

Comparative Analysis of Dental Enamel Polyvinylsiloxane Impression and Polyurethane Casting Methods for SEM Research

JORDI GALBANY,¹ FERRAN ESTEBARANZ,¹ LAURA M. MARTÍNEZ,¹ ALEJANDRO ROMERO,² JOAQUÍN DE JUAN,² DANIEL TURBÓN,¹ AND ALEJANDRO PÉREZ-PÉREZ^{1*}

¹Sec. Antropologia, Departament de Biologia Animal, Universitat de Barcelona. Av. Diagonal 645, 08028 Barcelona, Spain

²Departamento de Biotecnología, Facultad de Ciencias, Universidad de Alicante, 99. E-03080 Alicante, Spain

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ABSTRACT Dental casting is a very common procedure for making high-quality replicas of paleo-anthropological remains. Replicas are frequently used, instead of original remains, to study both fossil and extant Primate teeth in morphological and metrical analyses. Several commercial products can be used in molds. This study analyzed SEM image resolution and enamel surface feature definition of tooth molds at various magnification levels and obtained, with both Coltène[®] and 3M[™] low-viscosity body polyvinylsiloxane impression, materials and polyurethane casts. Results, through comparison with the original teeth, show that both the negative molds and the positive casts are highly reliable in replicating enamel surfaces. However, positive cast quality is optimal for SEM observation only till the fourth consecutive replica from the original mold, especially at high SEM magnification levels. *Microsc. Res. Tech* 69:246–252, 2006. © 2006 Wiley-Liss, Inc.

INTRODUCTION

Although teeth constitute the most abundant remains of the paleontological record of *Homininae*, they are not easily accessible to all scientists. As repeated handling and measurement of fossils are among the main causes of deterioration, museum curators tend to limit access to specimens. Wild-caught primate collections, irreplaceable evidence of primate ecology and adaptation (Albrecht, 1982), are more easily accessible to researchers, but tend to suffer from increasing relapses due to the great interest of large-skeleton collection “hunters” (Almquist, 1973). Tooth cast availability offers a solution to this problem.

Although plaster molds of fossil remains are suitable for museum exhibition, high-quality tooth casts are required if replicas need to be studied instead of original specimens. This is especially so if high-resolution electron microscopy procedures are to be applied, as in dental microwear research. Original specimens are too valuable to be studied directly by SEM (Beynon, 1987), particularly when such procedure requires irreversible gold coating. Environmental microscopy (ESEM) can be used for isolated teeth or small specimens whenever this technology is available (Romero et al., 2004). Therefore, high-quality replication of fossil teeth is an effective way of providing the scientific community with access to rare and important specimens (Goodwin and Chaney, 1994), as well as an alternative method of studying microfossils or microscopic details (Purnell, 2003). Museum curators can then loan tooth casts to researchers for their analyses without any risk of further damage to the original specimens.

Science would certainly benefit from the existence of high-quality cast collections, and contesters and colleagues could independently test scientific hypotheses.

However, accurate tooth crown molding is difficult (Teaford and Oyen, 1989) and may cause unwanted damage to the original teeth if it is not done properly (Hillson, 2002). High-quality, easy-to-use tooth molding materials are required for optimum and durable results. Silicon-base replication procedures are widely used to obtain negative impressions of original teeth, especially in odontological practice and dental microwear research. Hydrophobic polyvinylsiloxanes, either epoxy-resin or polyurethane replicas, produce high-quality tooth casts (Goodwin and Chaney, 1994) that are very precise replicas of the original specimens (Galbany et al., 2004b) and which provide the detailed resolution necessary in quantitative studies of tooth morphology and wear (Teaford and Oyen, 1989). Dental casts are widely used both for classical morphological/topographic analyses (Dennis et al., 2004; Egocheaga et al., 2004; Jernvall and Selänne, 1999; M’Kirera and Ungar, 2003; Pérez-Pérez et al., 2003a; Ungar, 2004; Ungar et al., 2001; Ungar and Williamson, 2000; Zucconi et al., 1998) and in dental microwear research (Galbany et al., 2002; Galbany and Pérez-Pérez, 2004; Gordon, 1982, 1984a; Grine, 1986; Hojo, 1991, 2002; Martínez et al., 2004; Pérez-Pérez et al., 1994, 1999, 2003b; Puech et al., 1983; Ryan, 1979a,b; Teaford,

*Correspondence to: Dr. Alejandro Pérez-Pérez, Sec. Antropologia, Departament Biologia Animal, Fac. Biologia, Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain. E-mail: martinez.perez-perez@ub.edu

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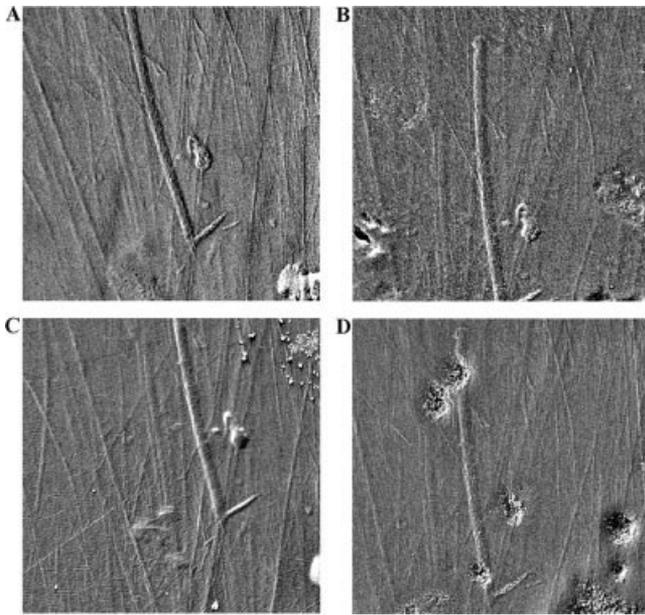


Fig. 1. SEM images at 100 \times of the buccal surface of the left M₃ tooth from Cueva del Molinico, obtained with various polyvinylsiloxane impression materials: (A) 3M Imprint II Light Body; (B) 3M Imprint II Heavy Body; (C) Coltène President Plus Jet Light Body; and (D) Coltène President Plus Jet Heavy Body. The SEM working distance ranged between 13.3 and 22.7 mm. The heavy-body molds show reduced detail resolution, which affects molding reliability.

1985, 1988a,b; Teaford and Glander, 1991; Teaford and Oyen, 1989; Teaford and Ungar, 2000; Ungar, 1992, 1998; Ungar et al., 1995, 2004; Ungar and Grine, 1991). Independent molding of dental specimens by each research group is the norm in such studies, but there are serious difficulties in the sharing of casts. Most of these difficulties, such as the useful life of the impression materials, the reliability of successive impressions from the same mold, the accuracy of different replicating materials for SEM research, the standardization of molding procedures for a variety of purposes, or researchers' diverse interests, have never been thoroughly analyzed.

President microSystem™ (Coltène®) polyvinylsiloxane impression material is widely used among dental researchers (Ungar, 1996) because it has excellent dimensional stability and reproduction detail (Andritsakis and Vlamiš, 1986) and replicates surface features with resolutions to a fraction of a micron (Teaford and Oyen, 1989). Negative molds made of polyvinylsiloxane maintain SEM resolution for many years (Beynon, 1987), with over 99.5% recovery after deformation (Coltène, 2002). Nevertheless, the ability to make several accurate casts from a single mold (Gordon, 1982) is what determines the usefulness of curating polyvinylsiloxane collections of tooth casts that are easily accessible to researchers, along with the original specimens. Some research has been done in this regard by comparing the reliability of different impression materials (either 3M™ or Coltène, among the most common ones). Beynon (1987) used Coltène Putty to build a primary base holder where Coltène President Light Body was

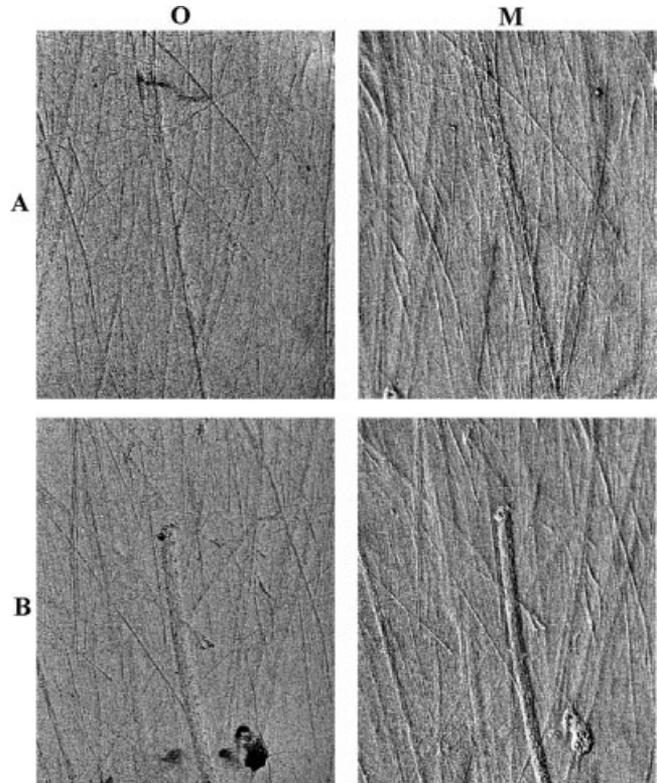


Fig. 2. 100 \times SEM images of the buccal enamel surface of a left M₃ tooth from Cueva del Molinico, with comparison of original surfaces and silicon-base molds. O, SEM images obtained from original tooth surfaces with BSE in an ESEM; M, images obtained from silicon-base molds with SE. Molds were obtained with 3M Imprint II Regular Body (A) and Coltène President Plus Jet (B). Regular body molds compared enamel replicas with molds of the original tooth surface and found them highly reliable.

poured to obtain the final negative impression. Hillson (1992) indicated that application of Light Body material directly on the crown sufficed for accurate surface replication, simplifying the molding technique and preventing possible changes in surface details. However, Hillson (1992) pointed out the difficulties in examining negative molds directly under SEM, i.e., negative molds tend to crack when pouring out the impressions, they are difficult to attach to the stub and to coat with gold-palladium, and linear enamel features are difficult to observe on them (Hillson, 1992). Although some observations indicate that the flexibility and porosity of negative silicon-base molds tend to cause mold shrinking under SEM vacuum (Galbany et al., 2004a,b), silicon negatives are adequate for SEM characterization of linear enamel hypoplasia on tooth surfaces (Guatelli-Steinberg, 2004; Guatelli-Steinberg and Mitchell, 2002). Positive casting from negative molds might be a more stable and lasting solution, though little has been published yet on the accuracy and reliability of casting materials. This study focuses on some of the methodological drawbacks of tooth cast sharing among researchers and institutions, such as the accuracy of SEM research on tooth replicas as against origi-

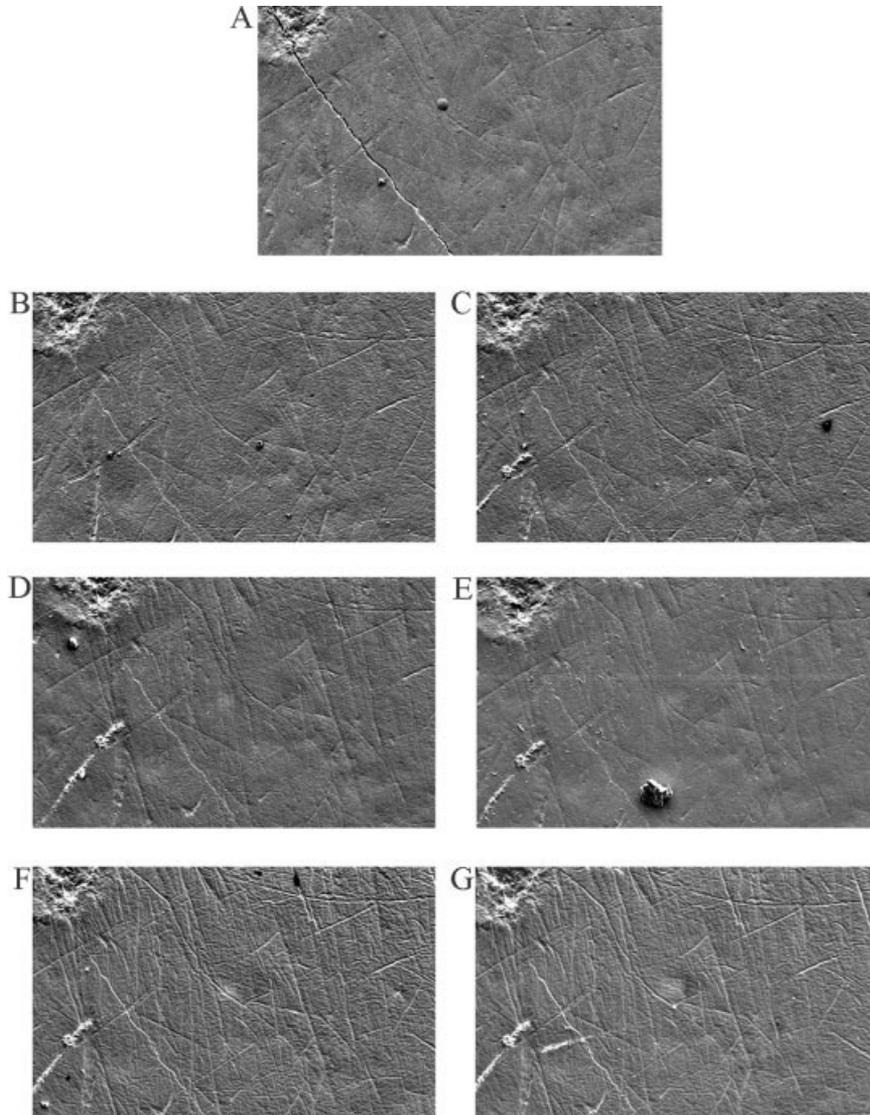


Fig. 3. SEM images at 100 \times magnification of the same buccal enamel surface of a left M¹ tooth replica from La Olmeda. The mold was obtained with President microSystem (Coltène) polyvinylsiloxane Regular Body. (A) Original teeth. (B) First replica. (C) Second replica. (D) Third replica. (E) Fourth replica. (F) Fifth replica. (G) Sixth replica. No differences in surface replication were found between the original tooth surface and the first four replicas obtained.

nal teeth, the reliability of consecutive casting obtaining several replicas from a single mold, and the useful life of tooth molds and casts.

MATERIALS AND METHODS

Four isolated human teeth (an upper left first molar LM¹, a lower right second incisor RI₂, a lower left third molar LM₃, and a lower left canine LC₁) from the medieval site of La Olmeda (Palencia, Spain) c. XII-XVII AD (Pérez-Pérez et al., 1991), and one isolated lower left first molar (LM₁) from Cueva del Molinico (Villena, Alicante, Spain), dating back to the IIIrd millennium BC (Soler García, 1993), were selected for analysis. Original teeth, as well as tooth molds and casts, were studied. Most negative tooth molds were obtained by using Regular Body President microSystem polyvinylsiloxane, since this impression material is widely used in dental studies. For comparative purposes, some molds were also obtained with President Light and Heavy

Body bases, as well as with 3M ESPE Imprint IITM polyvinylsiloxane. Tooth-crown molding procedures followed brand indications in all cases and are also described in detail elsewhere (Galbany et al., 2004a, 2005; Pérez-Pérez et al., 1999).

Preparation and Analysis of Negative Molds

Negative impressions of the buccal enamel surface of the lower first molar from Cueva del Molinico were obtained by using both polyvinylsiloxane impression brands (3M[®] and Coltène). The buccal tooth surface was cleaned with cotton swabs soaked in pure acetone, was then rinsed in 95% ethanol, and was finally air-dried. Two negative impressions were obtained with each silicon brand, and the enamel surface was cleaned again prior to obtaining the second impression. Silicone molds were stuck to brass discs with plastic carbon cement (Leit-C-Plast, Plastic Conductive Carbon Cement, Electron Microscopy Sciences, Washington, PA) and sputter-coated with ~15 nm of gold-palladium. A

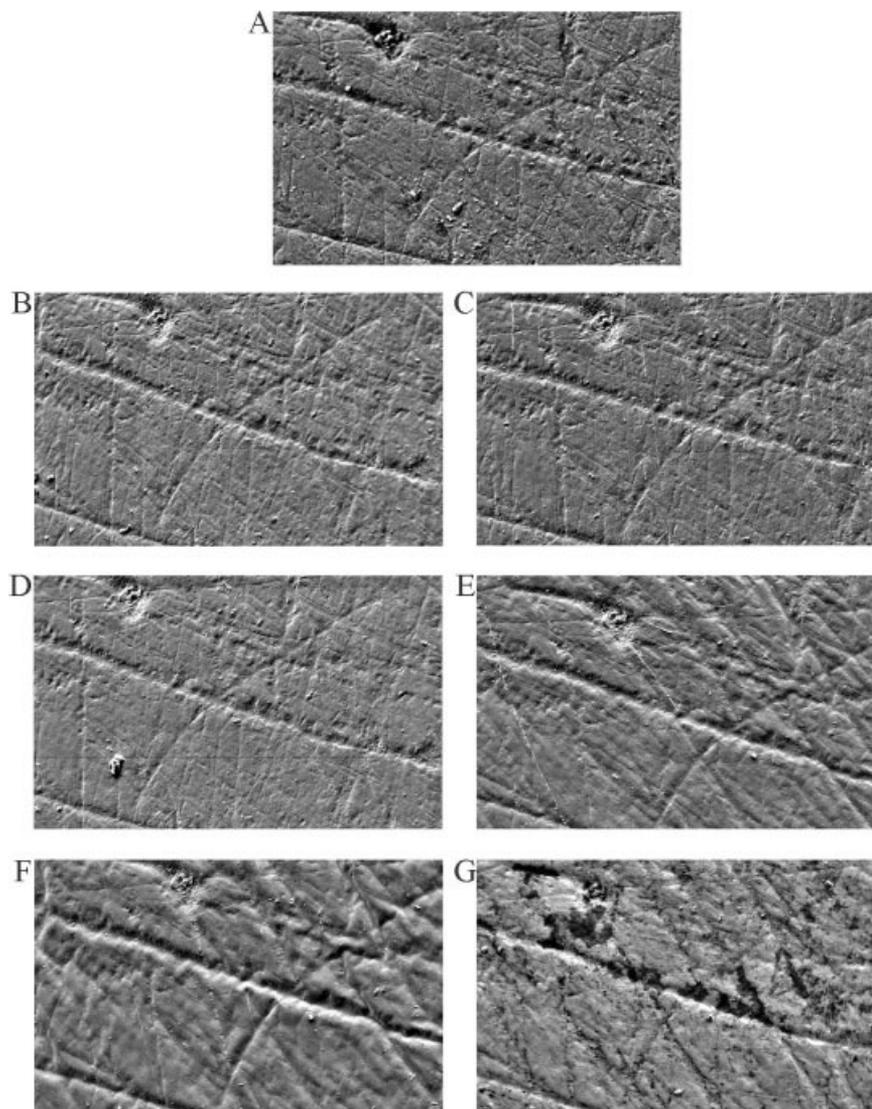


Fig. 4. SEM images at 500 \times magnification of the same buccal enamel surface of a right I_2 tooth replica from La Olmeda. The mold was obtained by President microSystem (Coltène) polyvinylsiloxane Regular Body. (A) Original teeth. (B) First replica. (C) Second replica. (D) Third replica. (E) Fourth replica. (F) Fifth replica. (G) Sixth replica. As in Figure 3, decay in surface resolution was found from the fourth successive replica obtained.

colloidal silver solution (Silver Conductive Adhesive 416, Electron Microscopy Science) was applied to improve conductivity and the preparations were mounted on SEM aluminum stubs. The original tooth and the molds were examined with a Hitachi S3000N SEM. The buccal enamel surface of the original tooth was observed without gold layer coating, using back-scattered electrons (BSE) at 20 kV accelerating voltage in a low-vacuum, variable-pressure mode (Romero et al., 2004), whereas the tooth impressions were sputter-coated with 15 nm of gold-palladium and observed using secondary electrons (standard error (SE)) and 15 kV accelerating voltage that recorded SEM images at the same location areas and with identical resolution as for the original teeth. All enamel surfaces were placed perpendicular to the electron beam and SEM images (1,280 \times 960 pixels resolution) were obtained at 100 \times magnification on the medial third of the buccal surface under the protoconid cusp.

Preparation and Analysis of Positive Replicas

Negative molds of buccal surfaces were obtained for the four teeth from the La Olmeda site. Only Regular Body President microSystem impression material was used, and six consecutive high-quality polyurethane positive casts were obtained for each mold with Ferropur power ratio (PR)-55 (Feroqa SL, Spain). A total of 24 casts were obtained and mounted on a brass disc with fusible glue (CeysTM). A colloidal argent belt (Electrodag 1415M-Acheson Colloiden) solution was applied to prevent electrostatic charges from accumulating during the SEM observation (Rose, 1983). Finally, the samples were sputter-coated with a gold layer and kept clean, dry, and dark inside a dust-free collection cabinet. Three SEM pictures at 100 \times , 500 \times , and 1000 \times magnifications were obtained for each cast with a Cambridge Stereoscan 120 SEM. In all cases, casts were placed in a horizontal position, with zero degree of tilt, 12 kV acceleration voltage, and 10–20 mm

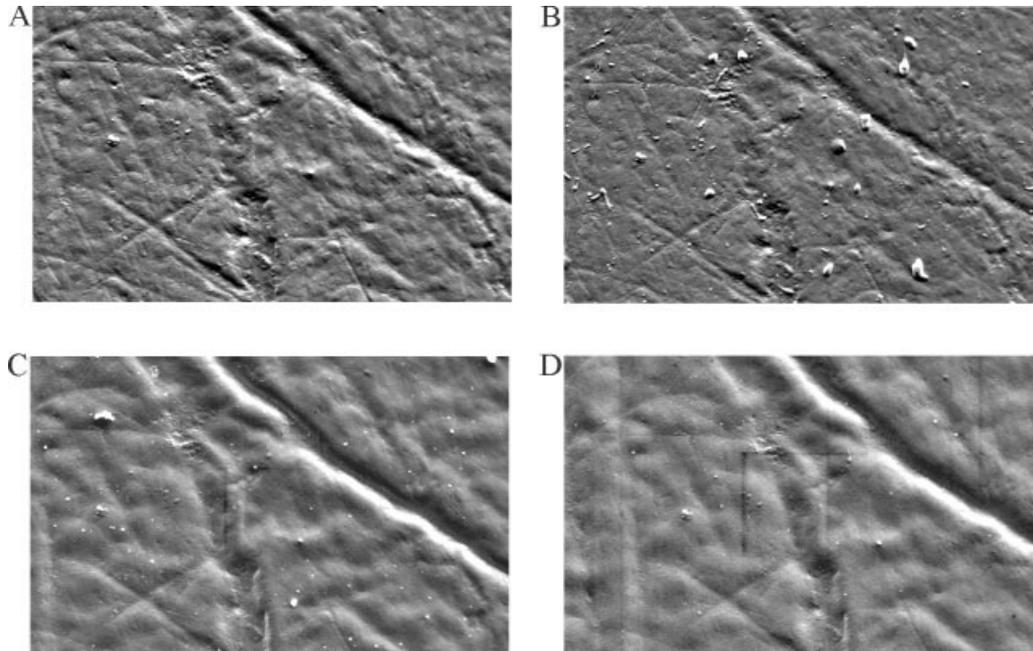


Fig. 5. SEM images at 1000 \times magnification of the same buccal enamel surface of a left M¹ tooth replica from La Olmeda. The mold was obtained with President microSystem (Coltène) polyvinylsiloxane Regular Body. (A) Third replica. (B) Fourth replica. (C) Fifth replica.

(D) Sixth replica. The first two replicas of the original tooth are not included, since no differences in cast resolution were found until the fifth replica was studied.

working distance. Digital images (1024 \times 832 pixel resolution) were obtained with the SEM Image Slave software. Prior to analysis, all images were enhanced with Adobe PhotoshopTM v 7.0 using a 50-pixel, high-pass filter and automatic gray-level adjustment (Galbany et al., 2004a, 2005).

RESULTS

Some differences in surface quality were observed between high- and low-viscosity 3M materials (Fig. 1). Areas of low casting resolution tended to form when high-viscosity (Heavy Body) materials were used (Figs. 1B and 1D), which gave lower detail resolution than when low-viscosity materials (Light and Regular Body) were used (Figs. 1A and 1C).

However, direct observation of the low-viscosity, negative polyvinylsiloxane impressions under SEM showed no damage caused by handling or by anything methodologically related to handling. Negative molds offered good stability and image resolution (SE mode), when compared to the actual enamel surfaces (BSE mode), for the two impression materials used (Fig. 2). Although longitudinal cracks or stress features may form if pressure is applied to the negative molds after coating (Hillson, 1992), vacuum in SEM chamber during observation seems not to have affected the mold stability or shape in this case.

Coltène polyvinylsiloxane Regular Body impression materials always produced high-quality tooth molds of enamel surfaces, and the positive epoxy tooth casts obtained from them showed good feature resolution when observed at 10 \times to 40 \times magnifications, with a VMT binocular magnifying glass. SEM observation of

these dental casts at 100 \times magnification yielded great image fidelity of both the original tooth surfaces (Fig. 3A) and the first four consecutive replicas. Comparisons were made at the same enamel spot (Figs. 3B–3E). However, the fifth (Fig. 3F) and sixth (Fig. 3G) replicas showed a noticeable reduction in surface detail and some degree of background noise due to mold deterioration after repeated casting. At 500 \times magnification, loss of resolution in replicating enamel surfaces is evident from the fourth replica (Figs. 4E–4G), where striation borders appear increasingly less defined and enamel surface roughness is increasingly evident. Finally, micrographs of dental casts obtained at 1000 \times magnification showed a loss in resolution from the fifth replica onwards (Fig. 5).

DISCUSSION

The comparisons of enamel resolution details in all the SEM images obtained clearly show that low-viscosity silicones are excellent materials for making impressions of buccal tooth surfaces (bubbles tend to appear only when heavy bodies are used), even though the viscosity of the replicating material may vary, depending on the surface to which it is applied (Chee and Donovan, 1992) or the shearing forces placed on it (Mandikos, 1998).

Although obtaining impressions with silicone materials may not always be a predictable process (Gordon, 1984b), in the present study all the low-viscosity, Regular Body polyvinylsiloxane dental molds provided high-quality casts for SEM analysis up to at least the fourth consecutive replica obtained from the same mold, at 100 \times , 500 \times , and 1000 \times magnification. From the fifth cast on, feature definition decreased in all casts: tooth

striations became thicker, with less defined borders, and enamel surfaces appeared rough and irregular. Feature modification after several consecutive casts may be caused by pull-out forces affecting negative molds when the positive cast is removed. Microwear analyses, essentially based on statistical methods, are greatly affected by the methodological approach used (Gordon, 1988) and extra-casting from a single mold needs to be carefully considered. Only a few, most probably from 1 to 4, good-quality casts can be obtained from the same original mold, with enough fidelity to replace original specimens in SEM research. Thus, molds themselves should be considered valuable specimens for use instead of original teeth. Some precautions need to be taken during cast preparation and handling, such as not using latex gloves with sulfur compounds (Browning et al., 1993) or hand lotions (Goodwin and Chaney, 1994) that inhibit polyvinylsiloxane setting. However, if they are properly curated, successive positive casts are a reliable alternative to original specimen analysis, since SEM feature resolution after consecutive casting is independent of magnification level, at least up to 1000 \times . Cast deterioration shows up after the fourth replica at all magnifications.

In conclusion, SEM observation of tooth enamel surfaces at various magnification levels indicates that both negative, low-viscosity polyvinylsiloxane molds and positive epoxy casts can be used for SEM research instead of original specimens. In addition, at least four consecutive positive replicas can be obtained from a single mold, ensuring that several researchers can study a tooth's morphology without loss of detail resolution.

It would be of general benefit to researchers to produce digital 3D models of tooth crowns from valuable collections. This would give permanent access to virtual tooth replicas without any need of the original tooth. It is not clear yet whether such "replicas" could be used in all kinds of research. SEM analyses of both negative and positive replicas are reliable at resolutions lower than a fraction of a micron, whereas 3D surface topography shows resolution levels between 0.05 mm (mesh-point height-sensing 3D scanner) and 0.2 mm (laser 3D scanning). Unfortunately, as micro-computer tomography 3D models (MCT) are still difficult and expensive to obtain, 3D digital replicas of tooth crown surfaces cannot yet replace high-quality tooth casts in SEM research. Detailed analyses of MCT 3D models are still needed to determine whether they can be used instead of original teeth, replacing traditional high-resolution SEM microwear research using tooth casts. In addition, automatic procedures for dental microwear research, based on roughness and anisotropy measurements of surface topography, still need to be proved significant in determining dietary-related behavior in Primates rather than postmortem enamel abrasion processes (Estebanz et al., 2005).

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REFERENCES

- Albrecht GH. 1982. Collections of nonhuman primate skeletal materials in the United States and Canada. *Am J Phys Anthropol* 57:77–97.
- Almquist AJ. 1973. More on primate skull collections in European museums. *Curr Anthropol* 14:264–265.
- Andritsakis DP, Vlamis KF. 1986. Polyvinylsiloxane. A new generation of the elastomeric impression materials. *Odontostomatol Proodos* 40:133–142.
- Beynon AD. 1987. Replication technique for studying microstructure in fossil enamel. *Scanning Microsc* 1:663–669.
- Browning GC, Broome JC, Jr, Murchison DF. 1993. Removal of latex glove contaminants prior to polyvinyl siloxane impressions. *J Dent Res* 72:129.
- Chee WWL, Donovan TE. 1992. Polyvinyl siloxane impression materials: a review of properties and techniques. *J Prosthet Dent* 68:728–732.
- Coltène. 2002. Instructions for use. Coltène® President microsystem™ regular body surface activated. Coltène AG, Altstätten/Switzerland. US Patent No 5,333,760; 5,400,925; 5,413,253.
- Dennis JC, Ungar PS, Teaford MF, Glander KE. 2004. Dental topography and molar wear in *Alouatta palliata* from Costa Rica. *Am J Phys Anthropol* 125:152–161.
- Egocheaga JE, Pérez-Pérez A, Rodríguez L, Galbany J, Martínez LM, Telles M. 2004. New evidence and interpretation of subvertical grooves in neanderthal teeth from Cueva de Sidrón (Spain) and Figueira Brava (Portugal). *Anthropologie* 42:49–52.
- Estebanz F, Galbany J, Martínez LM, Pérez-Pérez A. Correlation between scanning electron microscopy microwear patterns and interferometric microscopy surface roughness as indicator of dietary related behaviour in hominoid primates. In: Bailey S, Hublin J-J, editors. *Dental Perspectives on Human Evolution*. New York: Springer, in press.
- Galbany J, Pérez-Pérez A. 2004. Buccal enamel microwear variability in Cercopithecoidea primates as a reflection of dietary habits in forested and open savannah environments. *Anthropologie* 42:13–19.
- Galbany J, Martínez LM, Pérez-Pérez A. 2002. Variabilidad del patrón de microestriación dentaria en primates Hominoideos: una cuestión de especie o de entorno ecológico? *Rev Esp Antropol Biol* 23:77–83.
- Galbany J, Martínez LM, Pérez-Pérez A. 2004a. Tooth replication techniques, SEM imaging and microwear analysis in primates: methodological obstacles. *Anthropologie* 42:5–12.
- Galbany J, Martínez LM, Hiraldo O, Espurz V, Estebanz F, Sousa M, López-Amor HM, Medina AM, Farrés M, Bonnin A, Bernis C, Turbón D, Pérez-Pérez A. 2004b. Teeth: catálogo de moldes de dientes de homínidos de la Universitat de Barcelona. Barcelona: Universitat de Barcelona. p.193.
- Galbany J, Martínez LM, López-Amor HM, Espurz V, Hiraldo O, Romero A, De Juan J, Pérez-Pérez A. 2005. Error rates in buccal-dental microwear quantification using scanning electron microscopy. *Scanning* 27:23–29.
- Goodwin MB, Chaney DS. 1994. Molding and casting: techniques and materials. In: Leiggi P, May P, editors. *Vertebrate Paleontological Techniques*. Cambridge: Cambridge University Press. pp. 235–271.
- Gordon KD. 1982. A study of microwear on chimpanzee molars: implications for dental microwear analysis. *Am J Phys Anthropol* 59:195–215.
- Gordon KD. 1984a. The assessment of jaw movement direction from dental microwear. *Am J Phys Anthropol* 63:77–84.
- Gordon KD. 1984b. Pitting and bubbling artefacts in surface replicas made with silicone elastomers. *J Microsc* 134:183–188.
- Gordon KD. 1988. A review of methodology and quantification in dental microwear analysis. *Scanning Microsc* 2:1139–1147.
- Grine FE. 1986. Dental evidence for dietary differences in *Australopithecus* and *Paranthropus*: a quantitative analysis of permanent molar microwear. *J Hum Evol* 15:783–822.
- Guatelli-Steinberg D. 2004. Analysis and significance of linear enamel hypoplasia in Plio-Pleistocene hominins. *Am J Phys Anthropol* 123:199–215.
- Guatelli-Steinberg D, Mitchell J. 2002. Comparison of impression materials used on fossil teeth. *Am J Phys Anthropol (Suppl)* 34:78–79.
- Hillson S. 2002. *Dental anthropology*. Cambridge: Cambridge University Press. p. 373.
- Hillson SW. 1992. Impression and replica methods for studying hypoplasia and perikymata on human tooth crown surfaces from archaeological sites. *Int J Osteoarchaeol* 2:65–78.
- Hoyo T. 1991. Scanning electron microscopic analysis of dental wear on the heavily worn second molars of the wild Japanese monkey (*Macaca fuscata*). *Scanning Microsc* 5:505–508.

- Hojo T. 2002. Dental microwear of canines and M1s of late stone age compared with those of the modern age of Western Japan. *Am J Phys Anthropol (Suppl)* 34:86.
- Jernvall J, Selänne L. 1999. Laser confocal microscopy and geographic information systems in the study of dental morphology. *Palaeontol Electron* 2:18.
- Mandikos MN. 1998. Polyvinyl siloxane impression materials: an update on clinical use. *Aust Dent J* 43:428–434.
- Martínez LM, Galbany J, Pérez-Pérez A. 2004. Palaeodemography and dental microwear of *Homo habilis* from East Africa. *Anthropologie* 42:53–58.
- M'Kirera F, Ungar PS. 2003. Occlusal relief changes with molar wear in *Pan troglodytes troglodytes* and *Gorilla gorilla gorilla*. *Am J Primatol* 60:31–41.
- Pérez-Pérez A, Lalueza C, Hernández M, Turbón D. 1991. Análisis del patrón de estriación dentaria: variabilidad intrapoblacional en la serie medieval de La Olmeda (Palencia). In: Botella MC, Jiménez SA, Ruiz L, du Souich PH, editors. *Nuevas perspectivas en antropología*. Granada: Universidad de Granada. pp. 731–740.
- Pérez-Pérez A, Lalueza C, Turbón D. 1994. Intraindividual and intra-group variability of buccal tooth striation pattern. *Am J Phys Anthropol* 94:175–187.
- Pérez-Pérez A, Bermúdez de Castro JM, Arsuaga JL. 1999. Non-occlusal dental microwear analysis of 300,000-year-old *Homo heidelbergensis* teeth from Sima de los Huesos (Sierra de Atapuerca, Spain). *Am J Phys Anthropol* 108:433–457.
- Pérez-Pérez A, Farrés M, Martínez LM, López-Amor HM, Galbany J. 2003a. Correlación entre tamaño dentario y microestriación vestibular en homínidos Pli-Pleistocénicos de Kenia y Tanzania. In: Aluja M^aP, Malgosa A, Nogués RM^a, editors. *Antropología y biodiversidad*. Barcelona: Bellaterra. pp. 407–413.
- Pérez-Pérez A, Espurz V, Bermúdez de Castro JM, de Lumley MA, Turbón D. 2003b. Non-occlusal dental microwear variability in a sample of Middle and Late Pleistocene human populations from Europe and the Near East. *J Hum Evol* 44:497–513.
- Puech PF, Albertini H, Serratrice C. 1983. Tooth microwear and dietary patterns in early hominids from Laetoli, Hadar and Olduvai. *J Hum Evol* 12:721–729.
- Purnell MA. 2003. Casting, replication, and anaglyph stereo imaging of microscopic detail in fossils, with examples from conodonts and other jawless vertebrates. *Palaeontol Electron* 6:11.
- Romero A, Martínez-Ruiz N, De Juan J. 2004. Non-occlusal dental microwear in a Bronze-Age human sample from East Spain. *Anthropologie* 42:65–69.
- Rose JJ. 1983. A replication technique for scanning electron microscopy: applications for anthropologists. *Am J Phys Anthropol* 62:255–261.
- Ryan AS. 1979a. Tooth sharpening in primates. *Curr Anthropol* 20:121–122.
- Ryan AS. 1979b. Wear striation direction on primate teeth: a scanning electron microscope examination. *Am J Phys Anthropol* 50:155–168.
- Soler García JM^a. 1993. Guía de los yacimientos y del museo de Villena. Valencia: Generalitat Valenciana. p. 132.
- Teaford MF. 1985. Molar microwear and diet in the genus *Cebus*. *Am J Phys Anthropol* 66:363–364.
- Teaford MF. 1988a. A review of dental microwear and diet in modern mammals. *Scanning Microsc* 2:1149–1166.
- Teaford MF. 1988b. Scanning electron microscope diagnosis of wear patterns versus artifacts on fossil teeth. *Scanning Microsc* 2:1167–1175.
- Teaford MF, Glander KE. 1991. Dental microwear in live, wild-trapped *Alouatta palliata* from Costa Rica. *Am J Phys Anthropol* 85:313–319.
- Teaford MF, Oyen OJ. 1989. Live primates and dental replication: new problems and new techniques. *Am J Phys Anthropol* 80:73–81.
- Teaford MF, Ungar PS. 2000. Diet and the evolution of the earliest human ancestors. *Proc Natl Acad Sci USA* 97:13506–13511.
- Ungar PS. 1992. Dental evidence for diet in primates. *Anthrop Közl* 34:141–155.
- Ungar PS. 1996. Dental microwear of European Miocene catarrhines: evidence for diets and tooth use. *J Hum Evol* 31:335–366.
- Ungar PS. 1998. Dental allometry, morphology, and wear as evidence for diet in fossil primates. *Evol Anthropol* 6:205–217.
- Ungar PS. 2004. Dental topography and diets of *Australopithecus afarensis* and early *Homo*. *J Hum Evol* 46:605–622.
- Ungar PS, Grine FE. 1991. Incisor size and wear in *Australopithecus africanus* and *Paranthropus robustus*. *J Hum Evol* 20:313–340.
- Ungar PS, Williamson M. 2000. Exploring the effects of tooth wear on functional morphology: a preliminary study using dental topographic analysis. *Palaeontol Electron* 3:18.
- Ungar PS, Teaford MF, Glander KE, Pastor RF. 1995. Dust accumulation in the canopy: a potential cause of dental microwear in primates. *Am J Phys Anthropol* 97:93–99.
- Ungar PS, Grine FE, Teaford MF, Pérez-Pérez A. 2001. A review of interproximal wear grooves on fossil hominin teeth with new evidence from Olduvai Gorge. *Arch Oral Biol* 46:285–292.
- Ungar PS, Teaford MF, Kay R. 2004. Molar microwear and shearing crest development in Miocene Catarrhines. *Anthropologie* 42:21–35.
- Zuccotti LF, Williamson MD, Limp WF, Ungar PS. 1998. Technical note: modeling primate occlusal topography using geographic information systems technology. *Am J Phys Anthropol* 107:137–142.