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IN VITRO AND *IN VIVO* REPLICATION FOR SCANNING ELECTRON MICROSCOPY OF THE CERVICAL REGION OF HUMAN TEETH

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Abstract

A replica technique for scanning electron microscopy (SEM) of the cervical region of human teeth was evaluated on extracted premolar teeth by comparing the replicas and the original specimens in the SEM. For *in vivo* application, the technique was modified to circumvent contamination by saliva and gingival exudate. Impressions were taken with an addition silicone polyvinylsiloxane material and the replicas were poured in epoxy resin die material. A surface active dentine conditioner facilitated flow of the impression material into irregular surface areas; *in vivo* a scavenger impression was used to remove surface debris. Custom trays were made of light-cured acrylic resin for the *in vivo* impressions. The method faithfully reproduced surface detail in the amelocemental region. *In vivo* the scavenger impression followed by application of the surface-active conditioner effectively cleaned the tooth surface. The custom tray allowed selection and inclusion of landmarks and ensured reproducibility. The method meets the requirements of a simple, reproducible, non-invasive means of documenting the micromorphology of the cervical region of the teeth.

Key Words: replica, dental impression, scanning electron microscopy, cervical region, amelocemental junction, gingival recession, abrasion-erosion.

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Introduction

Scanning electron microscopy (SEM) has been extensively applied in studies of the dental hard tissues. However, preparation techniques for SEM may introduce artifacts in specimens of enamel (Fejerskov *et al.*, 1984) and cementum (Jones, 1987).

Many of the artefacts associated with specimen preparation may be circumvented by replication. Although obviously appropriate for *in vivo* longitudinal studies, replication is also applicable to *in vitro* investigations, because it is non-destructive, preserving the specimen for comparative study by other means.

Replication using dental impression and die materials is now an established and widely applied technique in SEM (e.g., Grundy, 1971; Barnes, 1978, 1979; Lambrechts *et al.*, 1981; Scott, 1982; Rose, 1983; Ekfeldt *et al.*, 1985; Vossen *et al.*, 1985; Walsh and Basu, 1987; Bromage, 1987; Beynon, 1987). However, the particular problems associated with replication of the cervical region have received relatively little attention (Cowell and Saxton, 1978; Absi *et al.*, 1989).

The relationship of the dental hard tissues at the amelocemental junction may be quite complex, with areas of high relief at the ultrastructural level (Akai *et al.*, 1976; Schroeder and Scherle, 1988). Air voids in the impression are therefore difficult to avoid; on the positive replica these appear as bubbles which not only obscure morphological detail, but are also a source of electrical charging artefacts on SEM images. Two additional factors which further complicate replication *in vivo* are the rapid deposition of a salivary pellicle on cleaned and dried surfaces (Silverstone *et al.*, 1985) and contamination by exudate from the adjacent gingival sulcus.

Recently, replication routines for anthropology have been refined by Beynon (1987) and Bromage (1987); and Teaford and Oyen (1989) have described replication of dental microwear in living primates. The present study, based on similar principles, describes a simple, reproducible method for application to the cervical region of human teeth. *In vitro*, the method was evaluated on young premolar teeth extracted on orthodontic

indications; for comparison, the original specimens were also photographed in the SEM. The method was then modified for *in vivo* application and applied in a preliminary study of the micromorphology of exposed cervical regions in the dentition of caries risk patients attending the Department of Cariology, Karolinska Institutet, Stockholm.

Materials and Methods

In vitro

The material comprised premolar teeth removed on orthodontic indications and stored in neutral buffered formaldehyde. Each tooth was decontaminated and denuded of organic material by immersion in 10% sodium hypochlorite solution for twenty minutes, followed by rinsing in distilled water for twenty minutes. The teeth were inspected at a magnification of 16X and any with obvious extraction damage in the cervical areas were discarded. Four teeth were finally selected for the study.

The bulk of the crown and root of each tooth was removed with a diamond disc under water coolant. The remainder, a few millimeters of crown and root hard tissue comprising the cervical region, was easily accommodated on an aluminum stub for SEM examination. Two of the four cervical samples were then separated into a buccal and lingual section and two into mesial and distal sections. The bases were planed flat on sandpaper. Debris was removed by gentle washing and brushing with warm soapy water. All specimens were stored in distilled water until impression taking.

Impressions were taken of two specimens at a time, mounted on a glass microscope slide. After gentle drying with compressed air for thirty seconds, a surface-tension reducing agent, Tubulicid Blue, (Dental Therapeutics, Nacka, Sweden) containing 0.2% EDTA and benzalkonium chloride, (Brännström *et al.*, 1980) was applied with a non-linting microbrush (CDB Huddinge, Sweden).

Automixing light body addition silicone impression material (President Jet, Coltene) was expressed through an extra fine nozzle along the amelocemental junction region and spread with a gentle blast of compressed air. The nozzle was then removed from the syringe and a layer of impression material, a few millimeters thick, was quickly expressed over the specimen. Finally, a copper band, sealed at one end with baseplate wax and filled with impression material, was gently superimposed over the specimen, expressing excess impression material onto the glass slide.

After a setting time of six minutes, the copper band was "snapped" away and the specimens were immediately returned to storage in water. The impressions were inspected at a magnification of 16X and then bench-cured overnight at room temperature.

The replica was made from an epoxy resin material (Epoxy-die, Ivoclar), specifically formulated for use with addition silicon elastomeric impression materials. The components were carefully measured and mixed according to the manufacturer's instructions, but not vibrated. Following the method described by Schelb (1988), the mixture was poured into a disposable plastic impression syringe, to which an extra fine bore nozzle had been attached. A fine stream of material was carefully expressed into the deepest part of the impression and spread with a gentle stream of compressed air. This process was repeated with a second application of epoxy material. The bulk of the impression was then filled from the syringe. The replica was placed in a fume cupboard for a minimum undisturbed setting time of 2 hours.

The replica was removed from the copper band impression 24 hours after pouring. The impression was then carefully washed with warm soapy water, rinsed with distilled water, and dried. A second replica was then poured and allowed to set for 24 hours. Just before removal, the base of the replica was carefully planed with sandpaper to the level of the edge of the copper band. Residual grit was removed by gentle brushing in warm soapy water. The replica was then removed from the impression and inspected at a magnification of 16X.

The original specimen and its replica were attached to aluminum stubs and air dried for 18 hours before sputter coating with 10 nm gold-palladium (Polaron, England). They were examined in a Philips 501 SEM at 15 kV and photographed at magnifications from 40X to 1250X (Kodak TMax 100).

In vivo

The subjects selected had exposed roots, without active caries. Some teeth showed loss of cervical contour, clinically denoted as early erosion-abrasion defects. The appointments for impression-taking were scheduled 1 hour after lunch and the subjects were instructed to brush their teeth and rinse thoroughly with water immediately after eating.

Custom trays covering the buccal surfaces of 3-9 teeth were made on stone study casts. The area to be included in the impression was pencilled on the model and a layer of baseplate wax moulded over the area. A sheet of preformed acrylic dough (Convertray, Wilde, Germany) was pressed over the wax and trimmed with a knife. The tray was light-cured for five minutes in the Traylight oven. Perforations about 0.75 cm apart were made with a 2 mm diameter flat fissue bur. A handle for thumb and finger grip was attached in the center of

the tray, to facilitate removal at right angles to the buccal surface. Two trays were made for each experimental area, one for a scavenger impression and one for the final impression.

The teeth were flossed, water sprayed and dried with compressed air. Following a scavenger impression Tubulicid Blue was immediately applied as in the *in vitro* study and the final impression was taken: the impression material was applied through the extra fine nozzle, as for the *in vitro* impression, but *in vivo* the initial application was into the gingival sulcus. As the total area of the impression encompassed only three or four teeth, the working time allowed spreading of the initial application with a gentle blast of air and then continued application without the extra fine nozzle. The custom tray, filled with impression material, was then superimposed. After six minutes, the set impression was removed by grasping the handle and snapping the tray away from the teeth.

The impression was inspected at a magnification of 16X and then bench cured overnight.

Before the replica was poured, the clinical impression was subjected to the following laboratory disinfection procedures: immersion in alkaline-buffered 2% glutaraldehyde (Cidex, Johnson and Johnson), removal and sealing in a plastic bag for 30 minutes, followed by rinsing in running water for 15 minutes and air drying.

The impression was carefully boxed in with baseplate wax and filled with epoxy die material as described for the *in vitro* technique. The replica was removed from the impression 24 hours later. The impression was washed with detergent, gently cleaned with a microbrush and repoured. The first replica was retained as a reference model and the second was prepared for examination in the SEM. The custom tray was cleaned and stored on the stone study model, for use in follow-up studies.

The replicas were inspected at a magnification of 16X; after gross reduction using "heatless stones" on a lathe, the replicas of the individual teeth were separated interproximally using a fine serrated steel disc in the laboratory handpiece.

Each replica was then washed and gently brushed with warm soapy water and inspected at a magnification of 16X; residual debris was removed with a microbrush. The replicas were mounted on aluminum stubs, air dried for 24 hours, and sputter coated as described for the *in vitro* procedures.

For comparison, replicas were also poured of some of the scavenger impressions.

Results

In vitro

At low magnifications, the morphological features of the cervical region were clearly reproduced. Figures 1a and 1b show a replica and the original buccal specimen. The gently undulating pattern of the amelocemental junction is clearly defined, with cementum overlapping the cervical enamel. Although the procedures for preparation of the tooth specimens were carefully selected to minimize artefacts due to dehydration, the root cementum in the tooth specimen in Fig. 1b is marred by cracks. At this level of magnification, the enamel surface does not appear to have been adversely effected by the preparation procedures.

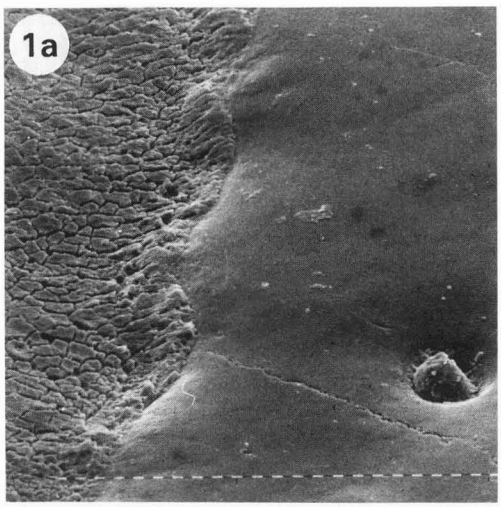
In Figs. 2a and 2b another region of the amelocemental junction is shown at low magnification. Compared to the region in Fig. 1, the border between root and crown is more tortuous. Cracking of the cementum in the original specimen (Fig. 2b) is marked. Cracks have also developed at the amelocemental junction, obscuring detail which was faithfully reproduced in the replica. In both photographs, the morphological detail of the coronal enamel is of comparable quality.

Figures 3a and 3b, at four times the magnification of the photographs in Figures 1 and 2, show a detail of the supracervical enamel, in a region with many enamel caps. The classical "cauliflower" appearance of the enamel cap seen in the tooth specimen in Fig. 3a, is not well reproduced in the replica: some deformation of the impression has occurred at the convoluted base of the enamel cap. At this magnification, the subsequent cracking of enamel in the original specimen is also marked.

In vivo

In order to compare the appearance of a tooth surface treated with a surface-tension reducing agent with that of a tooth from which debris had been removed by spraying with water only, replicas of some scavenger impressions were poured and examined in the SEM. Figure 4a is a low magnification view of the region near the gingival sulcus and Figure 4b shows a detail at higher magnification: plaque comprised of mounds of filamentous microorganisms in which cocci are embedded. In the foreground are several macrophage-like cells.

Figure 5a is a low magnification view of a replica of a mandibular incisor with gingival recession, but clinically no loss of root contour. To facilitate SEM examination of the region nearest the gingival sulcus, the replicated areas of the gingival tissues were trimmed off. Figure 5b shows that at higher magnification the topography of the root surface is crater-like and uneven. The micro-organisms, mainly in the craters, are coccoid in form.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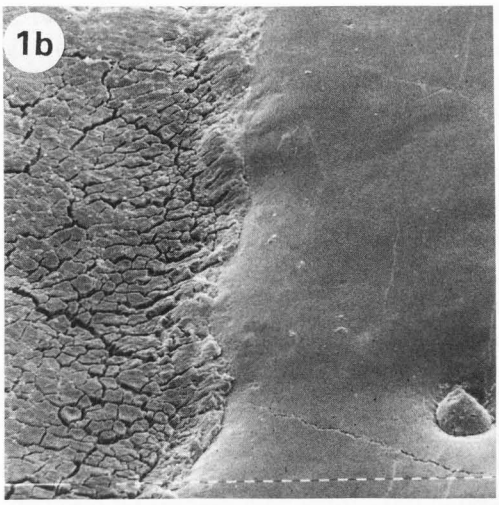
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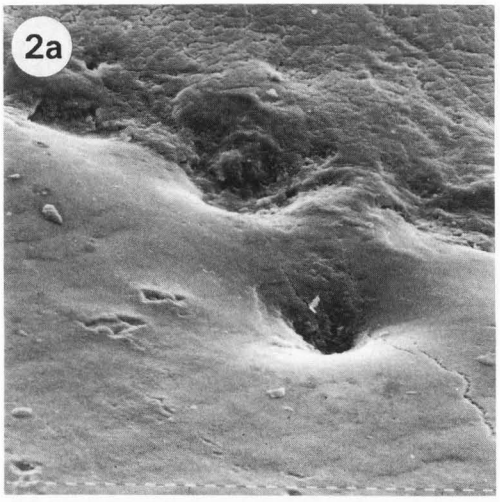
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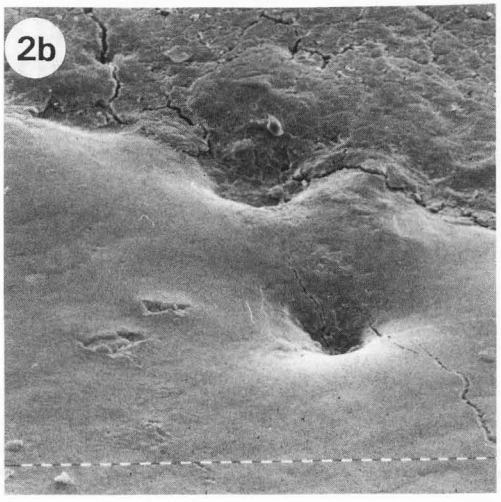
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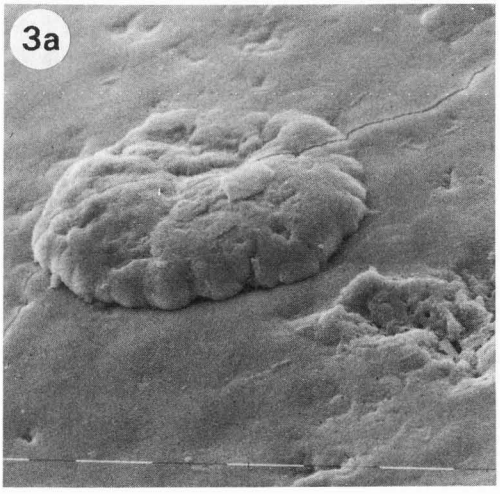
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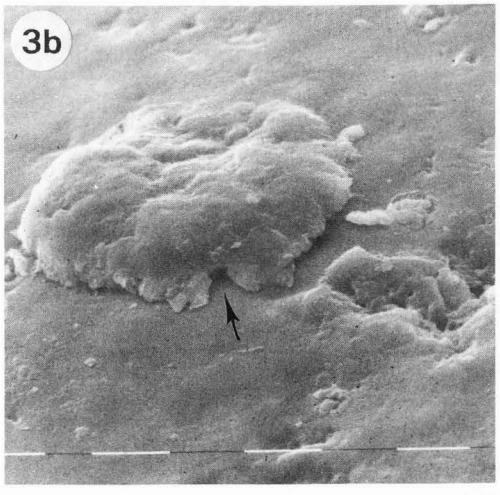
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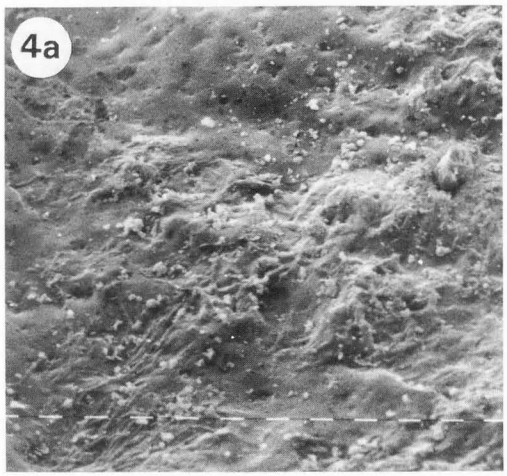

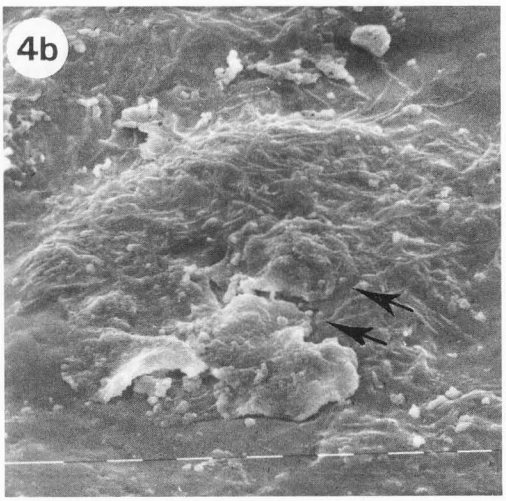

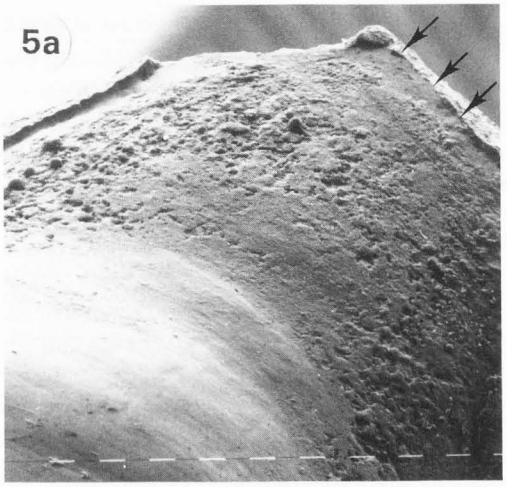

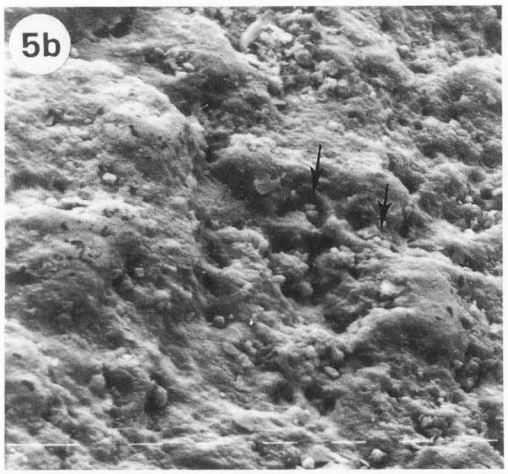

Figure 1a. *In vitro*. Replica of a buccal segment in which the enamel at the amelocemental junction is covered by cementum. Bar = 10 μm. **Figure 1b**. *In vitro*. Original specimen of replica in Figure 1a. Comparison of the two photomicrographs shows that the replica gives faithful reproduction of surface detail. Despite careful preparation after impression taking for replication, cracks have occurred in the cementum of the original specimen.

Figure 2a. *In vitro*. Replica of another cervical region. Bar = 10μm. **Figure 2b**. *In vitro*. Original specimen of replica in Figure 2a. Cracks in the cementum at the amelocemental junction obscure detail which has been faithfully reproduced in the replica.

Figure 3a. *In vitro*. Higher magnification of supracervical region of specimen in Figure1, showing an enamel cap. Bar = 10 μm. **Figure 3b**. Replica of specimen shown in Figure 3a. The impression has been torn at the convoluted base of the enamel cap (arrow), resulting in distorted replica. See text for discussion.

Figure 4a. *In vivo*. Replica of scavenger impression. Root surface near the gingival sulcus has been partly denuded of deposits, but the extremely adherent mature plaque is undisturbed. Bar = 10 μm. **Figure 4b**. *In vivo*. Detail of mature plaque in Figure 4a, showing the complicated mesh-like arrangement of filamentous microorganisms and cocci. In the foreground, arrows indicate several macrophage-like cells. Bar: 10 μm.

Figure 5a. *In vivo*. Low magnification view of replica of a mandibular incisor with gingival recession, but clinically no loss of cervical contour. The replicated gingiva was trimmed away to allow unobstructed viewing of the tooth surface in the sulcus. Arrows indicate limit of replicated tooth structure. Bar = 100 μm. **Figure 5b**. *In vivo*. Higher magnification of exposed root surface in Figure 5a. The surface is very irregular. Microorganisms, indicated by arrows, colonize mainly the craters. Bar = 10 μm.

Discussion

The results confirm that the replication method is suitable for documenting the morphology of the cervical hard tissues. It is simple and hygienic and artefacts have been minimized. Multiple replicas may be made from the same impression and the tray system facilitates reproducibility. The selection of the addition silicone elastomer impression material was based not only on favorable evaluations of its application for SEM replication (Lambrechts *et al.*, 1981; Ekfeldt *et al.*, 1985; Walsh and Basu, 1987) but also on the documented dimensional stability and excellent handling properties in clinical dentistry (Phillips, 1982).

Techniques requiring only equipment available in a modern dental surgery and laboratory were preferentially selected. Lambrechts *et al.* (1981) used an addition silicone elastomer impression material (President) which was subsequently electroplated and then viewed directly in the SEM, claiming excellent detail up to magnifications of 7500X. However, special laboratory equipment is required for electroplating and other researchers have been unable to duplicate the excellent results reported above, describing surface porosity in electroplated dies (Walsh and Basu, 1987).

Epoxy resins are commonly used for SEM replication, and the physical characteristics are well documented. The main advantages of Epoxy-die, the material selected for the present study, are compatibility with the addition silicone elastomer materials and relative ease of handling, with a minimum of equipment. The quantities of the components and the mixing procedures are individually specified for different size impressions, minimizing variations in quality with multiple pourings. The viscosity may be varied by the addition of thinner. The manufacturers state that setting contraction is 0.05%. The flexural strength is high, 75 N/mm^2. The rigidity and high edge strength of the set material permitted trimming with rotating instruments, sectioning with a hand saw and fracturing. The heat resistance of the material (120°) was also a positive factor. High beam tolerance allowed prolonged examination in the SEM. The opacity of the set material was also an advantage when viewing the replica against a dark background in the light microscope: debris was easy to see and surface detail was not obscured by internal air voids, as occurs in transparent epoxy resins.

Apart from faithful reproduction of surface detail, the method meets other important criteria for replication of biological specimens. Because of increasing awareness of the risk for infection to laboratory personnel, requirements for laboratory disinfection of impressions are becoming increasingly stringent (for review, see Bergman, 1989). Recent investigations by Peutzfeldt and Asmussen (1990) have confirmed that ability of addition silicone elastomeric impressions to reproduce surface detail is not adversely affected by the laboratory disinfection procedures described in this study.

As each stage of replication was evaluated, the importance of standardizing procedures was given priority, e.g., delivery of the impression material and the tray systems *in vitro* and *in vivo*.

The clinical advantages of an automixing delivery system for addition silicone elastomer impression materials have been described by Craig (1985) and Keck (1985). In this study, President Jet, the recently introduced automixing delivery system, was a marked improvement on the manual spatulation technique used in pre-study trials: the problem of incorporation of air bubbles during spatulation was eliminated and a uniform quality of impression material was ensured.

Particularly for the *in vivo* impressions, where maintenance of a dry field was difficult, the automixing system minimized delay between initial application of the impression material and superimposition of the tray.

Attachment of the extra fine nozzle to the impression syringe greatly facilitated the initial insertion of the impression material, both in *in vitro* and *in vivo*.

The copper band proved suitable as a tray for the *in vitro* impressions, ensuring uniformity of thickness of the impression material and providing a readily available, standard tray system for repeated impressions.

In vivo, clinically acceptable impressions may be obtained by the application of light body addition silicone material, supported by hand-kneaded, high-viscosity "putty" materials in stock trays. However, it is doubtful that the criteria for clinically acceptable accuracy and the quality of surface reproduction of prepared tooth structure are relevant at SEM levels of magnification. Techniques offering the greatest clinical accuracy are therefore self-evident.

Phillips (1982) recommended the custom tray for clinical impressions because an even thickness of impression material resulted in minimal shrinkage. Custom trays have been shown to give greatest accuracy, especially for pouring multiple impressions (Johnson and Craig, 1986; Tjan and Whang, 1987; Gordon *et al.*, 1990). For longitudinal studies, the custom tray contributes to reproducibility and aids orientation to landmarks. Earlier disadvantages, e.g., that tray fabrication was time-consuming and involved contact with allergogenic materials, have been overcome by the introduction of the light-cured acrylic tray kit. A recent evaluation by Wirz *et al.* (1990) has shown that trays made by this method are superior to autopolymerized trays with respect to stiffness, form and volume stability, have the required physical properties for accuracy and strength and are not subject to distortion in moisture.

The scavenger impression, used in some, but not all, earlier clinical replication studies, was not initially considered necessary. However, in a preliminary comparison of replicas made from scavenger impressions with replicas from a second impression as described in the method, regions in which the deposits had adhered to the scavenger impression, particularly in the gingival region, were difficult to interpret on the first replica, appearing patchy or smeared.

Various methods of cleaning tooth surfaces prior to impressions for replication have been described. In animal studies of occlusal wear, Teaford and Oyen (1989) swabbed the teeth with 3% sodium hypochlorite followed by a Water Pik; in human studies 3% sodium hypochlorite (Vossen *et al.*,1985) or sodium hypochlorite followed by hydrogen peroxide (Lambrechts *et al.*, 1981) has been applied to occlusal surfaces. Absi *et al.* (1989), in a study of dentinal hypersensitivity, applied 1% sodium hypochlorite to exposed cervical dentine to remove organic material and facilitate flow of the impression material into the dentinal tubules. As the teeth were extracted after impression-taking, the question of associated iatrogenic damage to the teeth or adjacent soft tissues did not arise. In the present study on healthy teeth, however, it was important that the cleaning procedures did not damage the exposed root surfaces and Tubulicid was therefore selected. An antimicrobial dentine conditioner containing 0.2% EDTA, Tubulicid is widely used not only in adhesive restorative dentistry for cleaning cut dentinal surfaces, but also for research purposes. It removes the smear layer, i.e., plaque, bacteria and cutting debris, without opening or widening the dentinal tubules (Brännström and Johnson, 1974; Brännström *et al.*, 1980). This factor is of particular importance for *in vivo* impressions of the root surface, where dentine may be exposed: Barnes (1979) has suggested that if the tubule apertures are widened, the contents may be aspirated during removal of the impression. Another property of particular relevance in taking *in vivo* impressions of the cervical region is that Tubulicid is non-irritant to the gingival tissues (Brännström, personal communication; van Dijken and Hörstedt, 1987).

Initially, the *in vitro* impressions were taken without any surface conditioning of the specimens. However, when the advantages of the surface-tension reducing treatment *in vivo* became apparent, this step was also included in the *in vitro* method.

The bench curing time for the impressions and the undisturbed setting time for the replicas were standardized. Pre-study trials with shorter bench curing of the impression, e.g., 3 hours, resulted in a replica with a "tacky" surface or a laminated appearance in the SEM. The setting time for the epoxy resin varied according to the size of the impression; attempts to remove the small *in vitro* replicas from the copper band impressions after only a few hours deformed the replica.

An interesting observation from the *in vitro* studies was that in specimens of relatively smooth surfaces and low porosity, a second pouring of the replica resulted in a very "clean" surface compared to the first replica. It is possible that despite prolonged bench curing of the impression, some degassing still occurred during setting of the first replica (Gordon, 1984), with the formation of a deposit at the impression-epoxy interface: after removal of the first replica, careful washing of the impression and brushing with a microbrush was necessary to achieve an optimal surface on the second replica. No such problem arose during pouring of subsequent replicas. As the impression material is dimensionally stable for at least a week and the trays are robust and not distorted by removal of the replica, multiple pours at 24 hour intervals are possible.

The observation in this study that tiny, irregular protuberances such as the cauliflower-like enamel cap in Fig. 3 may entrap and tear the impression material, may limit application of the method. Similar morphology might for example occur on etched enamel, on worn composite restorations, in enamel hypoplasias and at the margins of amalgam restorations. In studies of dental replication techniques, with the notable exception of Barnes (1978, 1979), artefact formation and interpretation are seldom considered.

During the present study, the question of an adequate control for comparison of *in vivo* replication of normal tissue has arisen. In earlier studies, replicas were made from impressions taken immediately prior to extraction and the extracted teeth were subsequently prepared for SEM as controls (Absi *et al.*, 1989). During extraction, however, application of the forceps traumatizes the root and induces fractures in the cervical enamel. As noted in the *in vitro* test in the present study, subsequent steps in preparing the specimen for SEM may cause further cracking, particularly in the amelocemental region. An animal model, comprising impressions (Teaford and Oyen, 1989) and atraumatic extraction procedures (Garnick and Dingle, 1988) under general anaesthesia, is currently under evaluation.

Although replica techniques are well-established, there is at present no "general purpose" method. The cervical region is of increasing concern in clinical dentistry: there is an urgent need for improved understanding of disease processes in the region. The replication method presented in this study is a simple, reproducible, non-invasive means of documenting micromorphological alterations to the hard tissues of the region. It is currently being applied *in vitro* to document the micromorphology of the amelocemental junction in

premolars and *in vivo* to monitor progression of wedge-shaped cervical defects in young adults.

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Discussion with Reviewers

I. Barnes: Why were the extracted teeth treated with hypochlorite? This is a destructive procedure and may well tend to superficially disorganize the surface of the cementum.

Authors: The specimens in this study were subjected to sodium hypochlorite treatment to decontaminate the tooth and to remove organic matter. An extracted tooth is contaminated by blood and saliva and a potential source of infection, particularly if rotary instruments are to be used during specimen preparation (Pantera and Schuster, 1990, additional references, see next page). Sodium hypochlorite is recommended by the Centers for Disease Control for decontamination of environmental surfaces. Coronal surfaces of extracted teeth have been sterilized by immersion in dilute sodium hypochlorite for 5 minutes (Pantera *et al.*, 1988, additional references, see next page).

The cervical region of an extracted tooth also retains remnants of periodontal tissue. The young premolar teeth in this study, extracted for orthodontics, were periodontally healthy, with an abundance of firmly attached tissue remnants in the amelocemental region. In initial trials it was found that immersion of the tooth in 10% sodium hypochlorite for twenty minutes was necessary to remove the tags of soft tissue. By comparison, the *in vitro* study of the cemento-enamel junction by Schroeder and Scherle (1988) was based on teeth immersed in 5% sodium hypochlorite for 8-10 hours.

The effect on the enamel of unerupted teeth of preparation techniques for SEM has been evaluated by Fejerskov *et al.* (1984). Specimens given short-term treatment with 5% sodium hypochlorite (10 minutes in 5% NaOCl) retained varying degrees of organic surface coating. The surfaces of specimens immersed for 16 or 24 hours in 5% NaOCl were free of coatings but porosity and crumbling were noted in some areas. Jones (1987) has indicated that NaOCl treatment of the root surfaces not only removes attached tags of periodontal tissue but also the unmineralized precementum. Clearly the effect of each step in specimen preparation must be taken into account in interpretation of the SEM appearance of the specimen, particularly in the cervical region where enamel, dentine and cementum are so closely apposed. Procedures for decontaminating extracted teeth and removing attached soft tissue warrant further attention.

A. Beynon: The impressions were disinfected with glutaraldehyde which is a highly reactive chemical. May this have an effect on the surface of the impression material?

Authors: Impressions taken in the Departments of Clinical Dentistry at the Karolinska Institute are routinely subjected to the disinfection procedures described in the present study. With the steadily growing frequency of AIDS impressions must be considered potential contamination pathways and the risk of transmission of infection to laboratory personnel cannot be discounted. Peutzfeldt and Asmussen (1990) studied the effect of disinfecting solutions on the surface texture of elastomeric impressions. Immersion of addition silicone elastomeric materials in 2% alkaline-buffered glutaraldehyde for up to an hour caused no deterioration in the quality of the impression with respect to reproduction of fine detail, as assessed by the surface roughness test.

A. Beynon: The authors describe using a scavenger impression to remove surface debris. Silicone rubbers are highly hydrophobic, and it is not to be anticipated that they would necessarily adhere to and remove the surface deposits from teeth. Indeed Figures 4a and 4b of scavenger impressions show microorganisms as elevations on the replica, which implies that they have been replicated and the original material remained on the tooth surface following removal of the impression (i.e., they are not extractive impressions). Please comment.

Authors: Although the impression material is hydrophobic, adherent debris can be seen under the light microscope! We interpreted Figs. 4a and 4b as indications that mature plaque (and other very adherent deposits) had not been disturbed by the scavenger impression and that the patchy, rather smeary background in these figures represented areas denuded of deposits which had adhered to the scavenger impression. Any impression which removed mature plaque might also damage delicate surface structures; Figs. 3 (*in vitro*) and 4 (*in vivo*) indicate that the risk for such iatrogenic damage is small. Alternate methods of "cleaning" the cervical region of the teeth, e.g., pumice in a rubber polishing cup (Christensen and Bangerter, 1987, additional references, see next page), have been shown to be

potentially damaging and at best "leave loose dentine on the root surface and pumice particles embedded in the dentine".

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