RepliSet: **High Resolution** Impressions **of the Teeth** of Human **Ancestors**

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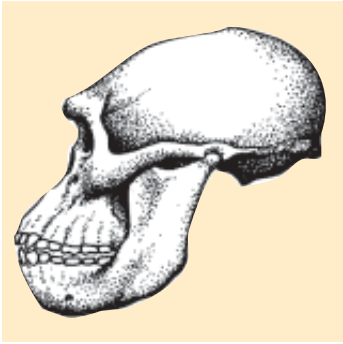


Introduction

As a physical anthropologist who studies the teeth of early human ancestors, the first author needs a high-resolution impression material that can be safely applied to fragile fossil tooth surfaces. Furthermore, the impression material must be stable under the electron beam of a scanning electron microscope (SEM).

Before traveling to Kenya and South Africa last Summer (2001) to make impressions of 3- 4 million year old fossil teeth, the first author contacted Struers about their RepliSet product. Both authors then conducted several experiments with RepliSet to test its safety for use on tooth surfaces, and to determine its resolving ability when different impression techniques are employed.

These experiments convinced us that RepliSet is an excellent choice for making impressions of fossil teeth, and the first author indeed used RepliSet for this purpose last Summer.

This Summer (2002), the first author will travel to Tanzania and Ethiopia to make more impressions of fossil teeth, and will again be using RepliSet. In this article, we describe the experiments we conducted prior to the first author's 2001 trip to Africa. These experiments document the usefulness of RepliSet for making high-resolution impressions of tooth surfaces.

Background and Research Questions

Hillson (1992) made several important observations and recommendations regarding methods for making impressions and replicas of tooth crown surfaces. He endorsed Beynon's (1987) two-stage impression technique in which: (1) an initial mold is made of a tooth tightly wrapped in aluminum foil, (2) the foil is removed after the mold hardens, and (3) Coltène President Light Body impression material is "introduced into the mould so that…impression material" flows "round the gap left by the foil" and is "forced into the fine surface detail" (1992:68). As Hillson further described, Beynon's Spurr resin replicas made from the impressions resolved "parallel sided depressions" (Beynon 1987) of widths as small as 0.2 microns. Hillson did not recommend direct examination of the impressions for most purposes, primarily because of "cracking and crazing" of the coated impression surface and because the steep "upcurved" sides of the impressions impede the view of the impression surface (1992:62).

Since the time of Beynon's (1987) and Hillson's (1992) writings, Coltène President Light Body impression material has become widely used by dental anthropologists. However, in 2001, Struers introduced a fast-curing silicon rubber impression material called RepliSet designed for use in engineering inspection applications (of bridges, aircraft, etc.). Struers advertised this material as being SEM-stable as well as dimensionally stable, leaving no residue, and having a resolution of 0.1 microns. RepliSet comes in two forms: a thixotropic one with high viscosity, suitable for use on sloping surfaces, and a less viscous form recommended for usage on flat, horizontal surfaces. Through our experiments, we sought answers to the following specific questions:

1) Can the RepliSet material be imaged directly without the "cracking and crazing" noted for Coltène President Light Body?

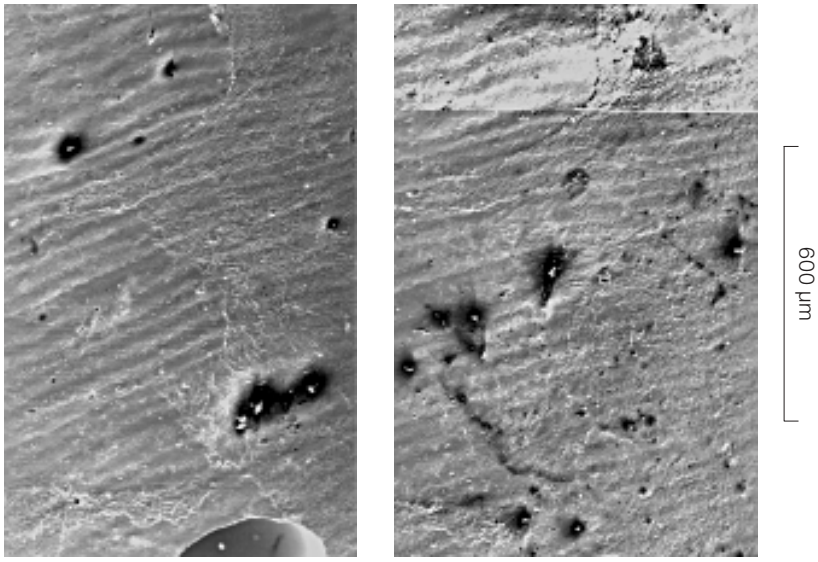
2) Is there a difference in quality between replicas made from Coltène President Light Body impressions and replicas made from RepliSet impressions?

3) Is there a difference in quality between impressions made from the high versus the low viscosity RepliSet materials?

Materials and Methods

Impressions were made of a single human upper third molar using: Coltène President Light Body, RepliSet F-5 (low viscosity) and RepliSet T-3 (high viscosity). Hillson (1992) cautions that fragile fossil specimens are not suited for the two-stage technique, which exposes specimens to mechanical stress, especially when the mold is removed. He thus recommends direct application of impression material on fragile specimens. Therefore, in this study, both "two-stage" and direct application impressions were made. "Two-stage" impressions were made in a manner similar to that described by

Figure 1: Replica made from a RepliSet impression compared to a replica made from President Light Body impression; Taken at 100 x



RepliSet replica *President Light Body*

Beynon (1987), using an initial mold, an aluminum foil spacer, and then the impression material. The direct application impressions are termed "single-stage" impressions in this study. Replicas were made using Epofix epoxy.

All samples were sputter-coated with a gold-palladium alloy, with a final coating thickness of 40 nanometers. A 2-nanometer per minute deposition rate was pulsed onto the samples at one-minute intervals, with one minute of recovery time after each pulse. The recovery period was necessary to prevent heating of the sample in the coating plasma. Epoxy replicas were adhered to stubs using carbon tape, while the RepliSet impressions required graphite adhesive paint for proper adhesion.

Images were taken using a JEOL JSM-820 Scanning Electron Microscope, with a working distance of 20 mm, an accelerating voltage of 12keV, and 0° tilt. An effort was made to maintain a consistent orientation of specimens to the detector during each SEM session. Micrographs were taken at magnifications often used in studies of fossil teeth (at 100x and 500x). In most cases, montages were made in order to obtain images of the same tooth areas for direct comparison.

Results

1. *Can the RepliSet material be imaged directly without the "cracking and crazing" noted for Coltène President Light Body?*

Yes: most of the time. Not only in the samples used for this study, but also in samples used in a study of hominin teeth (Guatelli-Steinberg, in press) the coating on the RepliSet material cracked infrequently. As can be noted in Figures 4,5, and 6, there is no discernable problem with cracking in the coating. Hillson (1992) notes that cracking may be due to "heating in the sputter coater and in the microscope," or by "slight pressure exerted during handling" (1992:69). In the few cases where coating did crack or craze, (samples used in a different study), it was usually the case that pressure had been applied to the specimen after coating in order to re-affix a loose specimen to its stub. Thus, heating in the coater or the microscope was not a problem here, using a low coating deposition rate, an accelerating voltage of 12 keV, and a relatively low beam current.

2. *Is there a difference in quality between replicas made from Coltène President Light Body impressions and replicas made from RepliSet impressions?*

Yes. Figure 1 compares micrographs of a replica made from a two-stage Light Body impression

Figure 2: Enlargement of areas in figure 1. At left replica made from RepliSet impression; at right replica made from President Light Body impression.

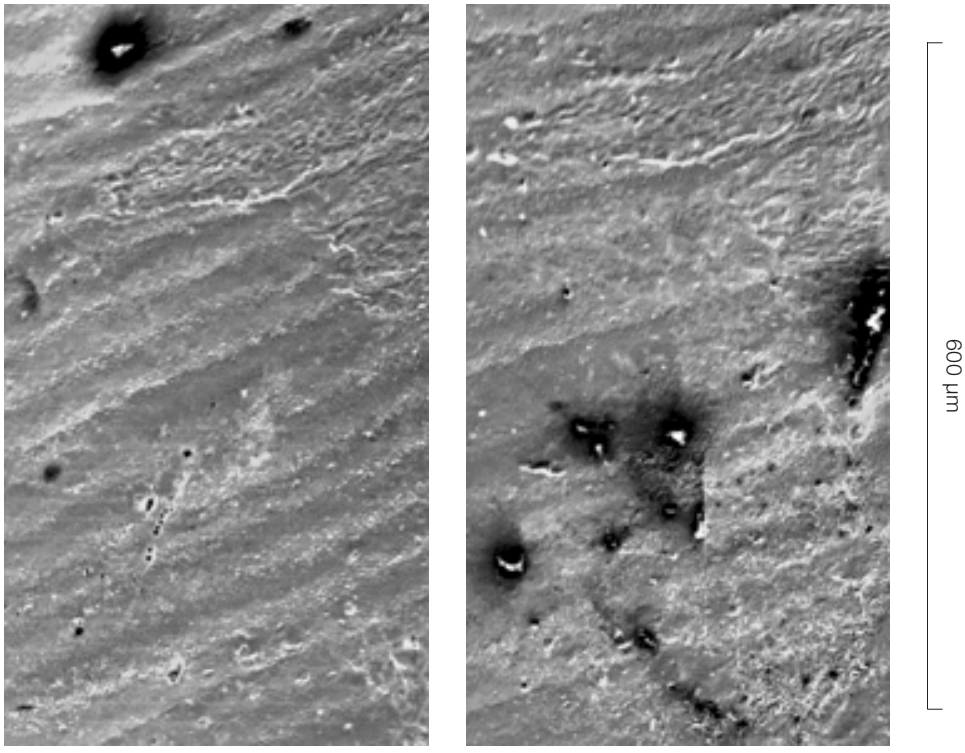
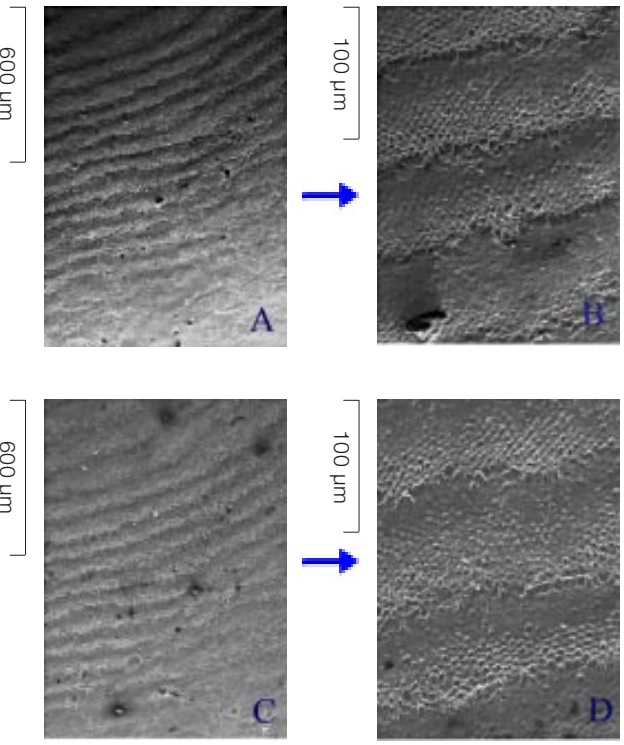


Figure 3, below:
A and C are micrographs from the same region taken at 100 x;
A is a replica made from a RepliSet impression; C is made from a President Light Body impression.
B is a micrograph from the region of A taken at 500 x;
D is a micrograph at 500 x from the region of C.



with a replica made from a two-stage RepliSet impression from the same area of the tooth taken at 100 times. Figure 2 is an enlargement of part of the area in Figure 1. Figure 3 compares micrographs taken at 100x and 500x: unfortunately, in this case, while the areas taken at 100x on the tooth are identical, those taken at 500x are not. However, the micrographs taken at 500x are within the region shown at 100x. Note that in all figures, the replica made from the RepliSet impression provides superior detail.

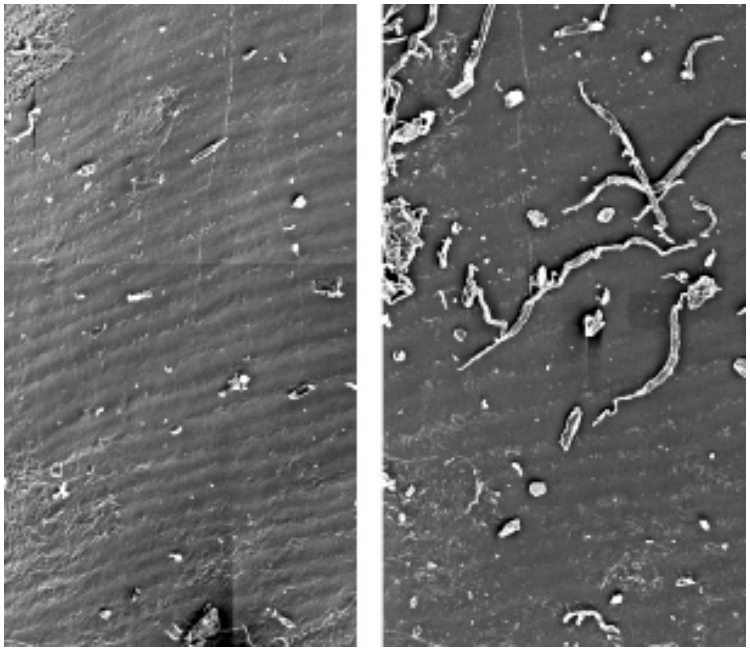
3. Is there a difference in quality between impressions made from the high versus the low viscosity RepliSet materials?

Yes, for single-stage impressions. No, for two-stage impressions. Figure 4 compares a single-stage F-5 RepliSet impression (low viscosity) and a single-stage T-3 RepliSet impression (high viscosity) from the same area of the tooth, taken at 100 times. The F-5 material is very flimsy and loses not only the overall tooth shape but also the topography of the perikymata. Figure 5 compares a two-stage F-5 RepliSet impression and a two-stage T-3 RepliSet impression form the same area of the tooth, taken at 100 times. Figure 6 is a close-up of part of the area in Figure 5. The quality of these impressions appears to be very close.

Discussion

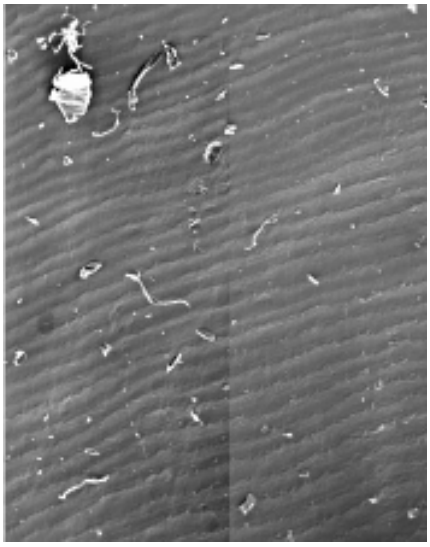
Hillson (1992) noted that cracking and crazing often afflict Coltène President Light Body impression surfaces examined under the SEM. Struers recommends a low accelerating voltage of 2 keV (www.struers.com), however, RepliSet impressions hold up well under an accelerating voltage of 12keV, with infrequent cracking or crazing of the impression surface. The superior performance of RepliSet under an electron beam suggests that for investigations in which complications are not introduced by examining a "negative" impression of the tooth surface, the standard additional time-consuming step of making an epoxy replica can be omitted. A further advantage of examining the impressions themselves is that they may preserve fine-scale detail better than replicas made from them; however, the present study did not explore this possibility.

This study presents preliminary evidence that replicas made from RepliSet provide higher resolution than those made from Coltène President Light Body; however, micrographs of additional samples would help to make a definitive assessment. Finally, the low and high viscosity materials were compared: using first a single-stage technique that is necessary on fragile specimens, and a two-stage technique that can be used

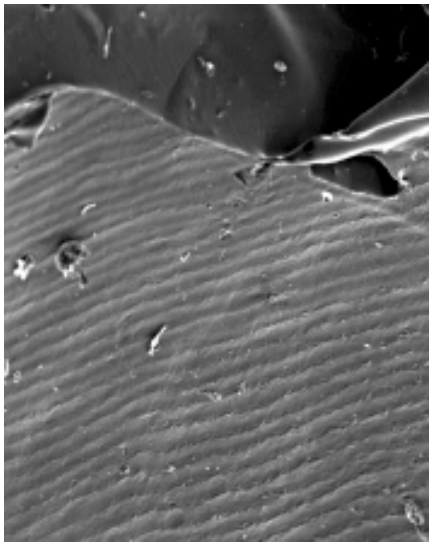


Single-stage T-3 Single-stage F-5

Figure 4: Single-stage T-3 impression compared to single-stage F-5; Taken at 100 x



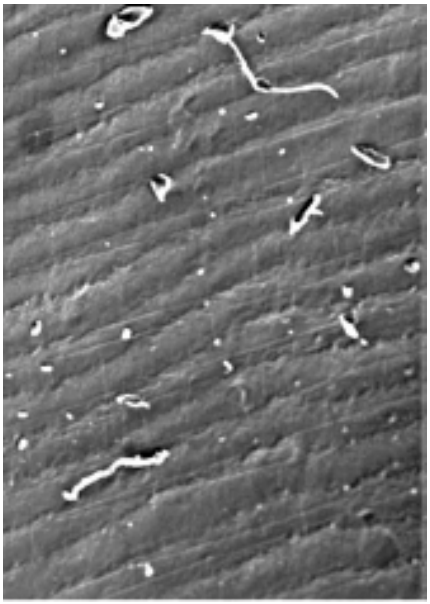
Two-stage F-5



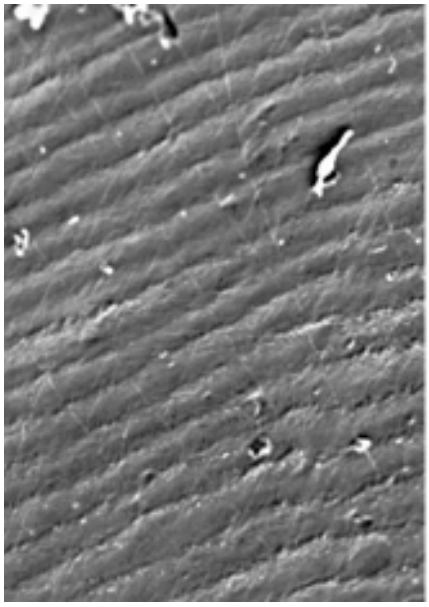
Two-stage T-3

Figure 5:
Two-stage F-5 vs. two-stage T-3 impressions. Taken at 100 x.

600 µm



Two-stage F-5



Two-stage T-3

600 µm

Figure 6: Enlargement of areas from figure 5.

on more robust specimens. If a single-stage technique is to be used, the thixotropic, higher viscosity substance, T-3 is superior. The higher viscosity substance allows the impression to retain the tooth shape and the tooth's surface topography. However, if the two-stage technique is used, there is little difference in the resolution obtainable by the low vs. the high viscosity compound.

Why there should be a difference in the resolving ability of the Coltène President Light Body and RepliSet compounds is not clear at the present time. The former belongs to a class of impression materials known as polyvinyl siloxanes, which are "addition reaction silicone elastomers" that were "first introduced in the 1970's" (Mandikos, 1998:428). Beynon (1987) reported a resolution of 0.2 microns for Coltène President Light Body, while Mandikos (1998) states that the very low viscosity polyvinyl siloxanes can reproduce lines that are 1-2 microns in width. Struers describes RepliSet as a "silicon rubber" compound, with a resolution of 0.1 microns. These reported differences in resolving ability, with RepliSet higher than Coltène President Light Body, are consistent with the differences in resolution found here.

Based on this study, the authors recommend the RepliSet material over Coltène President Light Body material for two reasons: the former can be coated and viewed directly under the SEM and apparently has better resolution than the latter when epoxy replicas are made from two-stage impressions. On fragile fossils, the higher viscosity, thixotropic T-3, substance is superior to the lower-viscosity substance.

Acknowledgments

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