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ORIGINAL RESEARCH

Determination of Inner Implant's Volumes: A Pilot Study for Microleakage Quantification by Stereomicroscopy and Spectrophotometry

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ABSTRACT

Aim: Microleakage quantification of fluids and microorganisms through the connections of different implant parts seems to be sparse. Moreover, no data exist regarding the determination of the volumes of inner parts of dental implant systems.

This study aims to determine the volumes of inner parts of three dental implant systems with the same interface and to evaluate the microleakage phenomenon.

Materials and methods: Three implant system sets (Euro-teknika®, Astra Tech® and Implantium®) were used in this study. Implants were inoculated with safranin, brain heart infusion and distilled water. After inoculation and assembly of the different parts, different inner volumes (V_1, V_2, V_3, V_4, V_5 and V_6) were measured and, the surfaces of the micro gaps were observed through a stereomicroscope. Implants containing safranin were immersed in vials containing distilled water. Samples then were taken to determine optical density using a spectrophotometer.

Results: Regardless the used substance, volumes of the 3-implant systems are different. Although volumes V_1, V_2, V_3 and V_5 appeared to be constant within the same system regardless the used substance, volumes V_4 and V_6 were not.

Conclusion: The determination of the volumes and the evaluation of leaked substance using stereomicroscopic and spectrophotometric methods showed the accuracy of these methods and the importance of their use in the study of microleakage.

Clinical significance: Leakage is an important factor for chronic inflammatory infiltration and marginal bone resorption. Studies have shown fluid and bacterial leakage into abutment-implant (A-I) assemblies of certain implants with 'closely locked' abutments and the creation of a constant bacterial reservoir in the empty space found between the implant and the abutment.

Keywords: Inner volumes, Implant, Microleakage, Safranin, Spectrophotometry, Stereomicroscopy.

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INTRODUCTION

Over the last 30 years implant osseointegration phenomenon has drastically changed dental treatment restorative modalities, making dental implantology one of the most successful rehabilitation techniques among medical and dental specialties with success rates reported above 90%.[1] The vast majority of dental implant systems comprise an endosseous implant surgically placed in bone and its transmucosal components (abutment screwed to implant), which are subsequently prepared for single tooth or multiple teeth replacement.[2] Maintenance and stability of screw-type connections can be jeopardized by unclamping forces between implant and abutment.[3] Implant-abutment connection can be an area where adverse biologic and mechanical consequences occur. Mechanical complications, such as increased incidences of abutment rotation and breakage,[4] screw loosening,[4] and preload reduction, have also been reported to occur with a poorly adapted implant-abutment interface (I-A-I).[3-7] Biologic complications such as tissue inflammation,[8,9] gingivitis,[9,10] and bone loss[11-13] have been reported to result from microleakage.[8,14-17]

Peri-implant pathology has been defined as 'peri-implant mucositis' with reversible inflammatory soft tissue reactions and peri-implantitis has been defined as inflammatory reactions with loss of supporting bone in the tissue surrounding a functioning implant.[18-20]

Increase in inflammation in regions in proximity to the I-A-I has been attributed to adhesion, colonization and

proliferation of bacteria on biofilms that are formed in the implant and at the implant abutment gap during soft tissue manipulation for prosthetic component installation.[19,21,22]

Microgap at I-A-I allows microorganisms in their sessile life style to proliferate close to the epithelial tissues, which often results in bone resorption approximately 2 mm apical to the microgap.[11]

In vitro studies have shown fluid and bacterial leakage into abutment-implant (A-I) assemblies of certain implants with 'tightly secured' standard abutments.[23-26]

Only few studies regarding quantification of fluid microleakage (color markers, small molecules or microorganisms) are available in the current literature. No data exist in relation between the determination of volumes (retention capacities) of different inner parts of dental implant systems and the effect of the volume of the inoculum (marker or bacteria) on the quantity of the leaked material and biofilm formation (*in vitro* and *in vivo*), and on the consequences of the latter 2 phenomena on the bone and the surrounding tissues. To be able to develop all these studies, it is logic and judicious to precise the threshold of the volume (s) to be inoculated in the used implant(s) system(s).

The aim of the present study was to determine the volumes (retention capacities) of the different parts of 3 sets of dental implant systems having identical interface configurations, and *in vitro* quantification of microleakage of 3 different substances using stereomicroscopy and spectrophotometry.

MATERIALS AND METHODS

Implants and Abutments

Three implant systems [Euroteknika® (E), Astra Tech® (A) and Implantium® (I)] were chosen to be used in this study. Twelve titanium implants with their respective abutments of each of the 3 systems were used. The 3-implant systems have the same internal hexagon implant-abutment connection configuration, but they have different lengths (12, 11 and 10 mm for E, A and I respectively) and different diameters (4.8, 5 and 4.8 mm for E, A and I correspondingly) (Table 1).

Substances and Color Markers used in This Study

Three substances [Safranin (SF), Brain Heart Infusion broth (BHI) and Distilled Water (DW)] were used to determine the volumes (keeping capacities) of implants and to quantify microleakage at I-A-I and screw abutment interface (S-A-I).

Calibration Curve

To accurately determine volumes (keeping capacities) of different implants belonging to the 3 systems (cited above) and quantify the amount of microleakage, a calibration curve was determined by placing increments of 0.1 to 1 µl of SF in 3 ml of DW contained in 15 ml vials (Corning Incorporated, NY, USA). The increments of color marker were transferred by means of a single channel micropipette (L322606, Pipetman, Gilson service, France) using ultra thin tips (1310A, 236, Ranin, USA) and the absorbance for each volume was acquired in a previously calibrated spectrophotometer (Gene Quant 1300, ref: 80-2120-00, Healthcare Bio-Sciences AB, Sweden) (n = 3 per volume). Calibration curve was determined by linear regression considering the absorbance as a function of color marker amount.

Sterilization of Implants, Abutments and Instruments

To avoid microbial contamination, all implants, abutments, torques, substances, all other instruments and heat stable consumables used in contact with the test materials were autoclaved at 121° Celsius during 30 minutes at 1 Kgf/cm^2.

All procedures regarding handling of sterile implants, abutments and collection of samples during experimental series were performed under sterile conditions in a microbiological hood with vertical laminar airflow. The stereomicroscope (Leica Zoom 2000, 13312596V, Leica Microsystems Inc., Buffalo, NY USA) and the micropipettes were cleaned by ethanol 70% before their sterilization by UV irradiation under the closed microbiological hood, all the manipulations being performed under the hood.

Table 1: Characteristics of the implants used in this study

Implant system	*Implant diameter (Reference)*	*Abutment diameter (Reference)*	*Screwing torque as recommended by manufacturers*	*Made in*
Euroteknika	6 mm (NID6062120)	5.8 mm (NPS PD 5826)	35 N/cm	France
Astra Tech	5 mm (24972)	5.5 mm (24235)	25 N/cm	Sweden
Implantium	5 mm (FX 4812)	5.5 mm (DAB 5515HL)	25 N/cm	South Korea

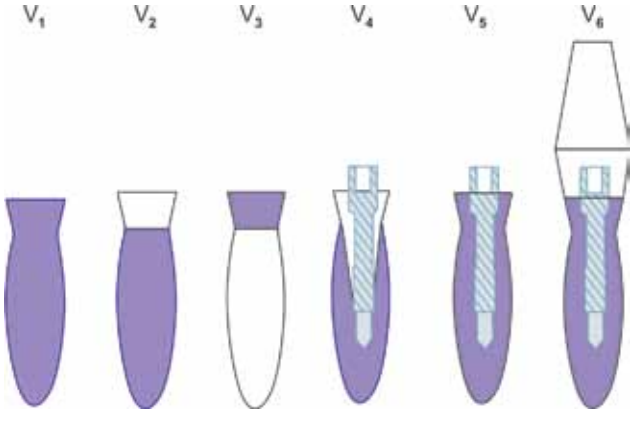
Preparation and Assembly of Implants

Each implant was inoculated with one of the three substances mentioned above starting with 0.1 µl. The volume was accurately pipetted into the deepest part of the internal lumen of implant viewed clearly in all its details through the stereomicroscope. The implant was then screwed into the specimen's holder to achieve an upright position. Then, two situations were considered:

1. The abutment-screw was connected to the implant according to the manufacturers' protocols using a calibrated torque controller of the respective implant manufacturer.
2. The screw alone was connected to the implant in the same manner as previously described.

Implant Volumes' (Keeping Capacities) Determination and Microleakage Detection using the Stereomicroscope

After connecting tight the abutment-screw or the screw alone to the implant, the whole surface of I-A-I, that of the screw-abutment interface (S-A-I) or that of the screw-implant interface (S-I-I) were accurately observed through the stereomicroscope with a full magnification of 300× in order to detect possible microleakage. This procedure (inoculation, assembly than observation) was repeated many times with at each time an increasing volume. We started with 0.1 µl till reaching the volume with which we detected a leakage in each system. In each stage, all the twelve implants of each system described above were used to confirm the volumes (keeping capacities) of the implant and the presence of microleakage. To this aim, all implants were inoculated with the corresponding volume and observed through the stereomicroscope.



Graph 1: The different volumes (keeping capacities) determined in this study. Dark part represents the volume to be determined. V_1—Volume (keeping capacity) of the whole empty part of the implant without any connection; V_2—Volume (keeping capacity) of the empty space of the implant without the common area between the implant and the abutment; V_3—Volume (keeping capacity) of common area between the implant and the abutment ($V_3 = V_1–V_2$); V_4—Volume (keeping capacity) of the empty space between the screw and the inner part of a screwed implant without abutment; V_5—Volume (keeping capacity) of the whole empty space of a screwed implant without abutment; V_6—Volume (keeping capacity) of the empty space of the screwed implant connected to an abutment

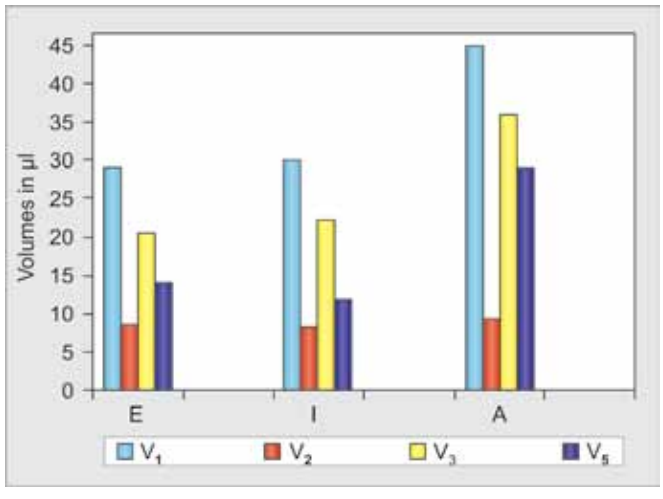
Implant Volumes' (Keeping Capacities') Determination and Microleakage Quantification by Spectrophotometry

After the assembly of different components of implant and their observation through stereomicroscope, each implant containing SF was immersed in 3 ml of DW contained in 15 ml vials. The vial was gently shacked then implant removed. Seventy µl were transferred from the vial into an ultra-micro silica (quartz) spectrophotometer cuvette (C1918, Sigma-Aldrich, France) and the optical density (OD) at $\lambda = 530$ nm for SF for each collection was measured using a spectrophotometer previously calibrated with DW. To accurately confirm volumes (keeping capacities) determined by stereomicroscopy and to quantify microleakage by spectrophotometry, all twelve implants belonging to each system were inoculated with the first volume with which a leakage was detected by stereomicroscopy. Then ODs of the leakages were performed as previously described.

RESULTS

Implant Volumes' (Keeping Capacities') Determination and Microleakage Detection by Stereomicroscopy

Volumes V_1, V_2, V_3 and V_5 (Graph 1) proved to be constant within the same implant system regardless the used substance. Nonetheless, these same volumes are not constant in all the different used systems (Graph 2).



Graph 2: Variation of V_1, V_2, V_3 and V_5 according to the used implant systems

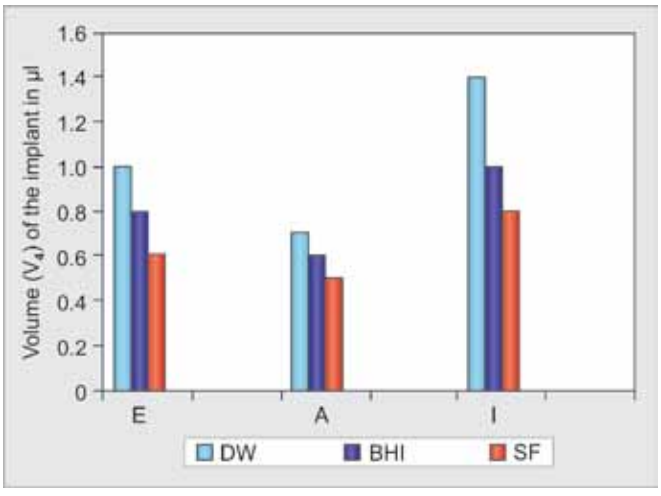
Regardless the used substance, V_1 showed the highest value (45 µl) in the A system; that was about 30 and 29 µl in I and E systems respectively. V_2 was about 9, 8.5 and 8 µl in A, E and I systems respectively (Graph 2).

V_3 is about 36, 22 and 20.5 µl in the A, I and E systems respectively. V_5 is about 29, 14 and 12 µl in the A, E and I systems respectively (Graph 2).

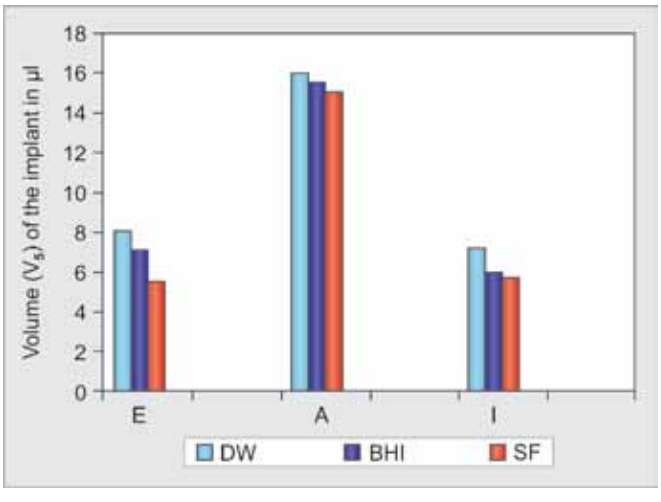
Surprisingly, we found that volumes V_4 and V_6 (see Graph 1) were variable inter- and intrasystem according to the used substance (Graph 3).

As shown in Graph 3, in case of the E system, V_4 reported different values according to the used substance. It is equal to 1, 0.8 and 0.6 µl when measured with DW, BHI and SF respectively.

In the case of A system, V_4 was equal to 0.7, 0.6 and 0.5 µl when measured with DW, BHI and SF respectively (Graph 3). While in the I system, V_4 was equal to 1.4, 1 and 0.8 µl when DW, BHI and SF were used respectively in its determination (Graph 3).



Graph 3: Variation of the volume (keeping capacity) V_4 according to the utilized implant systems and substances



Graph 4: Variation of the volume (keeping capacity) V_6 according to the utilized implant systems and substances

Furthermore, as shown in Graph 4, in the E system, V_6 has different values according to the substance utilized in its determination. It was equal to 8, 7.1 and 5.5 µl when measured with DW, BHI and SF respectively. In case of the A system, V_6 was equal to 16, 15.5 and 15 µl when measured with DW, BHI and SF respectively, whereas in the I system, V_6 had the following values 7.2, 6 and 5.7 µl when measured with DW, BHI and SF respectively (Graph 4).

Implant Volumes' Determination and Microleakage Quantification by Spectrophotometry

Determination of volumes (keeping capacities) of implant systems was based on the detection and quantification of the microleakage at I-A-I and S-A-I by spectrophotometry.

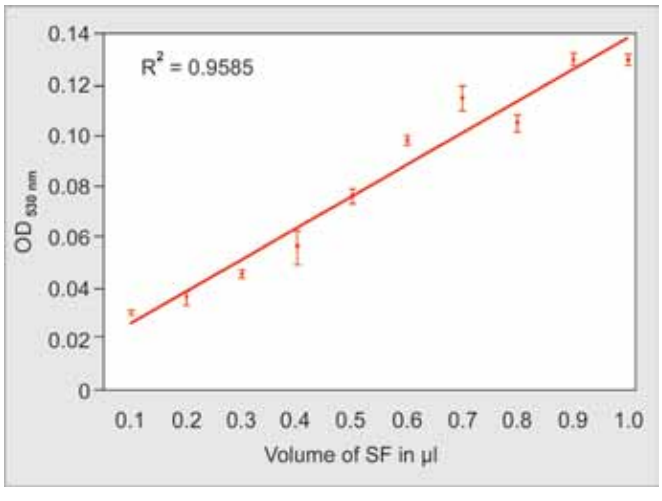
To quantify microleakage by this method, a standardized comparison was considered , using, a calibration curve carried out with increasing increments of the same color marker.

Calibration Curve

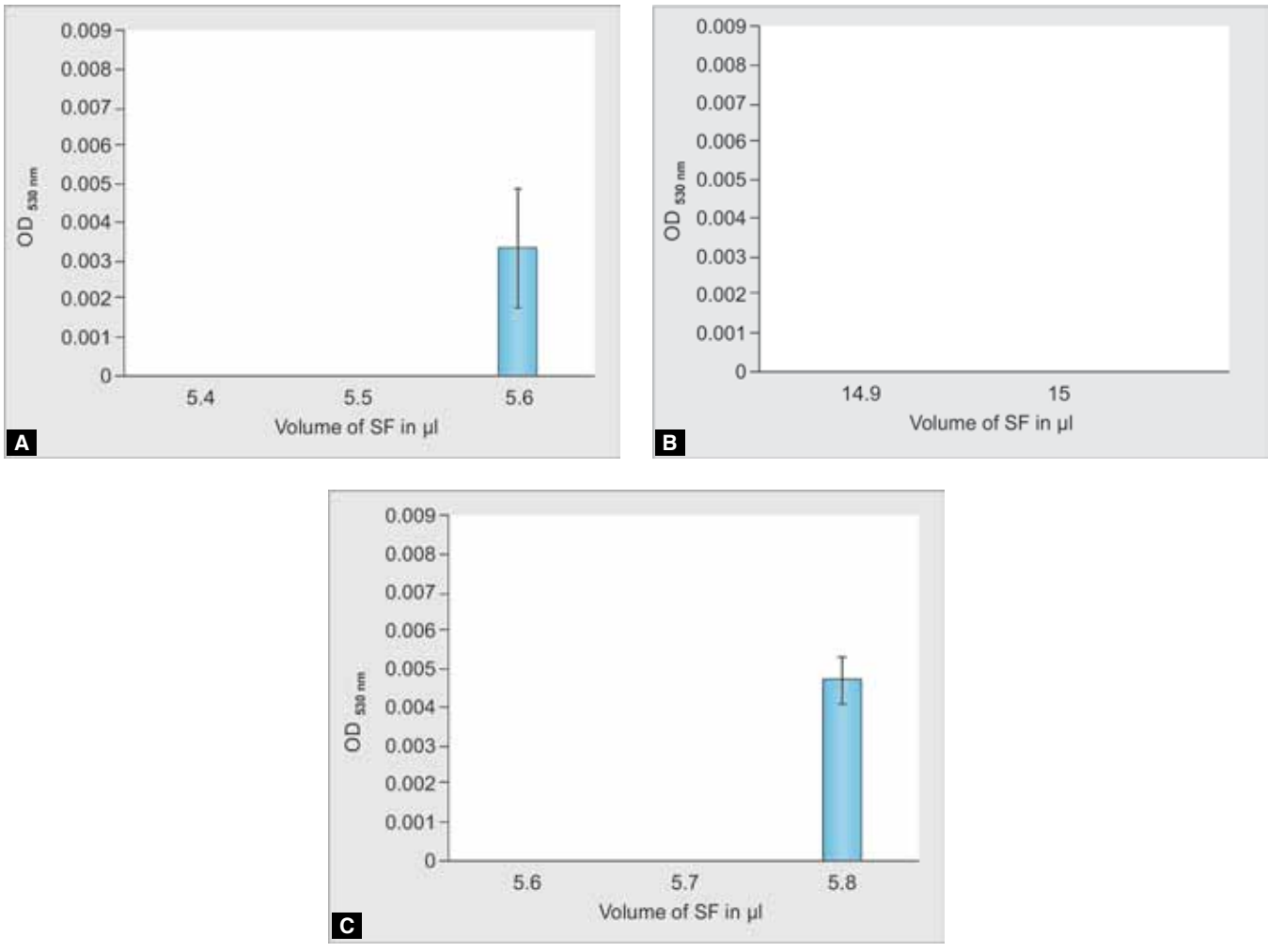
Graph 5 consists of a calibration curve obtained with safranin.

According to results obtained in the calibration curve, we were able to deduce the quantity of leakage trough the I-A-I and the S-A-I for each inoculated volume as described in material and methods. OD values of samples taken from vials containing connected implants with no microleakage were equal to zero, whereas the samples coming from the vials containing implants with microleakage had ODs > 0.

Graph 6 shows OD values of the samples taken from vials containing implants inoculated by increments of 0.1 µl of SF. The first sample presenting a positive OD value serves



Graph 5: This curve shows a linear relationship between the optical density at 530 nm ($OD_{530\ nm}$) and the amount of SF by placing increments of 0.1 to 1 µl of SF in 3 ml of DW contained in 15 ml vials. This curve provides a reference to quantify the SF released from the different implant systems through the I-A-I and the S-A-I



Graphs 6A to C: The OD values of the samples taken from the vials containing the Implants belonging to the E system (A), the A system (B), and the I system (C) inoculated by volumes lying between 0.1 and 5.6 µl (A), between 0.1 and 15.1 µl (B), and between 0.1 and 5.8 µl of SF (C) with increments of 0.1 µl. To simplify the figure, just two of all the volumes preceding that presenting the first microleakage are presented here

to quantify the microleakage by comparison of this value with those of the calibration curve. The sample immediately preceding this later could be used to precise the volume V_6. Thus, for the E system, when inoculating the implants (n = 12/each volume/each system) with a volume ≤ 5.5 µl of SF, there was no leakage neither through I-A-I nor S-A-I because $OD_{530nm} = 0$. At V = 5.6 µl of SF, $OD_{530nm} = 0.0033$ (Graph 6A). These values indicated that, for this volume, there was a microleakage indicated whose volume is < 0.1 µl. Furthermore, the assembled E system implants appeared to be capable to retain up to 5.5 µl of SF (or more precisely between 5.5 and 5.6 µl of SF).

For A system, when inoculating implants (n = 12/each volume/each system) with a volume ≤ 15 µl of SF, there was no leakage neither through I-A-I nor S-A-I (Graph 6B). For V = 15.1 µl of SF, $OD_{530nm} = 0.008$. These values indicated that there was indicated a microleakage whose volume is < 0.1 µl. Likewise, assembled A system implants appeared to be capable to retain up to 15 µl of SF (more precisely between 15 and 15.1 µl of SF).

For I system, when inoculating the implants (n = 12/each volume/each system) with a volume ≤ 5.7 µl of SF, there was no leakage neither through I-A-I nor S-A-I. At V = 5.8 µl of SF, $OD_{530nm} = 0.0047$ (Graph 6C). Thus, volume of the microleakage was < 0.1 µl. Moreover, assembled I system implants appeared to be capable to retain up to 5.7 µl of SF (more precisely between 5.7 and 5.8 µl of SF).

DISCUSSION

As the osseointegration phenomenon was first utilized to provide support to dental prosthesis through endoosseous implants,[18] substantial evolution has occurred in the design of implant body and prosthetic components. Marginal bone level and its maintenance were subject to both mechanical[27-29] and microbiological[30-33] aspects of implant abutment connection.

In implants where a microgap is present, microbial leakage and persistent bacteria in peri-implant zone could lead to inflammation and then to bone loss.[9,32-35]

Rationales for changing implant abutment connection design included an attempt to establish better prosthetic stability and decrease the implant abutment gap that have been reported to occur in many implant systems.[17,26,34,36-39] However, while an understanding of magnitude of the implant abutment misfit may provide an insight to the magnitude of bacterial colonization and proliferation, it does not provide any information about fluid transfer between internal and external regions of implant abutment connection.

Some studies compared the release of a color marker or bacteria through the microgap (I-A-I) at different conditions (different implant systems, various closing torque values, static or dynamic position…).[14,38,40]

Internal volumes of dental implant systems generally studied were never determined and the volumes of different substances (color markers, culture media or bacterial suspensions) used in the inoculation of implants to study the microleakage through their connections were chosen arbitrarily, 0.3 µl,[41] 0.5 µl,[17,25] 3.0 µl[26] and 5 µl.[40]

Volume hosting bacterial colonization and proliferation may influence the microleakage rate through the I-A-I. Bacterial colonization is a key step in bacterial biofilm formation.[42-45] This important phase depends on at least three factors:

(1) The bacterial surface (charge, presence of organelles), (2) the surface of the implant (electrostatic forces, steric forces, mechanical stability, elasticity, roughness, topography …)[46-52] playing a role in the different stages of the biofilm and (3) the environment (pH, temperature, nutrients, ionic strength, fluid, solutes …)[53-62] involved not only in the colonization, the physiology of the biofilm, the regulation of gene expression and the cell-to-cell communication within the biofilm but also in the formation and the eradication of the biofilm.

It is obvious that the dental implant colonization leads to a biofilm formation. Some environmental factors involved with biofilm formation are related to the parameters discussed in our present study. In nearly all the studies related to the implant dentistry, the effect of the identity of the inocula, the chosen inoculating volume, the biofilm as well as the volumes of the different parts of the used implant on the microleakage were never studied. The volumes of the inocula used in the above mentioned studies are chosen arbitrary in the literature and none of these studies reported any specific method for determining the inoculating volume. Major questions like the relation between the inoculating volume and the overall (V_6) internal implant volume were never raised.

In our study, we proceeded to evaluate internal volumes of 3 implant systems with the same interface in order to study microleakage of these systems on I-A-I and S-A-I. To reach this aim, we used two methods to determine the volumes (or more specific, keeping capacities) of the different parts of the implants, which constitute a key step for the microleakage study.

We found that volumes of the 3-implant systems used here (A, E and I) were different regardless the used substance (DW, BHI and SF): the Astra Tech® system had the highest volumes (V_1 and V_3) and was followed by the Implantium® and Euroteknika® systems respectively.

While A system has also the highest volumes (V_2 and V_5), I system has the lowest ones preceded by E system.

Although volumes V_1, V_2, V_3 and V_5 appeared to be constant within the same system regardless the used substance, volumes V_4 and V_6 were not.

Regardless the used substance, I system had the highest V_4 followed by E and A systems respectively. V_4 had the maximum value when measured with DW and lower values with BHI and SF respectively.

The system A had the highest V_6 and according to this system, highest volume value was obtained with DW and lower values were registered with BHI and SF correspondingly.

Although E system has a V_6 higher than that of I system when DW and BHI were used (with higher value obtained when using DW), I system had a V_6 higher than that of E system when SF was used.

While the variations of V_4 and V_6 (keeping capacities) of assembled implants according to the used substance are still not well understood, nevertheless results obtained in this work showed the importance of determination of volumes (keeping capacities) of assembled implants before studying microleakage with a given substance. Furthermore, our preliminary results in another study show that there is a relationship between the volume of the inoculating product and the microleakage (data not shown). Of equal importance, the study of the physicochemical interactions between the used substance(s) and the material(s) of the different parts of the implants in contact with the fluid as well as other factors may help us to explain variations in the keeping capacities of the implants.

Despite these variations, volume of the detected leakage (≤0.1 µl) using both stereomicroscopic and spectrophotometric methods showed the accuracy of these methods and importance of their use in these studies.

CONCLUSION

Findings described in our study helped us to make a better choice regarding the volume to be inoculated into a given implant system: this volume, which varies according to the used system and the used substance, has an impact on the microleakage and should not exceed the V_6 determined with the substance or the solution to be inoculated in that system.

CLINICAL SIGNIFICANCES

Microbial leakage at the implant-abutment connection is a major contributing factor for peri-implant inflammatory reactions. The internal volumes of the dental implant systems generally hosting the bacterial colonization and proliferation may influence the microleakage results thought the I-A-I. Prevention of microbial leakage at the implant-abutment connection is a major challenge for the construction of modern two-stage implant systems in order to minimize inflammatory reactions and to maximize bone stability at the implant neck and thus to maintain the clinical results specially in the esthetic zone.

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