Video Article

Mimicking and Measuring Occlusal Erosive Tooth Wear with the "Rub&Roll" and Non-contact Profilometry

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Abstract

Chewing, drinking, and occasional tooth grinding will result in physiological tooth wear during a lifetime. Extreme challenges, such as bruxism or habitual chewing on foreign objects, may lead to excessive wear. Recently, the role of erosion in accelerating mechanical tooth wear has been recognized, but the interplay between chemical and mechanical wear processes has not been extensively studied. Our laboratory recently introduced a novel oral wear simulation device, the Rub&Roll, that enables the user to perform wear and loading studies separately or simultaneously in an erosive and/or abrasive environment. This manuscript describes an application of the device: the combined mechanical and erosive loading of extracted human (pre)molars in a simulated chewing movement, with a controlled application of force, velocity, fluid, and time, and the application of non-contact profilometry in visualizing and measuring the resulting wear pattern. The occlusal morphology that was created in the experiment with the highest loading level is very similar to the clinical presentation of erosive wear.

Video Link

The video component of this article can be found at https://www.jove.com/video/56400/

Introduction

The oral cavity could be considered a harsh environment: humidity, temperature changes due to hot and cold food intake, and mechanical loading with some of the strongest muscles in the human body. Teeth, however, are eminently equipped to withstand these challenges. The enamel is very hard, and the dentin underneath prevents the relatively brittle enamel from fracturing. The mineral component of both materials, hydroxyapatite is of very low solubility and in equilibrium with the supersaturated saliva. Chewing, drinking, and occasional tooth grinding will result in physiological tooth wear during a lifetime[1,2,3]. Extreme challenges, such as bruxism or habitual chewing on foreign objects, may lead to excessive wear. Recently, the role of erosion in accelerating mechanical tooth wear has been recognized. Tooth erosion has been extensively studied *in vitro*, but the models used have generally been simple, and mechanical factors have largely been ignored. The clinical interplay between chemical and mechanical wear processes is therefore not fully understood[4].

Many *in vitro* erosion and erosive wear studies have used simple acid immersion of flat polished enamel or dentine samples, using hardness loss or profilometry as the measurement approach[5]. The introduction of an abrasive component has usually involved tooth brushing action, or sometimes tongue or enamel cusp sliding contacts[6]. Such studies have shown that enamel erosion results in a softened surface layer, which is easily abraded. Flat surfaces are usually needed because the mechanical loading device cannot handle uneven surfaces, and the measuring techniques for uneven surfaces are also more complicated. However, most erosive tooth wear in adolescents is seen on occlusal cusps, and abrasion by chewing food is expected to be the most relevant mechanical factor in occlusal erosive wear. The ideal oral wear machine that mimics the oral environment in all details does not exist, and most *in vitro* models will not allow for natural occlusal surfaces of teeth to be either exposed or measured[7,8].

Our laboratory recently introduced a novel device, which conforms to many of Heintze's[7] specifications and tolerances of oral wear simulation models, and that enables the user to perform wear and loading studies separately or simultaneously in an erosive and/or abrasive environment. The new device (Rub&Roll) consists of a stirring machine and a container (**Figure 1a).** In the container, a cylinder with specimens can be mounted. Between the cylinder and the inner wall of the container one of more rods are placed (**Figure 1b**). By starting the stirring motor, the rod rotates over the specimens in the cylinder (**Figure 1c**). Using shims, different forces can be applied on the specimens. For a comprehensive description of the design, construction, operation mechanism, and features of the device refer to the paper introducing and discussing the device[9]. The device is robust, not technically demanding, and can apply loads to 32 specimens simultaneously. The antagonist force is moving over the specimen surface while maintaining smooth, continuous contact, which is comparable to normal chewing[10]. Here we present an application to model erosive wear of the occlusal surfaces of natural teeth, and we demonstrate the clinical relevance and versatility of the method.

Protocol

Collection of the teeth used in this experiment was performed in accordance with the guidelines of the regional Medical Ethics Committee.

1. Specimen Collection and Sample Preparation

1. Collect 24 sound extracted human (pre)molars from dental practices, and brush with pumice in a low-speed handpiece to a clean (no debris, no gingival remnants) and smooth tooth surface, and finally rinse for 15 s under running tap water.
2. Embed the samples to make them fit into the recesses (12 mm x 15 mm x 27.5 mm) of the cylinder of the device.
    1. Melt the impression compound (one stick of 113 g) on a hotplate (50 °C) for approximately 10 min and dip the occlusal part of the molar in the molten substance, covering the occlusal surface. Place the molar upside down on a microscope slide, and press down until all cup tips touch the glass and wait ± 30 s until the impression compound has cooled and set, fixing the tooth.
    2. Use a syringe to pour 10 mL of polymethylmethacrylate (PMMA) mixture into a silicone mold with inside dimensions of 12 mm x 15 mm x 27.5 mm. Mix PMMA in a fume hood (ratio 13 g polymer: 10 mL monomer) for roughly 25 s using a spatula. Leave to stand for 15 s so that any air bubbles can escape before using.
    3. Turn the microscope slide upside down and suspend the molar in the silicon mold filled with PMMA. Press the slide down until it touches the mold. The pouring phase lasts approximately 1.5 min at room temperature.
    4. After setting of the PMMA at room temperature and 1,000 hPa for 20 min, remove the microscope slide and remove the embedded tooth from the silicon mold.
    5. Measure the total height of the embedded molar and adjust the height to exactly 27.3 mm by incrementally removing the cured PMMA from the bottom with a milling machine equipped with a milling cutter of 16 mm.

2. Preparing Demineralization Solution

1. Add 0.1 M lactic acid (50 g), 1.5 mM $CaCl_2$ (1.103 g), 0.9 mM KH_2PO_4 (0.612 g), 10 mL 1% chloramine, 0.5 ppm F (2.5 mL of 1,000 ppm Fluorid Standard solution) to 4,900 mL of deionized water on a stirring plate.
2. Titrate with 10 M KOH (± 50 mL) to pH 4.8 with calibrated pH glass electrode.

3. Sample Mounting and Machine Settings of the Rub&Roll (Figure 1)

1. Remove the cylinder from the container and place 24 specimens in the recesses in the cylinder of the Rub&Roll.
2. To adjust loading force, adjust the protrusion of the specimen from the cylinder by placing a shim in the recess underneath the specimen. For no load (0 N) of 8 specimens, use no shim, and for 30 N (8 specimens) and 50 N (8 specimens), use a shim of 1 mm and 1.5 mm, respectively.
3. Mount the 2 parts of the cylinder and secure it with a M6 bolt and place the cylinder in the container.
4. Fill the container with 500 mL demineralization solution.
5. Place the loading rod: a PVC tube (Hardness 73 Shore A) with an outer diameter of 14 mm and an inner diameter 10 mm with an insert of a stainless steel 316 rod (Hardness 130 - 150 HB) with a diameter of 9 mm.
6. Set the rotation speed to 20 rpm, to simulate clinical chewing frequency, and let the device run uninterruptedly.
7. During the experiment, replace the demineralization solution and PVC tube, and check the pH with a calibrated glass electrode twice a week.
8. After 3 months (corresponding to about 1,500,000 cycles) disconnect the cylinder from the container and remove the specimen by disassembling 2 parts of the cylinder, and store the specimen in deionized water.
   NOTE: All specimens are scanned before and after loading in the Rub&Roll, using a non-contact profilometer.

4. Profilometric Scanning, Analysis, and Subtraction

1. Generate a topographical measurement of the sample using a profilometer.
2. Switch on equipment: computer, PSU module, and sensor controller. Place the appropriate sensor and secure with thumbscrew. Then, carefully insert the optical fiber in the sensor controller.
3. Select the correct sensor on the sensor controller. The sensor controller will show 4 options (F1 - F4). Press F4 twice and the confocal sensor menu is displayed.
    1. Press F3 (sensor choice) and scroll to 2 - 10,000 µm. Select (10 mm) and press F4. Press F1 and choose F4 (yes) to save settings to EEPROM. Select LED intensity and turn to position "± 9 o'clock" and press F4 - F2 - F4 taking a "dark reference" of the sensor.

4. Open the software and choose the option "connect" to the device. Be aware that the measuring table will automatically move to the "home position" search. The home screen appears on the display. Press Tools in the menu bar followed by sensor selection and choose Sensor S29 | 10 - 10,000 µm.
    1. Press Tools in the menu bar followed by sample rate and choose 300 Hz. Press Tools in the menu bar followed by speed sensor and choose 0 - 100%.

5. Select Scan in the menu bar. Then select Key Move Stage. Press the yellow area in the middle of the screen to move the measuring table to the center.
6. Position the specimen in the center of the measuring table, followed by setting the correct height within the range of the sensor. Place the sensor above the area of interest of the specimen and adjust the distance of the sensor in such a way that the complete sample area to be

scanned is located within the focus range of the sensor. The sensor controller indicates whether the height is within the range of the sensor showing a green area in the live data height.

7. Choose Settings. Set the average to 2, to ensure each recorded data point is the average of 2 measurements. This will slow down scanning speed, but increase scanning quality. After finishing the settings press OK to return to main scanning setup.
8. Position the sensor beam on the upper left corner of the specimen. Set the total scan area to 15 mm x 12 mm with a step size in X and Y direction of 40 µm (0.04 mm), number of steps in X = 375 and Y = 300. Again, smaller steps will increase scan resolution, but also scanning time. Press Scan now to start scanning.
9. When the scan is finished after about 10 min, select File in the menu bar, followed by choosing Save As. The scans are standardized in such a way that the scales are always on the same levels.
10. Select File in the menu bar, followed by choosing Open file. Select Warpage in the menu bar. Apply a warpage filter of 1 to eliminate the noise of the scanning table and sensor. Select Highest point in the menu bar, and find the highest point on the molar.
11. Select Tools in the menu bar, followed by choosing option-scale to the scan configuration. In the scan configuration, set the offset in mm calculated by Z value highest point (measured in 4.10) - 3500. Set the Range in manual, from 0 to 3.6 mm, and press OK.
12. Select Load Area in the menu bar to reset the scale. Select File in the menu bar, followed by choosing Save As.
13. Subtract scans taken at two different moments in time.
    1. Select Open in the menu bar in the software. Locate the original scan and the modified scan file in the directory, select files, and press OK. The Options screen will appear, choose in the leveling option: manual leveling option; and in the compensation offset the options respectively, apply to original and apply to modified.
    2. Select Window in the menu bar, followed by the option create view and finally the option cross section view.
14. Select the modified surface and move the modified surface over the original surface in horizontal, vertical, and Z direction (height) by holding the control key and press the left and right arrow key for horizontal direction; holding the control key and press up and down arrow key for vertical direction; holding the shift key and press up and down arrow key for Z direction in such a way that the subtracted volume and height shown in the difference view are as low as possible.
15. Select File in the menu bar, followed by choosing Save As. Output is read as mean volume loss and mean height loss.

Representative Results

We exposed human molar teeth (n = 8 per group) to an acidic aqueous solution at pH 4.8 in the Rub&Roll, for 3 months. This corresponds to a clinical functioning time of about 6 years. The mechanical load applied was: no load (0 N), 30 N, or 50 N.

Mean occlusal surface height loss for the three groups was: 76 ± 20 µm for 0 N; 161 ± 40 µm for 30 N; and 266 ± 101 µm for 50 N (**Figure 2**). The erosive wear with the mechanical loading resulted in saucer shaped lesions on the occlusal cusp tips closely resembling the clinical phenomenon associated with erosive tooth wear called "cupping" (**Figure 3** and **Figure 4**).

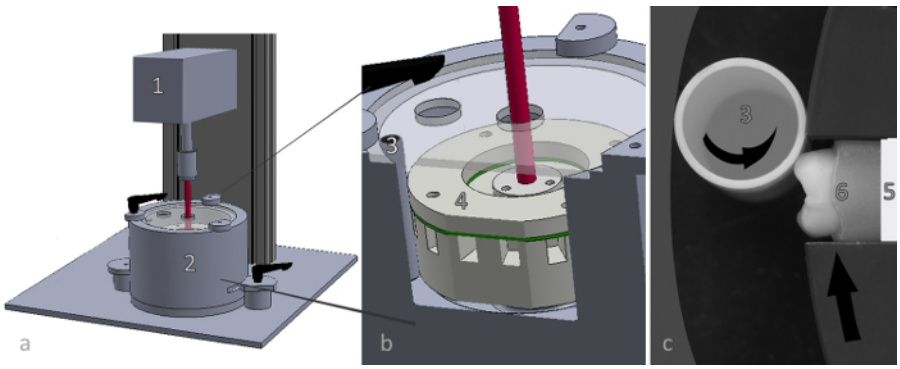

Figure 1. Schematic presentation of the Rub&Roll. (**a**) Overview of the apparatus: 1. stirring motor, 2. Container. (**b**) Inside view of container: 3. rod, 4. cylinder. (**c**) The rod contacting the specimen and the outside of the container: 3. rod, 5. Shim, 6. Embedded molar. Please click here to view a larger version of this figure.

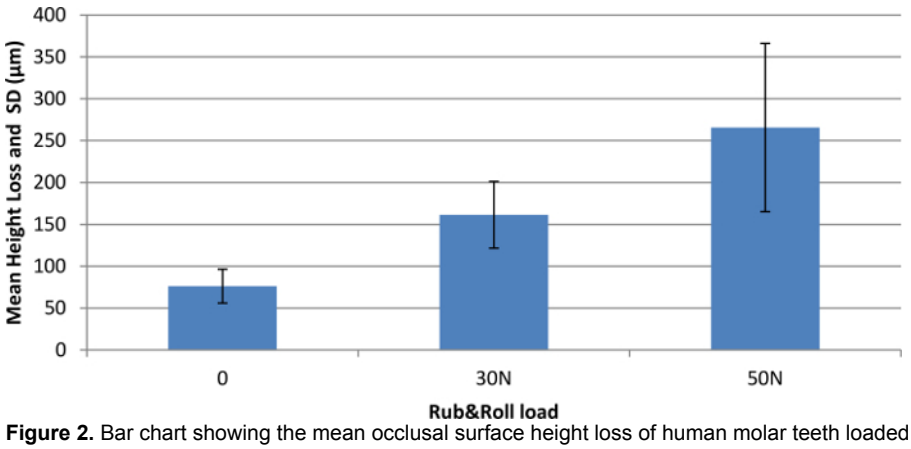

Figure 2. Bar chart showing the mean occlusal surface height loss of human molar teeth loaded with 0, 30, or 50 N in a demineralization solution at pH 4.8 using the Rub&Roll device. Error bars indicate SD. Please click here to view a larger version of this figure.

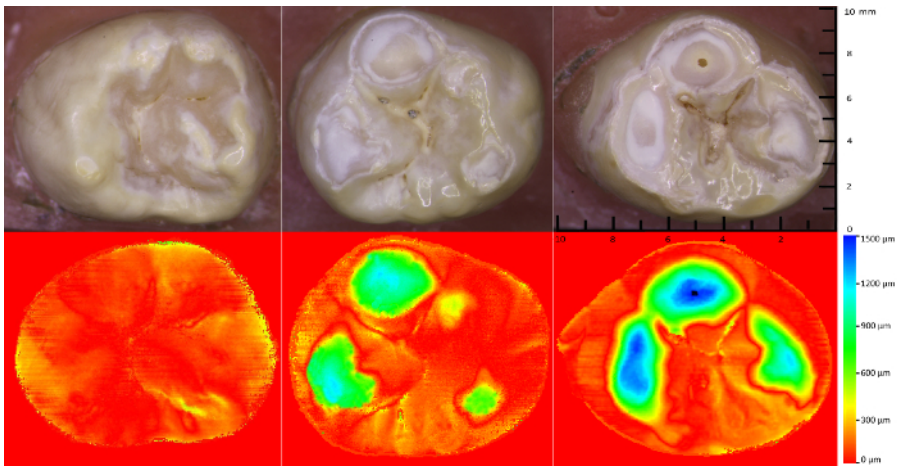

Figure 3. Typical example of molar teeth after 3 months in the Rub&Roll in a demineralization solution with pH 4.8. From left to right, the tooth was mechanically loaded with 0 N, 30 N, or 50 N. Top row shows stereomicroscopic light photographs (magnification 10X), and the bottom row shows corresponding subtraction images. The colors in the subtraction images indicate height loss from no loss (red) to 1,500 µm loss (blue). Please click here to view a larger version of this figure.

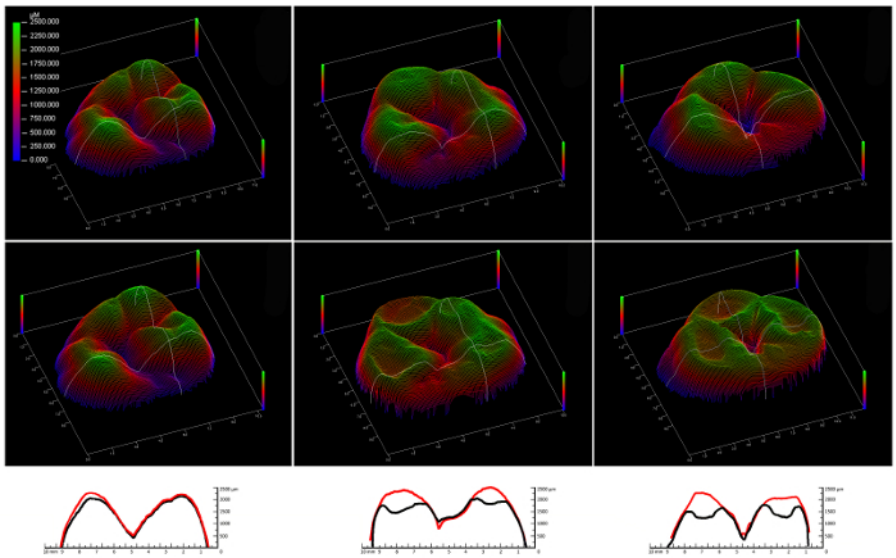

Figure 4. Examples of profilometric scans of selected samples, before (top row) and after 3 months exposure (middle row). The bottom row shows line cross sections of the two superimposed scans (red before and black after exposure). Please click here to view a larger version of this figure.

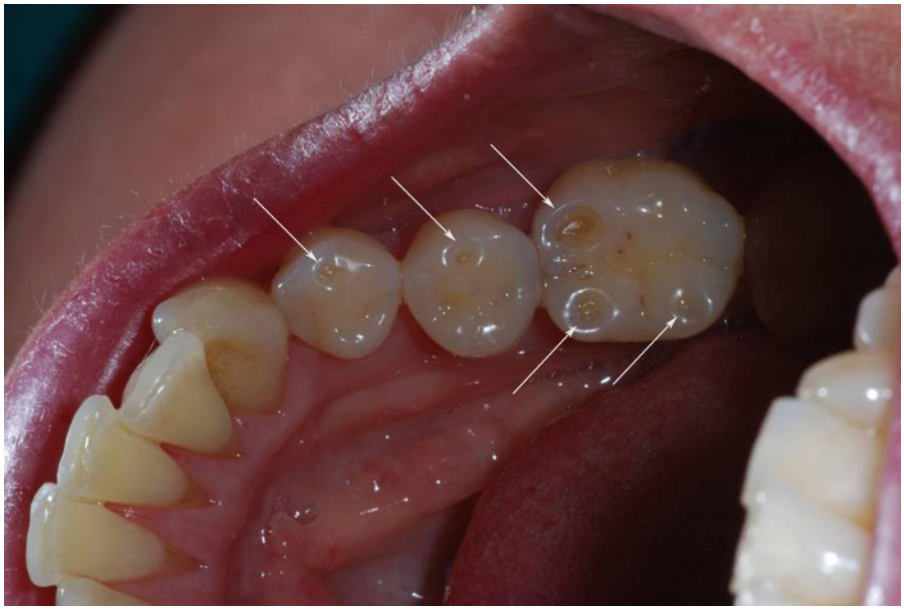

Figure 5. A clinical example of occlusal erosive tooth wear. Note cupping of the cusps (courtesy of Dr. R. Kuijs). Please click here to view a larger version of this figure.

Discussion

The application presented here gives a good impression of the clinical relevance of the Rub&Roll. The occlusal morphology that was created in the experiment with the highest loading level is very similar to the clinical presentation of erosive tooth wear (**Figure 5**)[11,12].

The versatility of the set up lies first of all with the solutions used. In the simplest model, water may be used. Loading samples in a water medium may be used for sample ageing, for instance of composite resin restoration adhesive bonding, before bond strength testing[13]. In a more clinically relevant model, water may be replaced by artificial saliva. Abrasive components such as fibers may be added to simulate the chewing of abrasive foods. Even whole foodstuffs may be used in situations where masticatory wear is the outcome under investigation. For pure erosive wear, the solution may be formulated to mimic soft drinks or juices.

Secondly, as shown in the example, the loading may be adjusted by changing the position of the samples. The loading is limited to about 75 N, but this lies well within the range of normal chewing forces[14]. By choosing physiological loading levels, the rate of the ageing process is clinically relevant. Total experimental time is still reduced, due to the continuous exposure in the rotating unit, and the high number of specimens that can be exposed at the same time.

More modifications of the device may be envisaged. By adding a thermal control unit, the aspect of thermocycling could be introduced, another important aspect of intraoral ageing. By changing the medium cyclically, pH-cycling could be introduced, for simulation of a caries process (de- and remineralization) of enamel. The rod surface may be modified to simulate different antagonist situations, such as porcelain or composite restorations. By placing a visco-elastic material underneath the samples, the action of the periodontal ligament may be simulated. The device is relatively simple and may easily be customized by the user, as desired.

There are some details that must be considered when operating the device. When using uneven contact surfaces, sample positioning is complicated, as movement of the rod across the surface may be hampered by protruding shapes. This may cause slipping of the rod and unwanted vibrations. At the start of each experiment, it is therefore necessary to closely monitor the running of the device. After about 8 hours it usually will run smoothly. It is recommended to replace the rod after this period and from that point onwards replace it twice per week.

Before teeth are embedded for occlusal loading, the positioning should be carefully considered so that the direction of the movement of the rod simulates the articulation or chewing motion as close as possible. The force on the occlusal surface may lead to wear, which in turn will decrease the load. Regular monitoring of sample protrusion is recommended in order to keep the loading within the intended range. In experiments using acidic media, for erosion or caries modeling, the pH value should also be monitored. It may change during the course of longer experiments as a result of the dissolution of the enamel.

Disclosures

The authors have nothing to disclose.

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