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CLEANING FOSSIL TOOTH SURFACES FOR MICROWEAR ANALYSIS: USE OF SOLVENT GELS TO REMOVE RESISTANT CONSOLIDANT

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ABSTRACT

Fine-scale surface texture analysis of teeth has become increasingly useful for anthropologists and palaeontologists to infer diet and jaw mechanics in fossil animals. We describe a fast, non-abrasive and residue free method for the removal of resistant consolidant from fossil teeth. The method utilises solvent gels, and its use is a significant improvement over previous techniques, particularly where microwear analysis is to be performed. The method adapts techniques originally developed by art conservators for the removal of varnish from oil paintings without damaging the oil paint beneath. A combination of Carbopol (a water soluble acrylic polymer) and Ethomeen (a polyoxyethylene cocoamine detergent) allows solvents such as acetone and ethanol to be suspended in a gel for application to consolidant coated tooth surfaces. Key advantages are that dissolved consolidant is lifted away from the tooth surface into the solvent gel and a high degree of control is possible such that small discrete areas can be cleaned of consolidant. Because the solvents are held within a gel, cleaning of the tooth surface can be performed without the need for a fume hood.

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KEY WORDS: solvent gel; microwear; consolidant removal

INTRODUCTION

Microwear analysis requires images or 3D data to be acquired from tooth surfaces at relatively high magnification, sampling data from very small areas, typically only a few hundred micrometres across. In order for the microwear features and textures to be accurately detected or replicated it is imperative that the tooth surface be thoroughly cleaned prior to imaging or moulding. Any surface contaminant or coating that could potentially mask microwear must be removed. The cleaning process must not abrade or etch the tooth surface or leave residue that might obscure the original microwear.

Quantitative analysis of tooth microwear has been applied extensively to mammals (Walker et al. 1978; Gordon 1984; Teaford 1988; Organ et al.

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2005; Ungar et al. 2007) and is starting to be applied to dinosaurs (Williams et al. 2009); prior studies of dinosaur tooth microwear have been qualitative (e.g., Fiorillo 1991, 1998; Upchurch and Barrett 2000; Schubert and Ungar 2005). The cleaning methods employed by most of these researchers involve soft brushing or gentle swabbing (using cotton swabs) with either distilled water or a solvent such as acetone or ethanol. Whilst these methods work well on material that has been treated with modern consolidants such as the methacrylate co-polymer Paraloid, they have proven time consuming and laborious where more traditional consolidants such as shellac or glyptal have been used and totally ineffective where the shellac has aged.

Material from the older museum collections (19th and early 20th century), particularly dinosaur material, has often been treated with one or both of two consolidants: shellac and animal resin. As shellac ages it darkens and becomes cross-linked (bonds develop that link one polymer chain to another) making it extremely resistant to solvents. As microwear analysis is increasingly applied to dinosaurs, more researchers are likely to discover this problem.

Problems with Brush-Based Cleaning:

When attempting to remove consolidant from the occlusal surface of a tooth by the brushing on of a solvent and continual cleaning of the brush, or by the use of disposable swabs, the whole tooth and surrounding area tends to become soaked in a combination of the solvent and dissolved consolidant. Given that consolidants like shellac typically form a coating rather than penetrate a surface when they are originally applied, this is a backward step. The brushing process also tends to move consolidant around, smearing it over microwear and making it difficult to determine when all vestiges have been removed. This technique is especially problematic if SEM analysis is to be performed on moulds and casts rather than the original specimens (brush marks in remaining consolidant are a particular hazard). Use of this technique also results in a high rate of solvent evaporation requiring the use of a fume cupboard. It has also been suggested that repeated applications of solvents such as alcohol and acetone can dehydrate enamel and dentine leading to surface damage (Fernandez-Jalvo and Monfort 2008) although this damage is questioned by dental researchers who claim dentine in particular becomes more resistant (e.g., Nalla et al. 2005). The possibility of acetone-caused cracking is of particular concern as the process can be time consuming and requires repeated application of brushed-on solvent where the consolidant is shellac, as the older shellac is, the more resistant it tends to become.

Figure 1 shows a tooth surface after each of two consecutive attempts to remove the consolidant coating via the brushing on of ethanol. Figure 1.1, 1.3 and 1.5 show the first attempt. Brush strokes are clearly visible in the higher magnification images Figure 1.3 and 1.5. Figure 1.2, 1.4 and 1.6 show the second attempt. Whilst an underlying pervasive and dominant near vertical microwear pattern is emerging, sufficient varnish remains to in-fill and partially obscure this pattern. Figure 2 shows a tooth surface that was cleaned whilst being viewed under a stereo microscope (at x40 magnification giving a 5 mm field-of-view) by brushing on ethanol until it appeared to be clear of consolidant. The higher magnification SEM images (Figure 2.2 and 2.3) clearly show that the tooth surface is still coated in consolidant.

It is both time consuming and frustrating to complete a sequence of brush cleaning, moulding, casting and SEM imaging only to discover that a tooth surface is not clean, especially if the original tooth is in a remote museum collection and a return visit must be arranged. A more reliable cleaning method is needed.

Solvent Gels

Art conservators wanting to clean varnish from paintings without damaging the oil paint beneath discovered that by suspending the solvent in a gel, they could limit evaporation and control both contact time and the pH. The addition of soaps and detergents to the gel allowed the dissolved varnish to be sequestered by the gel and thus easily removed from the painting (Southall 1988). They found that overpainted areas could be dealt with by the addition of xylene to the gel, causing partial dissolution and swelling of the paint layer (Wolbers et al. 1990). It is this solvent gel formulation, used for varnish on paintings, which we have adapted for the removal of various consolidants from dinosaur teeth, including aged shellac.

This paper describes the cleaning method used by the authors, developed from a technique pioneered by museum conservators. This technique, for the removal of varnish from oil paintings via the application of solvent gels, is not widely known, and papers describing its use (Hedley 1980; Burnstock and White 1990; Eastaugh 1990;

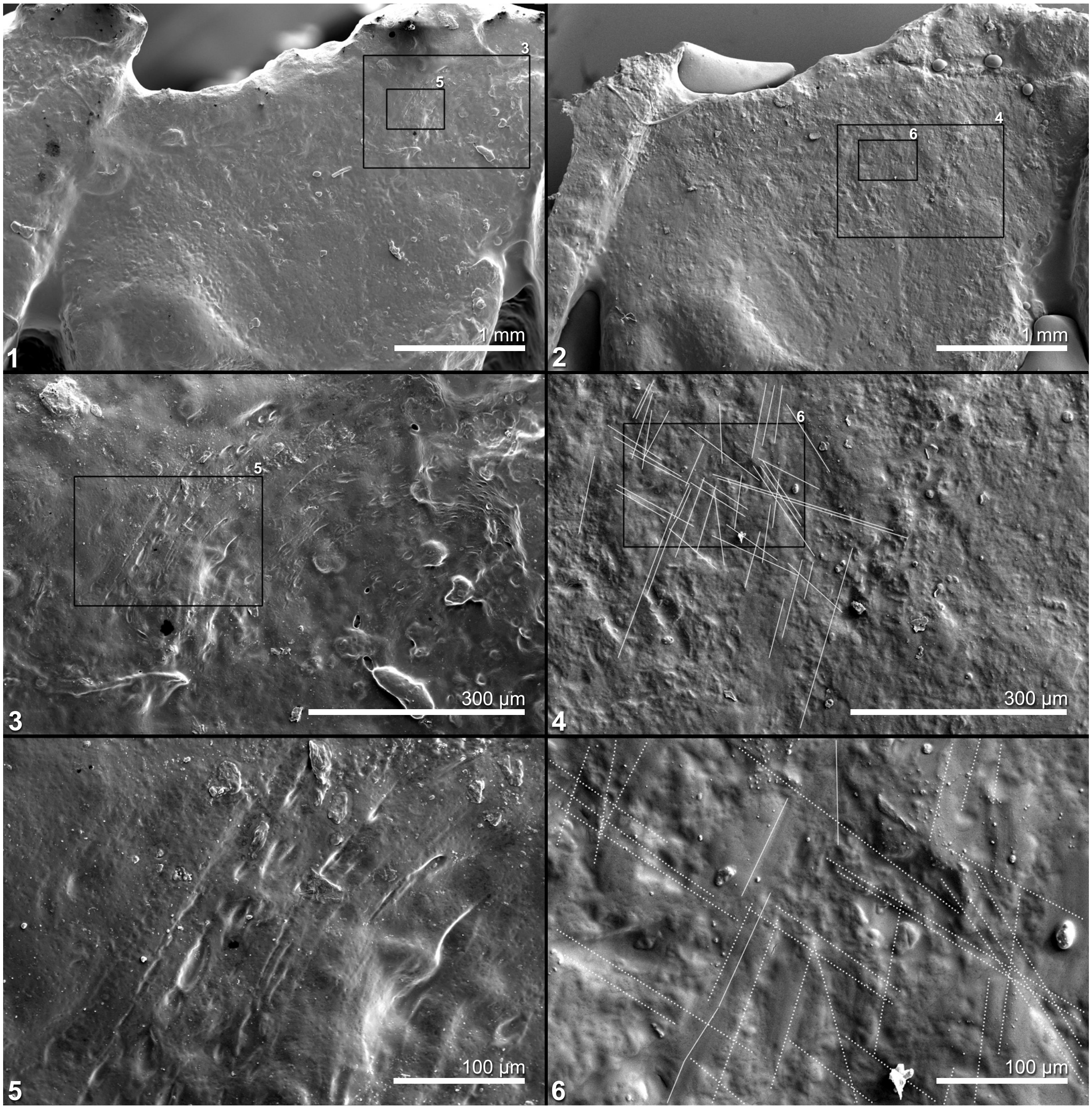

FIGURE 1. Occlusal surface of tooth of a *Heterodontosaurus* (SEM images of casts of SAM PK-1332 right dentary tooth, 3rd from posterior) illustrating the difficulties of cleaning by brush and solvent (ethanol). **1.1** Cast 1 made after first attempt to remove varnish from tooth surface; boxes show areas illustrated in 1.3 and 1.5. **1.2** Cast 2 made after second attempt to remove varnish from tooth surface, at low magnification the tooth appears to be clean; boxes show areas illustrated in 1.4 and 1.6. Microwear patterns can be identified at this magnification. **1.3** Enlargement of area in box 3 of 1.1; varnish has been smeared across the tooth surface and brush marks can be seen clearly in it. **1.4** Enlargement of area in box 4 of 1.2; a dominant near vertical microwear pattern (part highlighted with solid white lines) is discernable but varnish still remains infilling and obscuring the pattern. **1.5** Enlargement of area in box 5 of 1.1 and 1.3; at higher magnification there is still no visible microwear beneath the brush marks in the varnish. **1.6** Enlargement of area in box 6 of 1.2 and 1.4; at higher magnification several different orientations of microwear (part highlighted with solid white lines (visible microwear) and dashed white lines (obscured microwear)) are just discernable through the obscuring varnish, highlighting the amount of varnish still remaining on the surface of a tooth that had been cleaned twice and appeared to be free of varnish at low magnification.

FIGURE 2. Occlusal surface of an isolated tooth of hadrosaurid (SEM images of cast of AMNH 21700) illustrating the difficulties of cleaning with brush and solvent (ethanol). **2.1** Low magnification image showing full width of tooth, at this magnification the tooth appears to be clean; boxes show areas illustrated in 2.2 and 2.3. Microwear patterns can be identified at this magnification. **2.2** Enlargement of area in box 2 of 2.1; at higher magnification it can be seen that the microwear (part highlighted with solid white lines) is largely obscured. **2.3** Enlargement of area in box 3 of 2.1 and 2.2; at higher magnification it is clear the surface is still coated in consolidants and the microwear patterns (part highlighted with solid white lines (visible microwear) and dashed white lines (obscured microwear)) are largely obscured.

Wolbers et al. 1990; Wolbers 1992) are not widely available.

MATERIAL AND METHODS

Ornithopod dinosaur specimens to which the cleaning methods described herein have been applied include teeth and jaw elements from the collections of the Natural History Museum, London, the American Museum of Natural History, New York (AMNH), the Peabody Museum of Natural History, Yale University, the Smithsonian Institute National Museum of Natural History, Washington DC (SM), the Carnegie Museum of Natural History, the Iziko South African Museum (SAM), the Dinosaur Isle Museum, Isle of Wight and the Oxford University Museum of Natural History, Oxford. All were cleaned using solvent gels and then moulded with a vinyl polysiloxane impression medium. Epoxy resin casts were taken from the vinyl polysiloxane moulds, sputter coated with gold and imaged in a Hitachi S-3600N Scanning Electron Microscope (SEM).

Shellac, glyptal and shellac/animal resin combinations were removed by the application of solvent gels from the occlusal surfaces of hundreds of teeth from 143 specimens, which consisted of individual teeth, teeth within jaw fragments and teeth within complete jaw elements.

Fossil teeth that were cleaned by the traditional brushing on of ethanol method were also moulded, cast and imaged.

Creating the Solvent Gel

Components:

- 200 ml ethanol (IMS)
- 200 ml acetone
- 50 ml xylene
- 20 ml Ethomeen C/25 (a polyoxyethylene cocoamine detergent; Akzo / Linden Chemicals)
- 6g Carbopol EZ2 (a water soluble acrylic acid polymer; Noveon / Linden Chemicals)
- 50 ml pure water (distilled or deionised)

Care should be taken to follow the manufacturer's instructions regarding safe use and storage of all of these products. Material Safety Data Sheets (MSDS) are available via the manufacturer's and distributor's web sites. Both Ethomeen and Carbopol can be obtained from Linden Chemicals (www.lindenchemicals.com), Ethomeen is a product of Akzo (www.akzonobel.com), and Carbopol is a product of Noveon (www.lubrizol.com). Ethanol, iso-propanol, acetone and xylene can be obtained from standard suppliers of laboratory chemicals.

The solvent gel should be prepared and stored in polyethylene or polypropylene bottles to prevent reaction between container and gel. Bottles with transparent sides allow progress to be monitored during preparation of the gel. Bottles with wide openings and tightly sealing lids are preferable.

The quantities listed above will produce approximately 500 ml of solvent gel; for smaller volumes reduce the component quantities on a pro-rata basis. The solvent gel can be created quickly for immediate use via a one stage method, but a two stage method (see below) produces a more consistent gel, allows the pH of the gel to be controlled and gives flexibility in the combination of solvents used. Standard laboratory procedures should be followed with reference to all relevant health and safety legislation. We recommend that the addition of ethanol, acetone and/or xylene to the solvent gel should be performed in a fume cupboard. However, once the solvents have been added to the gel, a fume cupboard is no longer required. The solvent gel can be used with standard air extraction systems or in a well ventilated area.

For the one stage method of gel preparation, sprinkle the Carbopol EZ2 powder onto the Ethomeen C/25 whilst stirring continuously until a uniform paste is produced. Stir in the required combination of solvents ethanol/acetone/xylene, and then add the pure water gradually whilst stirring continuously. Apply a tight fitting lid to the bottle, and shake the bottle vigorously.

For the two stage method, first prepare a Carbopol gel as follows: Sprinkle the Carbopol EZ2 powder onto pure water whilst stirring continuously, until a smooth, stiff 'wallpaper paste'-like mixture forms. This Carbopol gel can be used within a few minutes if necessary (as soon as it settles and takes on a uniform consistency) but will benefit from being left to stand overnight in a sealed bottle to allow the Carbopol to fully disperse. Next, pour the Ethomeen EZ2 into the Carbopol gel and stir until a smooth, colourless and transparent Carbopol/Ethomeen gel forms. The bottle lid can be screwed tight and the bottle shaken vigorously to aid mixing at this stage. The introduction of Ethomeen should neutralize the acidity of the Carbopol. Testing with pH paper strips should show a pH between 7 and 8. If these numbers do not result, adding more Ethomeen will increase the pH and adding more Carbopol gel will reduce the pH.

Next, mix the required combination of solvents ethanol/acetone/xylene in a second bottle, and then cut in the Carbopol/Ethomeen gel gradually. If the gel becomes cloudy or if a sticky white residue begins to form, water must be added to the gel to allow the solvents to be fully absorbed. Adding the above solvents should result in Solvent gel with a pH of around 8.5.

The advantage of using the two stage method of production is that stock Carbopol/Ethomeen gel can be made up as a first stage. Later, small samples of this gel can be cut into various combinations of solvents for testing on unknown consolidants.

Variations in Composition

Various formulations of the gel can be made by substituting one solvent for another. The above formula, which adds a small quantity of xylene to a 50:50 solution of acetone and ethanol, acts to break the cross-links in aged shellac and enables it to be dissolved. It is effective on glyptal as it takes the guess work out of which solvent was used in the formulation of the glyptal, and it will also work on young shellac (although not as effectively as ethanol alone) and so can be used as a universal formula. An alternative formulation substituting additional ethanol for the acetone and xylene (i.e., 450 ml of ethanol) is more effective on shellac that has not developed cross-links. Typically this is shellac that is less than two years old. However, depending upon the additives used in the shellac, it may still dissolve readily in ethanol after many years. It is worth testing a small area to assess how first to treat shellac.

When solvents are added to the Carbopol/Ethomeen gel, the pH can be tested. If it is too acidic, Ethomeen can be added to reduce the acidity. Whilst slight acidity of the solvent gel is not critical for use on the highly resistant enamel and dentine surfaces of teeth, this ability to control pH has great importance for the wider application of solvent gels to less resistant surfaces such as bone.

It is worth noting that linseed oil may be a component in shellac. Whilst we did not encounter a consolidant of this composition, in such instances the acetone/ethanol combination would not be as effective. Tests carried out on oleo-resinous varnish by Burnstock and White (1990) had greater success with iso-propanol.

Application of the Solvent Gel

Loose dirt should be brushed from the surface being cleaned and a thick layer (>8 mm) of solvent gel applied. A layer of stretch plastic wrap should be wrapped over the solvent gel and the teeth/jaw to hold the gel in place. This has a number of positive effects. The plastic wrap provides gentle pressure, keeping the gel in contact with the consolidant surface, it prevents the gel from spreading to adjacent surfaces, and it reduces the amount of solvent evaporation from the gel. We found that a 17 μm thick, commercial, food quality, polyethylene stretch film plastic wrap, with cling/tack on one side, worked well for our purposes, but thicker versions of the domestic ‘cling film’ (UK) or Saran™ Wrap (US) should be equally suitable.

Over a period of 15 to 20 minutes the consolidant will soften, dissolve and be drawn into the gel. Typically, the dissolved consolidant (particularly shellac) discolours the gel, and, as both plastic wrap and gel are transparent, the process can be monitored. One application of gel is usually enough. If the consolidant has been applied in multiple layers it may be necessary to repeat the process. The plastic wrap can be used to scoop the gel from the tooth surface, and both plastic wrap and gel can be discarded (subject to the relevant local regulations on the disposal of hazardous waste). With shellac, a very thin blistered film typically remains that should be ‘teased’ away from the tooth surface. A wooden spatula, wooden dental stick or latex block (to avoid introducing scratches) will work well for this purpose. Any remaining solvent gel can be brushed from the tooth surface with a little ethanol or water and a fine soft brush.

The Carbopol EZ2 gel allows a solvent or combination of solvents to be held in suspension, preventing immediate evaporation and allowing their action to be concentrated at a specific point and over a significant period of time. Being an amine with detergent properties Ethomeen C/25 serves two purposes when mixed with a Carbopol EZ2 gel. The slight alkalinity of the amine neutralizes the acidity of the gel and the detergent properties enable the gel to sequester material dissolved by the suspended solvents. This latter quality is of key importance in that the dissolved consolidant is taken away from the tooth surface and locked into the gel. As a layer of shellac dissolves into the gel it thins and eventually reaches the point where it will distort and blister, lifting away from the tooth surface cleanly. The clean separation virtually eliminates instances of remaining consolidant and the consequent need to re-clean and re-mould/cast.

Solvent gels need to be stored, handled and disposed of as hazardous chemicals, but no more so than their solvent components. The lower rate of evaporation from gels makes them considerably more pleasant to work with. Carbopol/Ethomeen gel is not hazardous until solvents are added, so it can be made up in bulk and stored for extended periods of time. Small volumes for immediate use can be measured out as needed and the solvents added moments before use. Alternatively, solvent gel can be made up from scratch within an hour and potentially within a few minutes.

RESULTS AND DISCUSSION

Figures 1 and 2 show casts taken from teeth that were cleaned by the traditional brushing on of ethanol method. These casts appear to be free of consolidant at low magnification (Figures 1.1 and 2.1), but at higher magnifications (Figures 1.2, 1.3, 2.2, 2.3) the microwear is clearly being obscured by smeared consolidant.

Figures 3 and 4 compare the quality of microwear obtained from tooth surfaces that have been cleaned via the application of solvent gel to that from adjacent uncleaned areas. It can be seen from these images that the use of solvent gel leaves little or no remaining consolidant. The ability of solvent gels to lift consolidant cleanly rather than dissolve it and allow it to in-fill microwear can be seen in Figure 5. Here we show a selection of teeth that were marginal to areas cleaned with solvent gel, such that only part of the tooth surface has been cleaned. In each case there is no smearing or blurring, the edge of the consolidant coating is sharp and well defined. All of these casts showed large areas of tooth surface that were either completely free of consolidant or that had only small, discrete patches of consolidant within them.

A combination of acetone, ethanol and xylene makes a good universal solvent gel, and holding the pH down to a value around 8.5 slows the action of the solvent gel, causing the shellac to swell and blister. This amount has proven more effective at removing thick coatings of shellac than using more alkaline solvent gels. These tend to act more rapidly, penetrate the shellac and remove it unevenly.

A note of caution on the removal of shellac: Conservators are aware that removing any consolidant from a fossil may cause damage. In the case of aged shellac that damage can be significant. This is due to the tendency of shellac to shrink over time, exerting pressure on the fossil. In some cases, if this pressure is removed the fossil can fracture and disintegrate (Davidson and Alderson 2009). For microwear analysis there is no alternative to removing the consolidant, but by using solvent gels the area of removal can be restricted to very small parts of the tooth, minimizing the risk. None of the hundreds of teeth to which our solvent gel cleaning method has been applied showed any evidence of resulting damage.

Solvent gels concentrate the action of the solvent on the specific part of the surface being cleaned and in doing so reduce the diffusion of the solvent into the surrounding area. Where a tooth or jaw has been reconstructed by gluing several parts together, use of a solvent gel will enable cleaning of a portion of the tooth without compromising the reconstruction.

By retaining the solvent within the gel, the volume of evaporate is greatly reduced, conserving the solvent and allowing cleaning to be performed with simple air extraction rather than a fume cupboard (subject to all relevant health and safety legislation, especially where xylene is used within a solvent gel). A more comfortable working environment is the result.

SUMMARY

Solvent gels remove consolidant more cleanly, more reliably, in considerably less time and in a more comfortable working environment than the brushing on of solvent method. Specific areas of interest can be cleaned without any adverse effects on adjacent areas and the flexibility in formulation allows various consolidants to be tackled. These advantages, coupled with the ability to control pH (and thus the level of aggression) allows the possibility of its wider application to less resistant consolidant coated surfaces.

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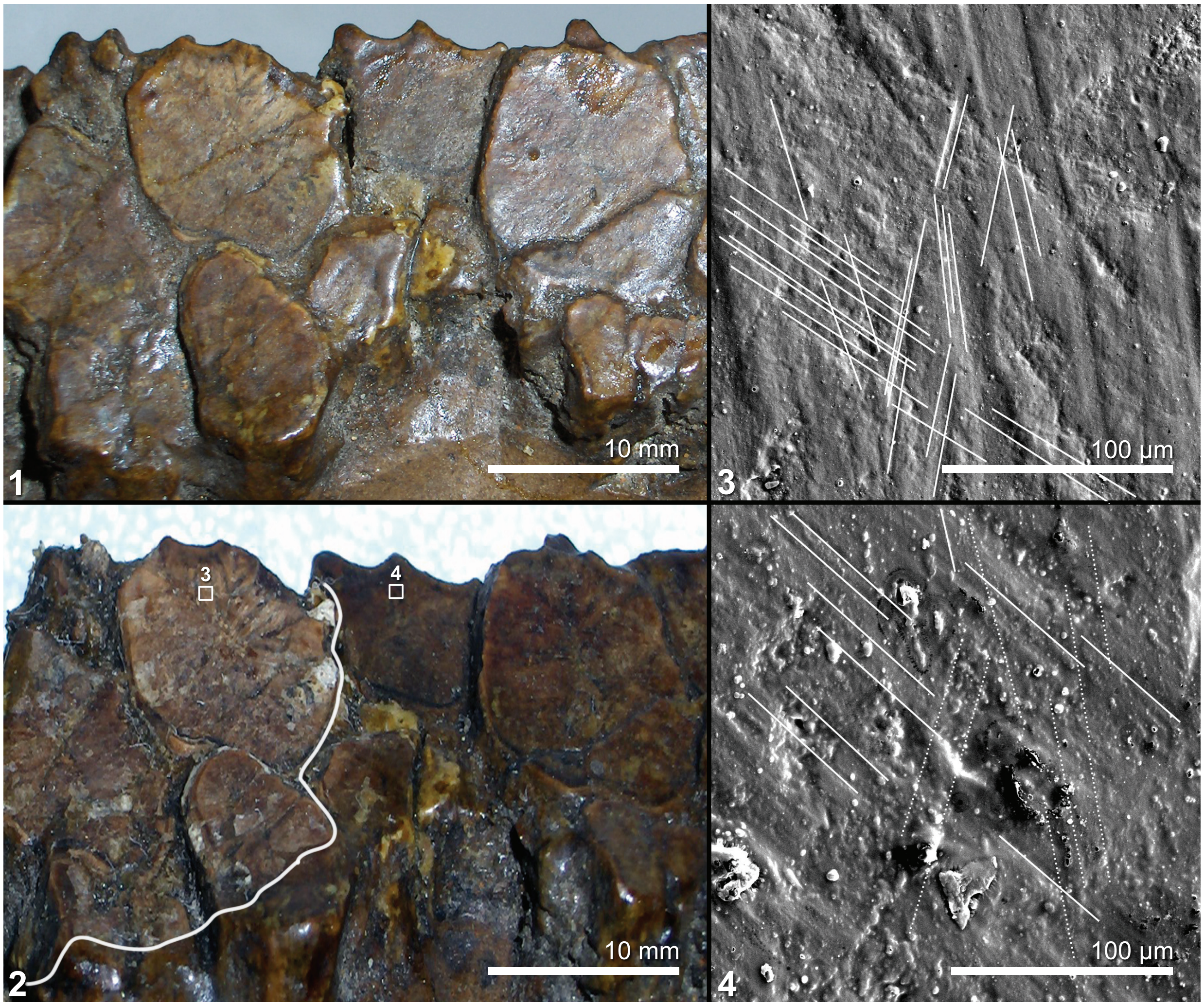

FIGURE 3. Occlusal surface of left dentary teeth of *Corythosaurus casuarius* (AMNH 3971), illustrating the results of cleaning with solvent gel. **3.1** Photograph of mesial fragment of left dentary prior to cleaning. **3.2** Post cleaning, shellac removed from teeth on the left by application of solvent gel; boxes show areas illustrated in 3.3 and 3.4. Broad white line drawn on to highlight the sharp boundary of the area from which shellac has been removed. **3.3** SEM micrograph of cast of central portion of a cleaned tooth (area in box 3 of 3.2); multiple microwear orientations (part highlighted with solid white lines) are visible with no remnant of varnish. **3.4** SEM micrograph of cast of central portion of an uncleaned tooth (area in box 4 of 3.2); microwear (part highlighted with solid white lines) is discernable but is largely obscured by varnish; in particular the near vertical microwear (part highlighted with dashed white lines), which is visible in 3.3, is barely noticeable here.

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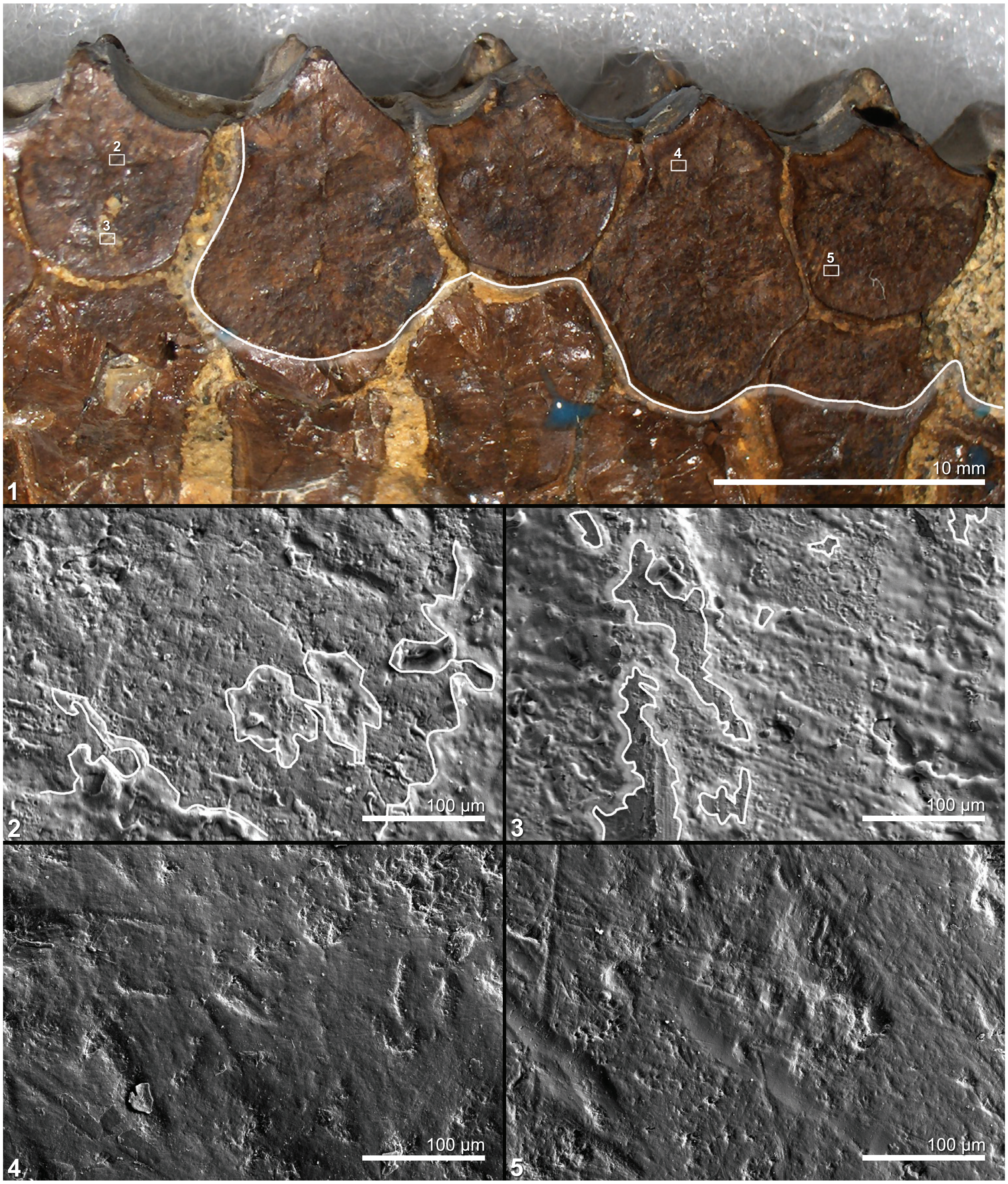

FIGURE 4. Occlusal surface of left dentary teeth of *Edmontosaurus* (SM 22102), illustrating the results of cleaning with solvent gel. **4.1** Photograph of distal section of a left dentary. White line drawn on to highlight the sharp boundary of the area from which shellac has been removed. The teeth on the top row and to the right have been cleaned by application of solvent gel. The teeth on the left and along the bottom row were not cleaned and remain coated in shellac. Boxes show areas of tooth illustrated in 4.2 to 4.5. **4.2** SEM micrograph of cast of site on uncleaned tooth (area in box 2 of 4.1); microwear is obscured by shellac. White lines drawn around areas of shellac, with shading in the shellac. **4.3** SEM micrograph of cast of site on uncleaned tooth (area in box 3 of 4.1); microwear is obscured by shellac. White lines drawn around areas of shellac, with shading in the shellac. **4.4** SEM micrograph of cast of site on cleaned tooth (area in box 4 of 4.1); showing microwear with no remnant of shellac. **4.5** SEM micrograph of cast of site on cleaned tooth (area in box 5 of 4.1); showing microwear with no remnant of shellac. (Note: the broad shallow grooves are typical of tool marks left by a vibro-tool during preparation of a fossil.)

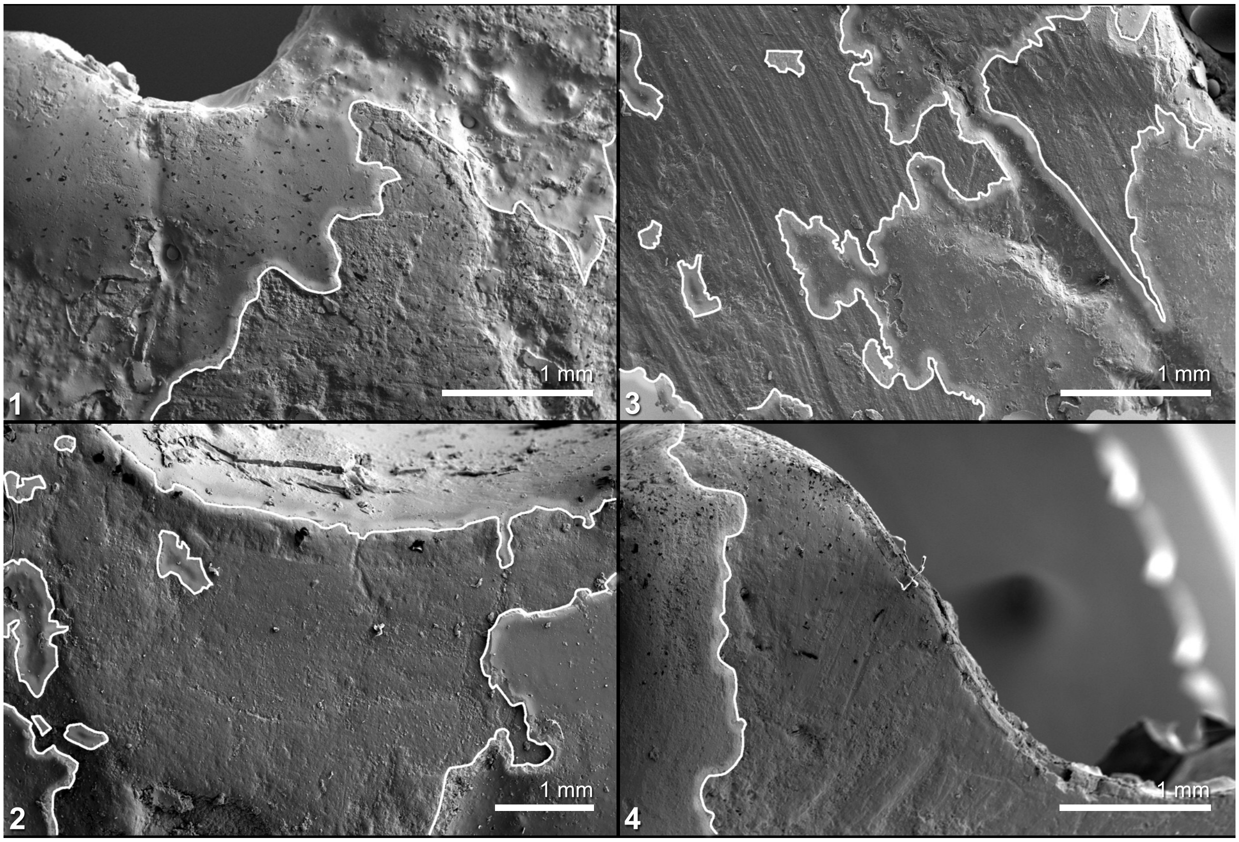

FIGURE 5. Occlusal surfaces of hadrosaurid teeth (SEM images of casts) illustrating the results of cleaning with solvent gel. These teeth were marginal to areas that were cleaned by the application of solvent gel prior to moulding and casting and show the clear boundary between the consolidant layer and clean tooth surface. White lines are drawn around areas of shellac, with shading in the shellac. **5.1** *Edmontosaurus* AMNH 5879 right maxilla tooth, right hand side of tooth has been cleaned. **5.2** Hadrosaurid AMNH 5896 maxilla tooth, left hand side of tooth has been cleaned; vestiges of consolidant remain on the left hand side in depressions. **5.3** *Hadrosaurus notablis* SM 5465 left dentary tooth, left hand side of tooth has been cleaned. **5.4** Hadrosaurid AMNH 6387 right maxilla tooth, right hand side of tooth has been cleaned.

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APPENDIX

Components and Suppliers:

Carbopol was originally manufactured by B.F. Goodrich (now Noveon). It was available in several formulations (e.g., 934, 940, 950 and 954), and it is these that are discussed by painting conservators (Southall 1988; Wolbers et al. 1990). EZ2 is a newer but comparable formulation of Carbopol and is more readily available today than the older versions. It is supplied as a white powder. When mixed with pure water it forms a clear, slightly acidic, viscous gel. The water must be pure, otherwise any minerals present (particularly calcium) will react with the Carbopol and precipitate out. The manufactures claim that the EZ formulations disperse more quickly, which certainly appears to be the case (EZ2 can be used within minutes of mixing whereas 954 took over an hour to achieve a uniform consistency). Carbopol 954 based solvent gels were used within the Palaeontology Conservation Unit at the Natural History Museum, London, and Carbopol EZ2 based solvent gels were used on specimens at all other sites.

Ethomeen is manufactured by Akzo. There are two forms suitable for use in solvent gels (C/12 and C/25), with C/25 being recommended for use with the more polar solvents (Wolbers et al. 1990). Ethomeen is an ochre coloured viscous liquid and is slightly alkaline. Both forms are available from Linden Chemicals.