Detecting the Implant *in vitro* Microleakage Using Spectrophotometry and Neutral Red Dye

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Implants are one of the most innovative and less invasive prosthetic procedures available. Almost all types of edentations can, nowadays, be treated with an implant-retained prosthetic piece, either fixed or removable. But regardless of the development in the industry over the last decades and the high success rate - estimated at over 95% - there are still some problems concerning single or multi-unit restorations[1-3]. One of the main concerns about implant therapy today is the microleakage phenomenon. The microleakage appears between the implant and the abutment and, due to masticatory forces, it creates a pump effect which can carry the oral bacteria inside the implant, where it's almost impossible to reach, thus, creating a local inflammation, called peri-implantitis[4-6]. Many authors have tried to identify and quantify the phenomenon by diverse means, but a consensus hasn't been reached on how or when exactly it appears. In the present study, Neutral Red dye was used to determine the sealing of the implant-abutment interface in dynamic conditions, with the help of the spectrophotometry.

Keywords: Implants, Peri-Implantitis, Microleakage, Spectrophotometry, Neutral Red

The implant-based therapy is very versatile. The prosthetic options are countless and the outcomes are highly predictable, thus, giving the patient a feeling of safety. And all of this without damaging the adjacent teeth at all, as all the support will be given by the implants, so no more healthy dental tissue will be lost. However, maintenance and oral hygiene are mandatory and play a major role in the success or failure of the implant treatment [7-11,17]

One of the most common reasons for failures, when dealing with dental implants, is the - so called pathology – peri-implantitis. Peri-implantitis basically describes the inflammation of the soft and hard tissues around an implant. Or as the Canadian Dental Association defines it: it's an *infectious disease that causes an inflammatory process in the soft and hard tissues surrounding an osseointegrated implant, leading to the loss of supporting bone* [12]. So, a mandatory condition for the local inflammation to be called *peri-implantitis i*s to have bone loss. If the inflammatory process only concerns the soft tissues, the term used is peri-mucositis.

One of the main reasons for peri-implantitis is the oral bacteria and the lack of oral hygiene. But even with disciplined patients, the inflammation can still occur, due to the microleakage phenomenon and the pump effect it creates. The microleakage appears because of the imperfect sealing capacity of the implant-abutment interface. Moreover, the masticatory forces tend to destabilize the interface even more and create the so called pump effect, which brings contaminated oral fluids inside the virtual chamber of the implant [13,14] (fig. 1).

Many authors have tried to determine the movement and the microleakage of the implant-abutment interface in static and dynamic conditions, but a consensus hasn't been reached on which type of implants or connections and surgical/prosthetic protocols can influence the treatment for a better outcome.

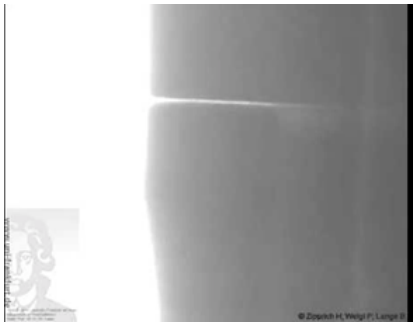

Fig. 1. The micromovement of the abutment (Copyright from Dipl.-Ing. Holger Zipprich Poliklinik für zahnärztliche Prothetik, Sektion Werkstoffkunde Theodor-Stern-Kai 7 / Haus 29, D-60590 Frankfurt am Main)

Experimental part

Materials and methods

To determine the sealing of the implant-abutment interface, an internal hex implant has been used. In order to mimic the physiological conditions of the oral mouth and mastication process, the implant was fixed in acrylate, inside of a cylindrical aluminum sample holder, just as an osseointegrated implant would be placed in the bone.

The top of the implant (together with the abutment, around 0.5 mm under the interface) was left outside the resin in order to be able to analyze its sealing efficiency.

Inside the implant, before applying the abutment, about 1 μL of an aqueous solution of 0.5 % w/v Neutral Red (3-Amino-7-dimethylamino-2-methylphenazine

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hydrochloride, acetate buffer, *p*H 5.2, preservative added (Sigma-Aldrich, USA)) was injected with a fine insulin syringe, in the space available between the inner part of the implant and the abutment screw, which is also visible on x-rays. Next, the abutment was applied with a torque wrench, following the manufacturer's indications, using a 30 cm N torque.

To mimic all the conditions of a normal implant used in the oral cavity, a zirconia crown was cemented on the abutment, designed with an oblique occlusal face (fig. 2).

Fig. 2. Zirconia crown fixed on the abutment, seen from above. Teflon tape was used to seal the cavity access

The reason for this was to transform vertical forces into oblique forces, much more similar to the ones that occur during mastication

After the crown with the abutment were set in place, the system was rinsed with distilled water 3 times to make sure that the dye did not contaminate any surfaces outside the implant. Distilled water was then introduced again in the sample holder and, after 5 min, it was tested with the spectrophotometer to confirm that it hasn't been contaminated by any traces of dye in the process, as the results showed the value 0.000 of initial concentration. After eliminating the possibility of dye contamination, 2 mL distilled water was added in the sample holder using a glass pipette, flooding the implant and the abutment. To prevent evaporation, the sample holder was hermetically covered with a plastic foil.

The sample holder, with the implant-abutment complex inside of it, was set in the testing machine, in order to start the dynamic load of the abutment. The tests were carried out in the Strength of Materials Laboratory from Politehnica University Timisoara, on a Walter+bai servo-hydraulic testing machine. The characteristics of the Walter+bai testing machine are as follows: dynamic load in tensile/compression ± 8 kN, accuracy class 0.5 (according to ISO 7500-1 and EN 10002-2), piston stroke ± 50 mm (fig. 3).

Also, a zirconia cap was cemented on the aluminum rod which loads the abutment, in order to avoid any cracks or abrasion that could affect the dynamic process (fig. 4).

Fig. 3. Walter+bai servo-hydraulic testing machine

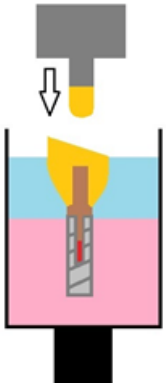

Fig. 4. Implant-abutment complex immersed in distilled water during dynamic load, while the dye was inside the implant

To check whether the implant-abutment interface allows the diffusion of the dye, a Cecil CE 1021 UV/Visible spectrophotometer (Cecil Instruments, UK) was used, at a wavelength of 450 nm (Sousa et al., 1996)[15]. To calibrate the spectrophotometer for concentration measurements, a standard solution was prepared of 0.5×10-3 % w/v Neutral Red dissolved in distilled water. (fig. 5).

Fig. 5.Cecil Spectrophotometer

In the first stage, after the sample holder system was set in place inside the Walter+bai servo-hydraulic testing machine, the abutment was loaded with 50-80 N force, 1 Hz, for 50,000 cycles (fig. 6).

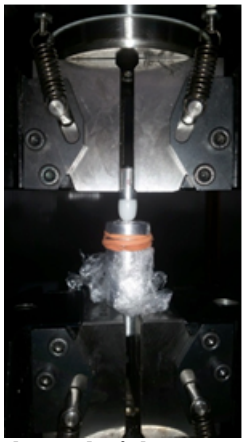

Fig. 6. Force loading on the implant-abutment complex

At the end of the 50,000 cycles, the water around the abutment was moved into the spectrophotometer's plastic cuvette, using a glass pipette, and analyzed with the spectrophotometer, to determine if the concentration remained 0.000 or if it changed due to the microleakage of the dye (fig. 7).

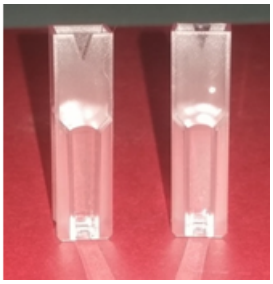

Fig. 7. Spectrophotometer plastic tanks

After the first spectrophotometric analysis, the results were record down and the water was placed back in the aluminum sample holder. The testing machine was then put back in function at a higher force – 120-180 N, 1 Hz, for another 50,000 cycles (fig. 8).

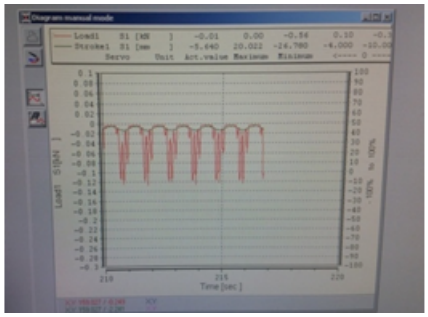

Fig. 8. Force loading of the abutment

Another determination was done afterwards with the spectrophotometer.

Results and discussions

At the end of the first 50,000 cycles of 50-80 N, 1 Hz, at the first determination, the concentration of the water around the implant has risen from 0.000% to 0.095%.

After the other 50,000 cycles of 120-180 N, 1 Hz, at the second determination, the concentration has more than doubled, the spectrophotometer showing a concentration value of 0.205% (fig. 9).

Fig. 9. Spectrophotometer display

The outcome of the present in-vitro dynamic testing of the internal-hex implant connection can be interpreted in many ways. The first theory, also suggested by other authors in the international literature, is that the interface between the implant and the abutment allows a certain degree of microleakage from and towards the inner part of the implant.

There have been many studies on this matter, most of them using bacterial suspensions to demonstrate the microleakage and mechanical fatigue followed by inspection of the interface.

Joao Paulo da Silva-Neto (2014)[16] has demonstrated a similar microleakage effect in static conditions. The author has inoculated the inner part of the implant with a bacterial suspension before tightening the abutment according to the manufacturer's recommendations. Afterwards, the implant-abutment complexes were immersed in sterile solution and left there for a number of days. After a 7-14 days period, at the biochemical examination of the sterile solutions, bacterial contamination was detected at all implant systems, regardless of the brand or the type of connection.

Probably one of the most notorious experiments was carried out by dr. Dipl.-Ing. H. Zipprich [13] in 2007. In his experiment, the author has used abutments which were loaded at an angle of 30° with a force of up to 200 N, while a constant and diverging X-ray device radiated the inspection pieces. With a special device the X-ray was transformed into visible light which was recorded, using a high speed digital camera. The experiment actually captured, live, the micromovements of the abutment in respect of the implant, when it is loaded.

As seen in literature, the problem of microleakage is still up to date and most implant connection systems are affected by it. The problem with the studies described above is that none of them gave a quantitive estimate of the microleakage phenomenon. The study using bacterial suspensions can detect if the microleakage occurs, but cannot quantify its extent. The studies using mechanical fatigue and examination of the interface, on the other hand, prove the existence of the micro-movements and, sometimes, the deterioration of the implant/abutment surface, but lack the microleakage evidence.

The present study aimed to combine the two methods to get a more precise result: a cyclic mechanical load was applied on the abutment, while the whole complex was immersed in distilled water and the inside of the implant (the empty space available, under under the abutment screw) was filled with dye. So, after a number of pre-determined cycles of micro-movements, the micro-leakage was determined quantitatively, by a spectrophotometric measurement of the concentration of the dye in the distilled water surrounding the abutment.

Conclusions

The microleakage phenomenon appears due to the imperfect fit of the abutment on the implant. While there are studies that have proven this happening in static conditions, many authors, together with the present study, have shown that, during loading, the space between implant and abutment increases and the microleakage amplifies.

In literature, this is also known as the *pump effect*. Meaning that, with the movement of the abutment, a negative pressure will appear inside the implant, causing the surrounding fluids (like saliva, contaminated with oral bacteria) to get inside the implant. After this point, there will be an exchange of fluids both ways, towards and from the inside of the implant. The clinical significance of this exchange can be linked to the inflammatory pathology regarding dental implants – peri-implantitis. This occurs due to the fact that the bacteria colonizing the inner part of the implant is almost impossible to reach and, thus, an acid environment will be present around the peri-implant tissues.

The present study confirms the results of other authors regarding the presence of microleakage at the abutment-implant interface. Nevertheless, there are still many biological variables to take into consideration. Not all patients have the same immune response, not all implant placements follow the same surgical and prosthetic protocol around the world and, also, not all patients have the same oral hygiene. This is why there are so many different outcomes in the clinical practice when it comes to implant therapy.

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