

This is a very interesting Dental Research Journal Publication from the University of Rome Italy School of Dentistry in collaboration with the Italian Ministry of Health Scientific Division.

The In-Vitro accelerated Wear/Abrasion study uses an Extra-Oral Simulated Servo-Hydraulic System that articulates Natural Teeth vs. several Materials/Crowns and depicts via MicroTomography the Volume and Surface changes that may occur subsequent to Long-term Cyclic Fatigue both Quantitatively and Qualitatively via Micro-Tomographic Imaging Technology.

The DiamondCrown -PEX Crowns performed Best and interestingly as well as if not better than Natural Teeth.

The Scientific-Clinical Research Literature still points to and reasserts the favorable Bio-Mechanical features of the PEX-DiamondCrown Formulae.

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Microtomography evaluation of dental tissue wear surface induced by *in vitro* simulated chewing cycles on human and composite teeth

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Summary. In this study a 3D microtomography display of tooth surfaces after *in vitro* dental wear tests has been obtained. Natural teeth have been compared with prosthetic teeth, manufactured by three different polyceramic composite materials. The prosthetic dental element samples, similar to molars, have been placed in opposition to human teeth extracted by paradontology diseases. After microtomography analysis, samples have been subjected to *in vitro* fatigue test cycles by servo-hydraulic mechanical testing machine. After the fatigue test, each sample has been subjected again to microtomography analysis to obtain volumetric value changes and dental wear surface images. Wear surface images were obtained by 3D *reconstruction* software and volumetric value changes were measured by CT analyser software. The aim of this work has been to show the potential of microtomography technique to display very clear and reliable wear surface images. Microtomography analysis methods to evaluate volumetric value changes have been used to quantify dental tissue and composite material wear.

Key words: 3D microtomography evaluation, composite materials, human teeth, *in vitro* fatigue test, dental wear surfaces.

Riassunto (*Valutazione microtomografica dell'usura dentale superficiale indotta da cicli di masticazione simulata in vitro su denti umani e in composito*). In questo studio è stata ottenuta una visualizzazione microtomografica 3D di superfici dentali dopo prove di usura dentale *in vitro*. Sono stati comparati denti naturali e denti protesici realizzati in tre differenti materiali compositi policeramici. I campioni di denti protesici, simili a molari, sono stati posizionati in opposizione a denti umani estratti per problemi paradontologici. Dopo l'analisi microtomografica, i campioni sono stati sottoposti a cicli di prove di fatica *in vitro* tramite una strumentazione per prove meccaniche servo idraulica. Dopo le prove di fatica, ogni campione è stato di nuovo soggetto ad analisi microtomografica per ottenere le variazioni dei valori di volume e le immagini delle superfici di usura. Le immagini sono state ottenute dal programma 3D *reconstruction* e le variazioni volumetriche dal CT *analyser*. Lo scopo di questo lavoro è stato quello di mostrare la possibilità della microtomografia di visualizzare in modo chiaro e affidabile le superfici di usura. I metodi di analisi microtomografica sono stati usati per valutare le variazioni volumetriche in modo da quantificare l'usura dei tessuti dentali e dei compositi.

Parole chiave: valutazione microtomografica 3D, materiali compositi, denti umani, prove di fatica *in vitro*, superfici di usura dentale.

INTRODUCTION

In the oral cavity, physical and biomechanical stresses on teeth and dental prosthetic elements, such as compression, tension and torsion strength are always present. Normal chewing activities occur during the day but also during sleep [1-3]. For example, bruxism, originated by the activation of reflex chewing activity, can result in abnormal dental wear surface and fractures. In this disease, the teeth of the opposing arches are moved against each other laterally, with a side-to-side action by the lateral pterygoid muscles.

This movement abrades tooth structure and can lead to the wearing down of the edges of the teeth. People with bruxim may also grind their posterior teeth, wearing down the cusps of the occlusal surface.

One important breakage feature of restorative and prosthetic material, after many chewing cycles, is fatigue wear.

Mechanical fatigue tests can be a great aid in the evaluation of materials' strength in very hard conditions like the oral cavity.

Now, composite materials used to manufacture

dental prosthetic elements show a higher resistance to surface wear fatigue, which is not only the same but better than that of natural teeth [4, 5].

Another important and critical factor is the dental manufacturing process that could lead to fracture and dental prosthetic element loss, if not performed correctly [5, 6].

In this study the most important purpose has also been to evaluate the dental tissue surface wear of natural dental element induced by opposite prosthetic tooth, which may damage dental enamel leading to tooth loss with problems for the oral health of the patient [5, 6].

The goal of this work has been to evaluate dental wear surface by microtomographic image reconstructions and volumetric value changes of natural and prosthetic tooth subjected to a biomechanical fatigue test, simulating 5 years of chewing activity. This test has been carried out for natural and composite prosthetic teeth placed in opposition during the simulation of chewing activity.

This work aims to show the usefulness of microtomography in choosing the composite material with ideal performance to be used in oral cavity, *i.e.* a strong material that can resist mouth stresses but doesn't cause excessive dental tissue destruction.

MATERIALS AND METHODS

Twenty-five human molars extracted from patients aged 30 to 50, due to paradonthologic disease were used for this experimental work.

Fifteen prosthetic molar tooth crown were manufactured according to manufacturer instructions with three different polyceramic composites: 5 with Gradia composite (hybrid micro-ceramic – GC America Inc., Chicago, USA), 5 with Diamond Crown composite (PEX – Phenolic EpoXine monomer – DL MEDICA s.p.a., Rome, Italy), and 5 with Ceramage composite (micro-fine-ceramic, PFS, Progressive Fine Structure – Shofu Inc., Kyoto, Japan).

Composite crown supports similar to the dental roots were manufactured with polyurethanic resin.

Before mechanical tests, the 25 human molars were stored in physiological solution and the 15 prosthetic tooth crowns in air.

By means of an *ad hoc* metallic cylindrical mould (internal diameter 20.8 mm, internal height 28.3 mm) all dental elements were glued in auto – hard-

ener acrylic Jet-Kit resin (Lang Dental Mfg. Co., Inc., Wheeling, IL, USA) to place these samples in the testing machine grips and in the microtomography instrument.

All samples, before and after the mechanical fatigue test, were also analyzed by a Skyscan 1072 instrument (SKYSCAN, Kartuizersweg 3B 2550 Kontich, Belgium).

Before a fatigue test with this technique, it is possible to observe the human and prosthetic teeth conditions, *i.e.* the healthy state of the human molars and the good manufacturing condition of the prosthetic molar teeth.

In this way, it was possible to obtain the pattern for each samples at the beginning.

Furthermore, for each sample volumetric values were measured and recorded to compare possible volumetric changes, before and after fatigue test.

Samples were grouped as follows:

- human tooth – human tooth (group 1);
- hybrid microceramic composite tooth – human tooth (group 2);
- PEX composite tooth – human tooth (group 3);
- PFS microceramic composite tooth – human tooth (group 4).

These pairs of samples were subjected to a fatigue test by means of the servo-hydraulic 858 MiniBionix testing machine (MTS System Co., Minneapolis, MN, USA) equipped by 5KN load cell and *ad hoc* manufactured system grips for this test. This system was composed of one bottom (lower) grip consisting of a stainless-steel disk with a mobile slide on board to make sample placement easier, and one top (upper) grip shaped like a cylinder (*Figure 1*) with a central circular low hole where the sample support is inserted, with four long screws and a metal plate with a hole to fix the sample support in place while leaving the tooth free.

On the testing machine, the mobile slide was used with an angle of 0° with respect to the horizontal plane to obtain a better contact occlusal surface of all tooth samples.

A sinusoidal compression load ranging from 34 to 340 N at a frequency of 6 Hz was applied to each pair of tooth samples for one million cycles. At the same frequency, a torsion strain ranging from -0,25° to +0,25° was also applied. This procedure is a simulation of 5 years of the *in vivo* aging process. These fatigue test parameters were inspired from an

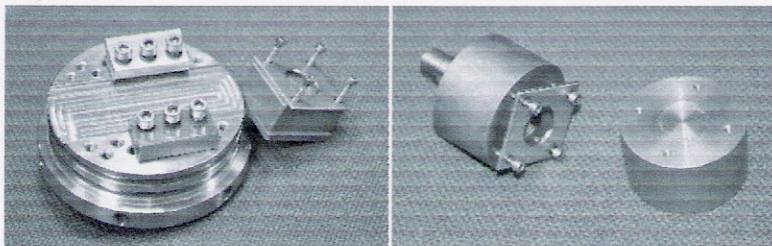


Fig. 1 | Components of the grip system manufactured for fatigue test samples: on the left a stainless-steel disk with a mobile slide on board (bottom grip) is shown; on the right two grips shaped like a cylinder (top grip) are displayed.

Table 1 | Results of the experimental work obtained for group 1, 2, 3 and 4

Sample groups	ΔV (mm ³)		ΔS (mm ²)	
Group 1	3.0 ± 1.4		6.1 ± 3.6	
Teeth	Prosthetic	Human	Prosthetic	Human
Group 2	2.9 ± 1.9	2.2 ± 1.4	2.1 ± 2.4	7.7 ± 6.3
Group 3	1.8 ± 1.2	3.1 ± 2.0	3.3 ± 4.3	1.0 ± 0.4
Group 4	3.0 ± 1.5	2.2 ± 0.8	4.4 ± 1.1	2.9 ± 2.6

analysis of the literature and some technical international regulations [7-10].

At the beginning of each test, all samples were accurately placed into the grips by a collaborating dentist. The best physiological occlusion was assured for group 1 of human teeth in the upper and lower grip.

Together with the collaborating dentist, an attempt was made to obtain the best possible occlusion for groups 2, 3 and 4 of prosthetic tooth in the upper grip and human tooth in the lower grip.

At the end of fatigue test each sample was subjected again to microtomography analysis in order to obtain a new set of data for comparison with microtomography results from before the fatigue test.

The dental wear surface images were obtained by superimposing the images obtained before and after the fatigue test using 3D Reconstruction software.

This process produced images with coloured areas displaying the dental wear zone which were evaluated and measured in square millimetres.

For each sample of all tested and analysed groups, the mean value of volumetric (ΔV) and surface difference (ΔS), before and after fatigue test, was obtained.

In this work the samples have been subjected twice to microtomography analysis and once to a mechanical fatigue test. During the time between tests, the prosthetic samples were stored in an air conditioned room while the human samples were stored in physiological solution and the tests were carried out with air conditioning maintaining a temperature of 22 °C and a relative humidity of H = 50%.

At the end, a one-way ANOVA test has been used to evaluate the statistical significance of differences among the groups.

RESULTS

Volumetric (ΔV) and surface (ΔS) difference mean values with the relative standard deviations, before and after fatigue test, are shown in *Table 1*.

The results obtained will be useful for evaluating dental tissue and polyceramic material destruction of dental occlusion surface due to simulated wear process induced by *in vitro* fatigue test.

Group 1 mean values, because they are a simulation of occlusal surface wear due to physiological dental activity, can be considered as reference value.

Due to the high standard deviations obtained, for the prosthetic and human samples group 2 and 4 show a ΔV and ΔS mean value similar to group 1.

In group 3, ΔV for both samples and ΔS for the prosthetic samples have a mean value similar to group 1, while for the human samples ΔS has a mean value very much lower than that of group 1.

Excluding group 1, for ΔV mean values prosthetic samples show the lower value in group 3, while the human teeth show similar values among groups 2, 3 and 4. For ΔS mean values, prosthetic samples show the lowest value in group 2 while the human teeth show the lower value in group 3. For this evaluation, groups 2 and 3 seem to show the best performance among prosthetic teeth manufactured with polyceramic composite material while group 3 the best



Fig. 2 | 3D microtomography reconstruction images of two human tooth samples; the circles show wear zone.

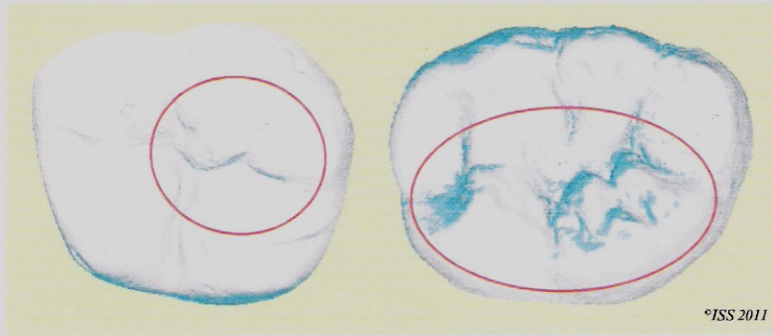


Fig. 3 | 3D microtomography reconstruction images of one human tooth sample (right) and one hybrid microceramic tooth (left); the circles show wear zone.

performance among human teeth. It is necessary to remember that these human samples were in opposition to prosthetic teeth during the fatigue test.

Two representative microtomographic images of dental occlusion wear surface for each group are shown.

Two images of dental occlusion wear surfaces for the group 1 of human samples are shown in *Figure 2*.

For group 2, pairs of one hybrid microceramic and one human tooth, two other images of dental occlusion wear surfaces are shown in *Figure 3*.

The human tooth shows a dental tissue wear surface similar, and perhaps greater than the wear surface shown in *Figure 2*, while the hybrid microceramic occlusion wear surface appears to be of very small dimensions (*Figure 3*).

For group 3, pairs of one PEX composite and one human tooth, two more images of dental occlusion wear surfaces are shown in *Figure 4*.

In the circles of *Figure 4*, dental occlusion wear surface for human and PEX tooth samples appear to be of very small dimensions.

For group 4, pairs of one PFS microceramic tooth and one human tooth, two images of dental occlusion wear surfaces are shown in *Figure 5*.

In the circles in *Figure 5* the human tooth shows a dental tissue wear surface similar to that of the two human tooth samples of *Figure 2*, while the PFS material occlusion surface appears to be of very small dimensions.

Comparing ΔV mean values for group 1 to those of the human teeth from and groups 2, 3 and 4, the result of ANOVA test showed no statistically significant differences ($p > 0.05$). Comparing ΔS mean value between group 1 and group 3, the result of ANOVA test showed a highly statistically significant difference ($p < 0.001$). Between group 1 and group 4, ANOVA test has showed statistically significant difference with $p < 0.05$.

No statistically significant differences were found comparing values of ΔV and ΔS among different groups of prosthetic teeth.

DISCUSSION

Composite materials used in the oral cavity are subjected to different stresses that can decrease their performance with time [11-15]. A good response to chewing load requires an oral cavity equilibrium that is changed by dental element loss.

Surface wear resistance is a very important property in this context. Its absence can lead to vertical dimension losses and temporo-mandibular joint dysfunction especially in patients with para-functional pathology, like bruxism or clenching, that can be an obstacle to reaching a healthy oral cavity equilibrium and may lead to myofascial muscle pain, dysfunction and headaches.

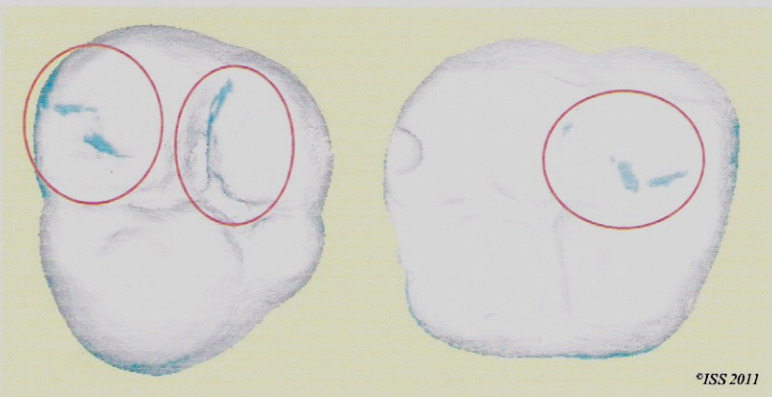


Fig. 4 | 3D microtomography reconstruction images of one human tooth sample (left) and one PEX microceramic tooth (right); the circles show wear zone.

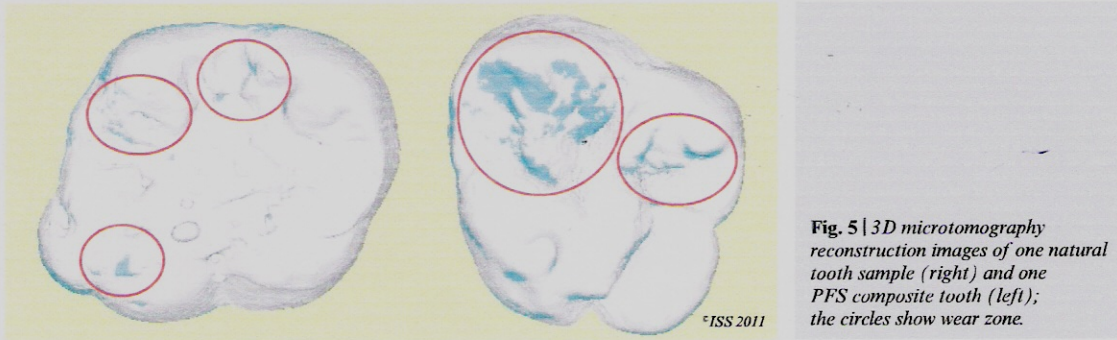


Fig. 5 | 3D microtomography reconstruction images of one natural tooth sample (right) and one PFS composite tooth (left); the circles show wear zone.

Before clinical investigations, the mechanical fatigue test is one of the methods to test *in vitro* biomaterial and prosthetic tooth performance simulating conditions in the oral cavity.

Medical literature, European and international regulations have defined the maximum and minimum simulated aging period, ranging between five years for dental implants and 20 years for hip prostheses.

One important factor is the stimulating frequency whose value depends on biomaterial or device conditions in use and may change from 2 Hz in the chewing cycle to 10-15 Hz in human walking.

Another factor to set is the stimulating load, *i.e.* the minimum and maximum strength that a sample should be subjected during the fatigue test. In the protocol used in this experimental work, for the oral cavity it has been set from 30 N to 300 N.

The pairs of human tooth, hybrid microceramic composite tooth- human tooth, PEX composite tooth- human tooth and PFS microceramic composite tooth- human tooth have been subjected to a test protocol in this work. By means of this test protocol a chewing cycle has been simulated with continuous occlusion surface contact and sliding that can generate a wear process for dental crown surface [5, 9].

After a mechanical fatigue test, the volumetric changing results of a reference human tooth sample compared to other groups, show similar or lower values as for composite teeth and opposite human teeth. These results show no relevant changes in groups 2, 3 and 4, compared to the performance of group 1 (reference) which also shows the limits of composite materials used in dentistry, even if these are of a new generation.

Dental occlusion wear surfaces between human tooth and human tooth is an aim which new composite materials should tend to. In fact, presently wear surface resistance is only due to filler characteristics, while resin matrix seems to always be a problematic factor for its highest power of wear surface [16-18].

3D microtomography reconstruction images of samples before and after chewing cycle simulation have shown a low alteration of surface design. The reference group of human teeth does not show a

large wear surface, on the contrary human teeth in opposition to prosthetic ones have shown more visible alterations while the composite wear surface has been very small.

From the obtained results it may be supposed that when placed opposite composite chemical compositions, human teeth have performed better than when placed in opposition to the other human teeth in group 1.

In fact, a statistically significant difference has been obtained in human teeth for ΔS between group 1 and group 3.

No statistically significant differences have been found comparing group 2, 3 and 4 prosthetic teeth in ΔV and ΔS .

These results may suggest that with some polyceramic materials, such as those used in this work, it is possible to obtain a dental tissue wear more similar to a physiological one and occlusion wear surfaces which are very small evaluated with the *in vitro* fatigue test simulating 5 years aging.

CONCLUSIONS

At the end of this experimental work, the obtained results may suggest that PEX composite prosthetic teeth in opposition to human teeth seem to show a good performance satisfying the requirements that are necessary for the correct use of prosthetic dental materials. In fact, such samples provided the lowest mean value of the volumetric change among prosthetic teeth, and for the relative opposite natural teeth, a volumetric mean value similar to the reference group. This can be explained by a lower volumetric change of PEX prosthetic teeth causing a tissue dental destruction on the opposite natural tooth similar to that obtained for natural tooth group.

In pairs of a PEX composite tooth with a natural tooth, the mean value of the surface change (ΔS) for both teeth exhibited a lower value compared to pairs of natural teeth. This can be explained if a lower surface change in PEX prosthetic teeth can cause a very low tissue dental destruction of the opposite human tooth, a result which was shown to be statistically significant in a comparison with the human tooth group by means of the ANOVA test.

This behaviour could not be due to the microfine ceramic component but can instead be caused by the organic polymeric matrix which makes the structure overall very strong and at the same time more elastic. As a result, this material shows characteristics similar to ceramic even though it has a very limited destruction power respect to dental tissues.

By means of 3D microtomographic reconstructions and relative superimposed images it has been possible to display the amount of wear surface for each sample. Therefore, it has been possible to verify alterations or irreversible modifications that have been caused by the wear, investigating the sample by means of dynamic scanning. This is a technique which has also been studied in human and prosthetic samples affected by fractures, fissures, cracks, creeps or other alterations.

In this experimental work an *in vitro* test protocol has been proposed to evaluate dental tissue wear and some parameters obtained by microtomographic analysis in order to quantify wear surfaces.

With these *in vitro* test methods, for the first time the performances of materials used in prosthetic dentistry in a simulation of oral cavity activity have

been obtained and at the end a material that can produce a lower dental tissue destruction has been evaluated.

In this study it is possible to observe that the values obtained for the group 2, 3 and 4 of human and prosthetic teeth seem to be always lower than group 1, which has been used as a reference group.

Superimposing two 3D image reconstructions has indicated wear surfaces by displaying different colours in some zones of the occlusion surface obtained with SkyScan 1072 processing software.

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Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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