7 *SEM micrograph of sample C4 with two ROIs highlighted, and grey-scale value against distance of selected region on left and on right*
a SEM micrograph
b Grey-scale value against distance of selected region on left
c Grey-scale value against distance of selected region on right

Table 1 Ratio *R* of peak amplitudes of etched and unetched zones for samples of cat enamel (groups A, B and C) and human enamel

R = etched peak amplitude/unetched peak amplitude

Human enamel	Group A	Group B	Group C
3.6	2.0	2.5	2.5
	1.7	3.4	2.4
	1.8	2.8	1.3
	1.2	3.0	3.3
	2.6	2.1	4.2
	1.8	1.5	3.3
	3.1	2.0	2.4
mean ± SD	**2.0 ± 0.6**	**2.5 ± 0.7**	**2.8 ± 0.9**

22% lower than that obtained on human enamel when the acid attack was conducted for 30, 45 and 60 s, respectively.

4. Conclusions: The enamel of 21 cat teeth etched with 40% orthophosphoric acid for 30, 45 and 60 s was studied by SEM. The SEM revealed that the acid etching made with the times and the concentration currently used in veterinary dentistry was not optimally effective with respect to the adhesion to cat enamel. Only after 60 s, few samples showed a deeper acid attack of the enamel. SEM images were also subjected to image analysis to quantify the efficacy of acid etching in the conditions used for human enamel, by an evaluation of grey levels. This analysis showed that acid etching on cat enamel was not as effective as for human enamel. In particular, the efficacy resulted in being lower by more than 22% (up to 44%) with respect to human enamel (with a better result shown by group C etched for 60 s).

The main cause of the inefficiency of acid etching was probably because of the presence of a thick prismless layer. The possible clinical solution could be to establish, as occurs in the human reconstructions, a bevelling or a chamfer of the extension of about 1 mm on the contour of the cavity to discover the prismatic enamel and make it attackable by the etching, and to increase the enamel surface of adhesion. In a future work, we will test the possibility to define, by means of a profilometer, a statistically reliable difference between cat enamel bonding surfaces obtained by etching (with no mechanical preparation) and cat enamel surfaces prepared and then etched.

5 References

[1] Holmstrom S., Frost P., Eisner E.: 'Veterinary dental techniques' (Philadelphia, PA, USA, 2004, 3rd edn)
[2] DuPont G.: 'Radiographic evaluation and treatment of feline dental resorptive', *Vet. Clin. North Am. Small. Anim. Pract.*, 2005, **35**, (4), pp. 943–962
[3] Gorrel C.: 'Small animal dentistry', in Nind F. (Ed.): 'Saunders solutions in veterinary practice' (Saunders Elsevier, St. Louis, MO, USA, 2008)
[4] Boyde A.: 'The structure and development of mammalian enamel'. PhD, University of London, 1964
[5] Kallenbach E.: 'Fine structure of differentiating ameloblasts in the kitten', *Am. J. Anatomy*, 1976, **145**, (3), pp. 283–317
[6] Hayashi Y.: 'Crystal growth in matrix vesicles of permanent tooth germs in kittens', *Acta Anatomy (Basel)*, 1983, **116**, (1), pp. 62–68
[7] Boyde A., Fortelius M., Lester K.S., Martin L.B.: 'Basis of the structure and development of mammalian enamel as seen by scanning electron microscopy', *Scanning Microsc.*, 1988, **2**, (3), pp. 1479–1490
[8] Sasaki T., Debari K., Higashi S.: 'Energy-dispersive X-ray microanalysis and scanning electron microscopy of developing and mature cat enamel', *Arch. Oral. Biol.*, 1984, **29**, (6), pp. 431–436
[9] DeLaurier A., Boyde A., Horton M., Price J.: 'Analysis of the surface characteristics and mineralization status of feline teeth using scanning electron microscopy', *J. Anatomy*, 2006, **209**, (5), pp. 655–669
[10] Skobe Z., Prostak K.S., Trombly P.L.: 'Scanning electron-microscope study of cat and dog enamel structure', *J. Morphol.*, 1985, **184**, (2), pp. 195–203
[11] Boyde A.: 'Enamel' in (Eds): 'Handbook of Microscopic Anatomy' (Springer-Verlag, Berlin, 1989)
[12] Hayashi K., Kiba H.: 'Microhardness of enamel and dentin of cat premolar teeth', *Jpn. J. Vet. Sci.*, 1989, **51**, (5), pp. 1033–1035
[13] Crossley D.A.: 'Tooth enamel thickness in the mature dentition of domestic dogs and cats – preliminary study', *J. Vet. Dent.*, 1995, **12**, (3), pp. 111–113
[14] Boyde A.: 'Electron microscopic observations relating to the nature and development of prism decussation in mammalian dental enamel', *Bull. Group. Int. Rech. Sci. Stomatol.*, 1969, **12**, (2), pp. 151–207
[15] Boyde A., Reith E.J.: 'Cyclical uptake pattern of tetracycline in post-secretory maturation phase enamel demonstrated in rooted teeth', *Calcif. Tissue Int.*, 1983, **35**, (6), pp. 762–766
[16] Colley P., Verstraete F., Kass P., Schiffman P.: 'Elemental composition of teeth with and without odontoclastic resorption lesions in cats', *Am. J. Vet. Res.*, 2002, **63**, (4), pp. 546–550
[17] Gallottini C., Di Mari W., De Carolis C., *ET AL.*: 'SEM analysis of the effectiveness of the acid etching on cat enamel'. Proc. 31st World Veterinary Congress, Prague, Czech Republic, September 2013
[18] Torres-Rodriguez C., Rodriguez Navarro A.B., Sanchez-Sanchez P., Gonzalez-Lopez S.: 'Selective removal of mineral and organic components of bovine enamel by phosphoric acid', *J. Adhes. Dent.*, 2012, **14**, (4), pp. 329–334

doi: 10.1049/mnl.2013.0729

[19] Gwinnett A.J.: ‘The ultrastructure of the “prismless” enamel of deciduous teeth’, *Arch. Oral. Biol*, 1966, **11**, (11), pp. 1109–1116
[20] Ripa L.W., Gwinnett A.J., Buonocore M.G.: ‘The “prismless” outer layer of deciduous and permanent enamel’, *Arch. Oral Biol.*, 1966, **11**, (1), pp. 41–48
[21] Kodaka T., Nakajima F., Higashi S.: ‘Structure of the so-called prismless enamel in human deciduous teeth’, *Caries. Res.*, 1989, **23**, (5), pp. 290–296
[22] Meola M.T., Papaccio G.: ‘A scanning electron microscope study of the effect of etching time and mechanical pre-treatment on the pattern of acid etching on the enamel of primary teeth’, *Int. Dent. J.*, 1986, **36**, (1), pp. 49–53
[23] Garcia-Godoy F., Gwinnett A.J.: ‘Effect of etching times and mechanical pretreatment on the enamel of primary teeth: an SEM study’, *Am. J. Dent.*, 1991, **4**, (3), pp. 115–118
[24] Eramo S., Pelino E., Jiabali S., Falivene O., De Carolis C.: ‘The cavity margin in the anterior direct composites. Experimental findings’, *Dent. Cadmos*, 2013, **81**, (6), pp. 325–329
[25] Nebot D., Goldberg M., Fortier J.P., Aldin P.: ‘Bonding and enamel prisms. Importance of cavity margin preparation for posterior composites’, *Actual. Odontostomatol.*, 1989, **43**, (167), pp. 609–661
[26] Pelino E., Jabali S., Genovesi C., Falivene O., Eramo S.: ‘La preparazione del margine cavitario in restauri diretti di denti anteriori: aspetti teorici’, *Dent. Cadmos.*, 2013, **81**, (1), pp. 36–42
[27] Xu H., Jiang Z., Xiao X., Fu J., Su Q.: ‘Influence of cavity design on the biomechanics of direct composite resin restorations in Class IV preparations’, *Eur. J. Oral. Sci.*, 2012, **120**, (2), pp. 161–167
[28] Davis M.J., Roth J., Levi M.: ‘Marginal integrity of adhesive fracture restorations: chamfer versus bevel’, *Quintessence Int. Dent. Dig.*, 1983, **14**, pp. 1135–1146
[29] Donly K.J., Browning R.: ‘Class IV preparation design for microfilled and macrofilled composite resin’, *Pediatr. Dent.*, 1992, **14**, pp. 34–36
[30] Poojary P.K., Bhandary S., Srinivasan R., Nasreen F., Pramod J., Mahesh M.: ‘Influence of restorative technique, bevelling and aging on composite bonding to sectioned incisal edges: a comparative in vitro study’, *J. Conserv. Dent.*, 2013, **16**, pp. 28–31
[31] Reis A., Francci C., Loguercio A.D., Carrilho M.R.O., Rodrigues Filho L.E.: ‘Re-attachment of anterior fractured teeth: fracture strength using different techniques’, *Oper. Dent.*, 2001, **26**, pp. 287–294
[32] Opdam N.J.M., Roeters J.J.M., Kuijs R., Burgerdijk R.C.W.: ‘Necessity of bevels for box only class II composite restoration’, *J. Prosthet. Dent.*, 1998, **80**, pp. 274–279