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Bacterial Leakage Along the Implant-Abutment Interface

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1. Introduction

Titanium implants have been successfully and increasingly used for the substitution of dental elements in the treatment of total or partial edentulism, exhibiting success rates frequently above 90%, since the earliest reports on this technique in the 1960 decade (Lang et al., 2004; Pjetursson et al., 2007; Jung et al., 2008). When treatment failures are calculated based on patients who lost implants and not on implant lost by the population in general, success rates may be considerably lower (Lambrecht et al., 2003; Stavropoulos et al., 2007; Esposito et al., 2010).

Excessive premature loading, occlusal trauma and poor bone support are considered the main factors associated with early implant loss (Esposito et al., 2000; Piattelli et al., 2003). Recent reports demonstrated that microorganisms in the oral cavity, especially the ones involved in periodontal diseases, together with unfavorable occlusal factors are considered as the main causes of unsuccessful treatment with implants (Mombelli & Lang, 1998; Covani et al., 2006).

A direct correlation between presence of microorganisms and disease of the peri-implant tissues has been demonstrated. Gram-negative, anaerobic species like *Fusobacterium spp*, *Prevotella spp* and spirochetes are frequently found in large quantities in affected sites. In contrast, healthy sites are predominantly colonized by Gram-positives (Mombelli & Lang, 1998; Quirynen et al., 2006). Periodontitis in proximity to implants and presence of periodontal pathogenic bacteria in the peri-implant sulci are considered risk factors to the success of dental implants (Mombelli et al., 1995; Mombelli & Décaillet, 2011). Surface characteristics, physical properties, as well as biological factors involved in this type of treatment may facilitate bacterial colonization and growth of potentially pathogenic microorganisms at the implant sites (Mombelli et al., 1995; Jansen et al., 1997; Covani et al., 2005).

Another risk factor to the peri-implant tissues is the presence of marginal discrepancies between prosthetic crowns and implants abutments, although it is a controversial issue. The assessment of these discrepancies varies largely depending on the material employed for crown fabrication, type of cement, measuring methods, etc. Studies using human extracted teeth have reported marginal discrepancies ranging from 5 to 430 μm (Abbate et al., 1989; Felton et al., 1991; Valderrama et al., 1995; Kosyfaki et al., 2010). The highest values, varying from 110 to 160 μm, are frequently associated to feather–edge cast gold crowns (Marxkors,

1980; Diedrich & Erpenstein 1985). In contrast, clinical evaluation of ceramic crowns have shown smaller discrepancies, ranging from 32-145 µm (May et al., 1998; Boening et al., 2000; Kokubo et al., 2005). Acceptable discrepancies between abutments and fixed prosthesis should not be higher than 120 µm (Jemt & Book, 1996; Kosyfaki et al., 2007), although some authors report success in patients having misfits of around 30 to 200 µm (Boeckler et al., 2005).

The great majority of current implant systems contain two parts connected by screws, the intraosseous cylinder and the abutment, showing, as a result, an interface and empty spaces between the components. Localization levels of the implant-abutment interface in relation to the alveolar bone crest during implant placement, as well as the type of connection may vary between the different implant systems. Depending on the system, the implant-abutment interface is positioned either at the bone or gingival level. In bone level systems, a second screw, or cementing agent, connects the prosthetic structure to the abutment, thus introducing a second union interface. Gingival level implant systems have the implant-abutment interface many times covered by the prosthetic structure, which results externally in a single implant-prosthesis interface that are positioned about the gingival level. Frequently, the implant and abutment connection surfaces are machine made while prosthetic structures may be machined or cast. In general, more regular surfaces and precise marginal adaptations can be achieved with machined components in comparison with in comparison with cast ones. On the other hand, independent parts connected by screws are prone to micro-movements, which may alter the adaptation between components and inevitably originate hollow spaces (Steinebrunner et al., 2005). In some systems the abutment is connected to the implant using a cementing agent, which fills the existing interfacial gaps (Piattelli et al., 2001; Scarano et al., 2005). Jansen et al. (1997) have demonstrated that the presence and sizes of gaps between implants and abutments may vary according to the type of connections and the structural characteristics of the abutments. Vertical marginal discrepancies between implant and abutment of approximately 0 to 10 µm (Jansen et al., 1997; Bondan et al., 2009) and horizontal average discrepancies of 60 µm (Byrne et al., 1998; Kano et al., 2007) have been observed. Even wider variations were found when methodological differences are considered, particularly in comparisons involving unitary and multiple prosthetic elements. Although considerably smaller than the spaces between implants and cast or synterized ceramic crowns, discrepancies between machined components are still larger than the corpuscular dimensions of various bacterial species.

The hollow spaces between implant and abutments may act as reservoir for commensal and/or pathogenic bacteria, especially anaerobic or microaerophilic species, representing a potential source of tissue inflammation. Hence, microbial colonization of the interfacial gaps may ultimately result in bone resorption (Quirynen et al., 1990; Quirynen et al., 1994; Mombelli et al., 1995).

Several *in vivo* and *in vitro* studies have shown bacterial leakage through the implant-abutment interface, either from the external sites to the inner parts of the implants or vise-versa (Quirynen et al.,1994; Jansen et al., 1997; Rimondini et al., 2001; Steinebrunner et al.,2005; Callan et al., 2005; do Nascimento et al., 2009a; Cosyn et al. 2009; Aloise et al., 2010). Other studies, have demonstrated the leakage of dyes and/or bacterial endotoxins through the implant-abutment interface (Gross et al., 1999; Piattelli et al 2001).

Bacterial contamination of two-part dental implants has been well described in non-loading conditions but several questions remain on the biomechanical principles that control the whole system during masticatory function (Brunsky et al. 2000). Lack of component

adaptation and passive connection are potential causes of mechanical damages, like screw loosening and fracture, as well as the development of mucositis and peri-implantitis due to biofilm retention (Quirynen et al., 1994; Mombelli et al., 1995).

Mechanical loading of the prosthetic abutments is another factor that may affect adaptation of implant and components and in consequence, leakage of microorganisms through the implant-abutment junction. Reports by Steinebrunner et al. (2005) indicate that bacterial penetration between implants and abutments connected by screws varies according to the number of loading cycles applied to the abutment. The leakage of microorganisms and fluids to the inner parts of the implants is considerably increased by load application, which generates micro-movements and interfacial gaps (Khraisat et al., 2006).

Bacterial species harboring the internal surfaces of the implants and components is not surprising, since the size of oral bacterial species ranges in average from 1.1 to 1.5 μm in length and 2 to 6 μm in diameter. Smaller species like spirochetes (diameter of 0.1 to 0.5 μm) may also be found in the oral microbiota (Jansen et al., 1997).

The presence of bacterial contamination of implants and components has traditionally been detected by conventional microbiological cultures, which have inherent deficiencies, particularly in the identification of fastidious species and strict anaerobes (Rolph et al., 2001; Moraes et al., 2002).

The last two decades witnessed the development and extensive use of new molecular techniques to detect, identify and quantify microbial species dwelling in the oral cavity. These rapid, sensitive and specific techniques revealed an enormous, hitherto unknown, microbiota. (Sakamoto et al., 2005; Haffajee et al. 2009). Improvements in new implants systems are being rapidly developed, for instance in their connection mechanism, surface treatment and physicochemical properties.

Considering the increased use of dental implants in restorative dentistry and recognizing the impact of bacterial leakage along the implant-abutment interface on the health of peri-implant tissues, this chapter intends to review and discuss the literature on the subject.

2. Biofilms and bacterial colonization of dental implants

2.1 Biofilm formation

Bacterial colonization of the the oral cavity in humans starts at birth and remains constant through life (Carlsson et al., 1970; Rosan & Lamont, 2000). A saliva film is initially formed composed basically of a cell-free matrix and proteins. Subsequently, bacterial colonization of this film is followed by selective adhesion on the different substrates (Gibbons, 1984). Large quantities of lactobacillus spp, responsible for biofilm adhesion, and streptococcus spp (mainly *S. sanguinis, S.oralis, S.mitis* and *S. sobrinus*),which promote biofilm growth, are initially found. Actinomyces spp and Gram-negative species are found in low proportion at this phase (Rosan & Lamont, 2000), and cell viability and adhesion capacity are fundamental requirements for the success and keeping of bacterial colonization (Gibbons & Van Houte, 1975).

A variety of bacterial species are transitory in the oral cavity. However, the characteristics of existing surfaces may facilitate adherence and prolong their permanence. Indeed, there are reports suggesting that some of the colonizing strains may remain stable for years while others may fluctuate (Alaluusua et al., 1994; Emanuelsson & Thorqvist, 2000). The profile of the oral microbiota is shaped, in addition to environmental factors, by significant interactions between bacterial species, inhibiting or stimulating each other (Grenier & Mainard, 1986). Bacterial antagonism with predominance of pathogenic species may be the

determinant of health or disease status of the supporting tissues (Hillman et al., 1985; Socransky et al., 1988).

According to Quirynen et al. (2002), gaps in the implant-abutment interface may act as a trap for bacteria, favoring the development of biofilm with varying composition and impact on periodontal tissues. *Agregatibacter actinomycetemcomitans*, *Tannerella forsythia and Porphyromonas gingivalis*, many times present in these biofilms, are pathogens intimately related to the development and maintenance of periodontitis and peri-implantitis. Other pathogens with relevant participation in these diseases are *Prevotella intermedia, Campylobacter rectus, Peptostreptococcus micros, Fusobacterium nucleatum, Eubacterium nodatum, Streptococcus intermedius* and spirochetes (Quirynen et al., 2002; Socransky & Haffajee, 2002).

Periodontal disease affects tissues that support teeth and is characterized by loss of periodontal ligament insertion, and resorption of adjacent alveolar bone. This disease has multifactorial etiology, which includes biofilms as having an essential role in its pathogenesis (Lamont & Jenkinson, 1998). The term peri-implantitis, established in the Periodontia European Workshop, 1993, characterizes diseased implant supporting tissues (Albrektsson & Isidor, 1994). The initial bacterial colonization of peri-implant sulci is characterized by an increasing number of facultative anaerobic *streptococcus*, although Gram-negatives anaerobes may be occasionally found but in smaller numbers (Monbelli et al., 1988). With time, strict gram–negative anaerobes as *Fusobacterium spp and Prevotella spp* become increasingly predominant (Mombelli & Mericske-Stern, 1990).

Several reports on the microbiota detected around dental implants were published in the last decade (Haffjee et al .,1998; Botero et al., 2005; Cosyn et al., 2009; Van Brakel, et al. 2010). Presence of potential periodontal pathogens colonizing the peri-implant grooves in unsuccessful implants has also been described (Laine et al., 2005; Shibli et al., 2007; Persson et al., 2010). Other studies suggested that periodontal pathogens and/or their metabolic products might be involved in peri-implant bone losses (Lindhe et al., 1992, Persson et al., 1996). However, information on the diversity of bacteria colonizing the internal surfaces of two-part dental implants, abutments and implant prostheses and on their correlation with periodontal and peri-implantar sulci species is still lacking. . Cosyn et al. (2009), in a short clinical evaluation, have found similarities in the microbiota of samples collected from the internal parts of implants and from their related peri-implant sulci.

Another investigations indicated that the existing oral microbiota prior to implant placement is determinant to the establishment and maintenance of the implant related microbiota.. Partially edentulous patients with a history of periodontal disease show high incidence of putative periodontal pathogens in implant sites (Mombelli et al., 1995; Quirynen et al., 2002, Quirynen et a., 1996a). Laine et al. (2005), reported that the bacterial biofilm composition in the peri-implant sulcus changes during the healing period following implantation and shows constant alterations subsequently. Pathogenic and non-pathogenic species have been found surrounding peri-implant sites (Callan et al., 2005; Quirynen et al., 2006). De Boever & De Boever (2006) suggested that periodonto-pathogenic species isolated from the implant sites during the first 6 months after placement there might be no clinical consequence. However, the presence of these pathogens in the immediate healing phase may have future consequences. Bacterial species found in the wound healing of implants that failed immediately after surgery are similar to the ones seen in acute infections (Laine et al., 2005). Lately, when the implants have been exposed to oral microorganisms for a long

period, the microbial biofilm changes into a pattern similar to the one found in chronic periodontal disease (Mombelli & Lang, 1998).

2.2 Design features and marginal fit of implant components

One of the most common causes of dental implants failure is poor adaptation of implant and prosthetic components, irrespectively of the implant system and connection design. Notwithstanding the constant efforts to overcome this drawback, the hollow spaces produced by poor adaptation act as traps for bacteria of the oral cavity resulting in inflammatory reactions of the peri-implant tissues (Mombelli et al., 1995; Quirynen et al., 2002). Both the microorganism and their metabolic products may be responsible for inflammation and bone loss (Lindhe et al., 1992; Quirynen & Van Steenberghe, 1993). Ultimately, uncontrolled local inflammation may lead to generalized peri-implantitis and compromise the long-term success with dental implants (Ericsson et al. 1995).

Two-part dental implant systems with screw-retained abutments are still largely used, principally due to their well reported protocol, high success rates, and broad spectrum of indications. However, gaps and cavities in the assemblies after abutment attachment are frequently related to peri-implant tissue problems. Figure 1 illustrates the gaps resulted from attached components of an implant system. These gaps favor bacterial biofilm accumulation between components, generally in the interfaces implant-abutment, abutment-prosthesis, and on the exposed surfaces of abutments, prosthesis and implants to the oral environment. Under loading, where the abutment components are subjected to eccentric forces, the number and size of gaps can be augmented. Moreover, micro-movements of implant components during function may allow the initiation of a pumping effect that facilitates bacterial leakage through the implant-abutment interface (Steinebrunner et al., 2005). Also, the abutment screw loosening may contribute to joint instability, screw fracture, and clinical failure. Increased percentages of abutment screw loosening and the consequent increase in micro-movements between components are more common in implants with external hexagonal connections (Binon et al., 2000).

Sahin & Cehreli (2001), in a revision study, evaluated the clinical significance of the passive fit on the final marginal fit of implant-supported restorations. The authors recommend that the implant-abutment assembly should result in a passive connection, not inductive of tension in implant components and adjacent bone. However, according to the authors, this is not possible since clinical and laboratory procedures utilized in the fabrication of the super structures are not adequate. The lack of dimensional precision and may introduce strains to the fixation screws, potentially causing screw or abutment fracture.

Studies on the adaptation between implant and components have been related their failed adaptive conditions to the presence of bacterial infiltration (Jansen et al., 1997; Steinebrunner et al., 2005). Quirynen & Van Steenberghe (1993) investigated the presence of microorganisms in the internal screw threads of Branemark implants. In nine patients, the apical part of two intermediate screws installed three months before was examined through contrast phase microscopy, showing a significant quantity of microorganisms, mainly coccus (86.2%) and nonmotile rods (12.3%). Motile species (1.3%) and spirochetes (0.1%) were scarce. Another similar study observed a microbiota mainly composed of anaerobes and facultative streptococcus, Gram-positive anaerobic rods such as *Propionibacterium, Eubacterium and Actinomyces* besides Gram-negative anaerobes, including *Fusobacterium, Prevotella and Porphyromonas* (Persson et al., 1996).

Several implant systems with different designs are currently available in the market. The Figure 2 and 3 illustrate three different implants and platform connections of an implant system. They have different shapes, surfaces, sizes and distances between screw threads, as well as different types of connections between implants and abutments. The external hexagonal platform proposed by Branemark et al. (1969), was until recently the most widely used and well documented. The system has a connecting hexagonal platform acting as an anti-rotational mechanism that is, together with the connecting screw, responsible for the mechanical stability of the implant-abutment set. Despite the wide acceptance and use, the system shows an external connection concentrating considerable more strength in the threads of the abutment screw, which may lead to fractures and implant failures (Norton et al., 1997; Finger et al., 2003; Bernardes et al., 2009). This type of connection shows the highest vertical and horizontal implant-abutment discrepancies described in the literature (Binon et al., 1996).

Studies on the precision of the connection between components of different systems showed that some can be interchanged with an accuracy similar to the one observed in connections with components of the same system (Hagiwara et al., 1997; Dellow et al., 1997).

Scanning electron microscopy examinations of the implant-abutment interface, showed vertical and horizontal maladaptations between implants with external hexagonal connections and their respective abutments varying, on average, from 1 to 100 µm, (Binon et al., 1996; Jansen et al., 1997). In general, machined abutments show superior adaptation in relation to laboratory cast ones, suggesting that stricter standard controls should be held on components obtained by casting procedures (Byrne et al., 1998). The type of metal alloy in cast components has also a relevant role on the final adaptation of the assemblies. Components cast in Ni/Cr or Cr/Co are more variable in respect to the marginal dimensional stability when compared with Au or Ag/Pd alloys cast components (Binon et al., 1995; Byrne et al., 1998).

Bone crest changes around implants were studied in dogs by Hermann et al. (2001), by observing the influence of marginal gaps in the implant-abutment interface and occurrence of micro-movements. Gap sizes were in the range of 10-100 µm and micro-movements were abolished in some groups by laser welding of interfaces The results showed that resorption in crest bone was significantly influenced by movements between abutments and implants but not by gap sizes along the interface.

New connection designs were proposed to overcome the drawbacks of the hexagonal precursor system. Hexagonal platforms with larger interfacial area as well as internal connection systems with varied shapes were developed to improve adaptation and stability of the implant components. However, in contrast to the well-standardized features of the external hexagonal precursor, the internal connections are usually unique for each developer and thus difficult to standardize.

Clinical and in vitro evaluations indicate that connections between components are more stable in the internal hexagonal system, in which the larger contact area between internal implant and abutments walls favors force distribution and preserves the abutment screw (Norton et al., 1997; Mollersten et al., 1997; Binon et al., 2000; Finger et al., 2003). Microbiological studies, however, do not show significant differences between hexagonal internal or external connections, when bacterial leakage is concerned (Jansen et al., 1997; Steinebrunner et al., 2005; Duarte et al., 2006).

Fig. 1. Histological Photomicrograph showing the gaps and hollow spaces (arrows) resulted from the poor adaptation of the implant and prosthetic components in the two-part implant systems (MKIII TiUnit and it respective abutment, Nobel Biocare- regular external hexagonal platform with 3.75 mm in diameter and 7 mm in length).
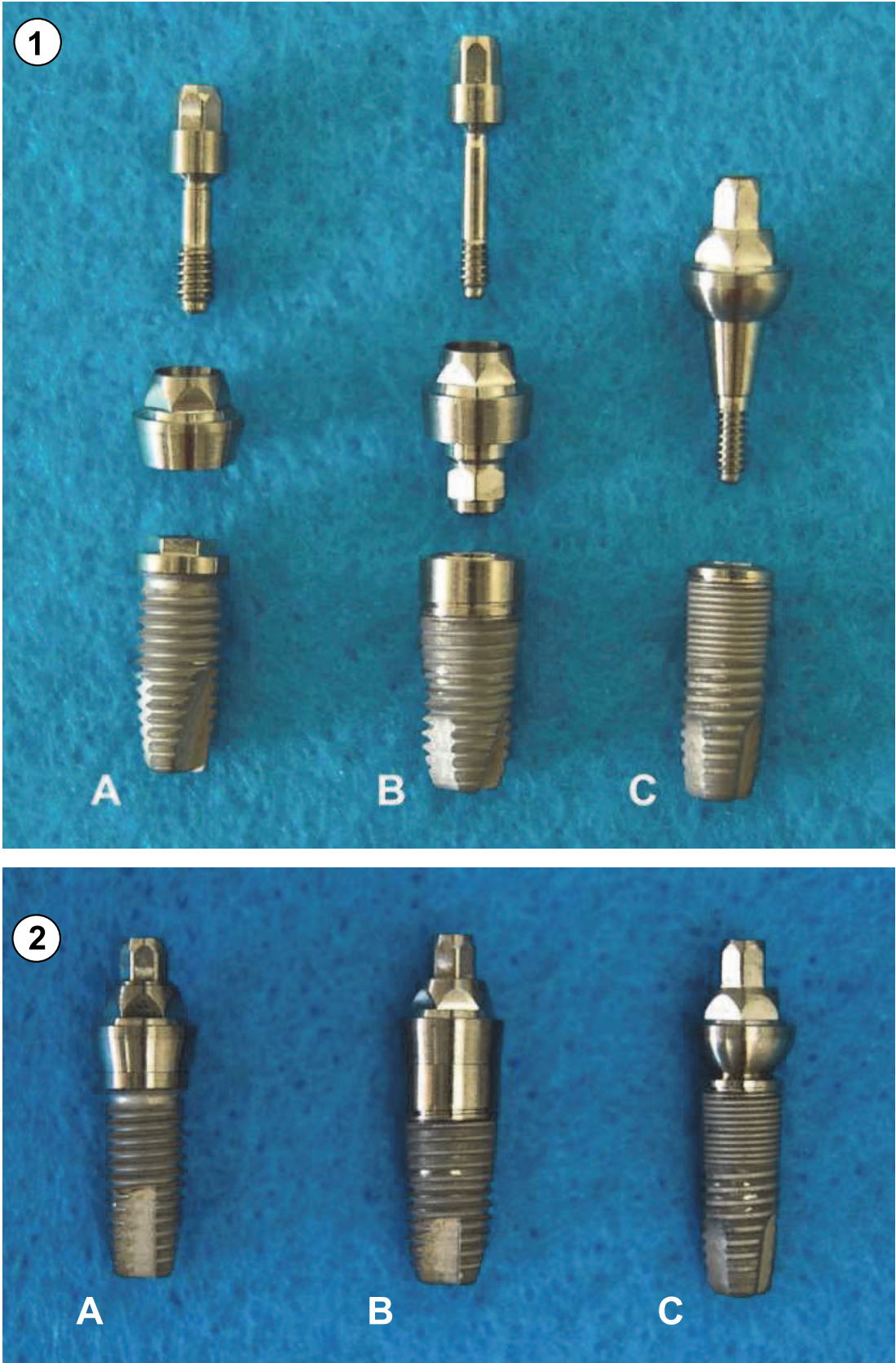
Fig. 2. Illustration showing 3 different implants and abutments before (1) and after (2) connection: (A) External Hexagonal, (B) Internal Hegonal and (C) Morse Cone.

The Morse cone connection system has recently been introduced and is becoming increasingly popular in implant dentistry. Due to its tapered design, the system is considered mechanically more stable and efficient in preventing bacterial leakage. The Morse cone connection, compared with other types, is more efficient in the dissipation of forces exerted on the prosthesis and consequently on the supporting bone tissue (Merz et al., 2000). It does not completely inhibit the passage of bacteria and fluids through the interface, but microbiological evaluations show that there is a more efficient bacterial sealing between the Morse cone implant and its abutment, revealed by lower bacterial counts in comparison to other connection systems (Pautke et al., 2009; Aloise et al., 2010). The intimate adaptation between contacting surfaces obtained with this type of connection seems to produce a frictional locking, which restrains micro-movements. Concurrently, minimized spaces at the interface seem to be related to decreased levels of bacterial contamination, probably by preventing leakage and reducing the available volume for bacterial growth. Further investigations are necessary, however, in order to determine the biomechanical behavior of these components in long-term studies, since most of these findings were obtained in short term studies.
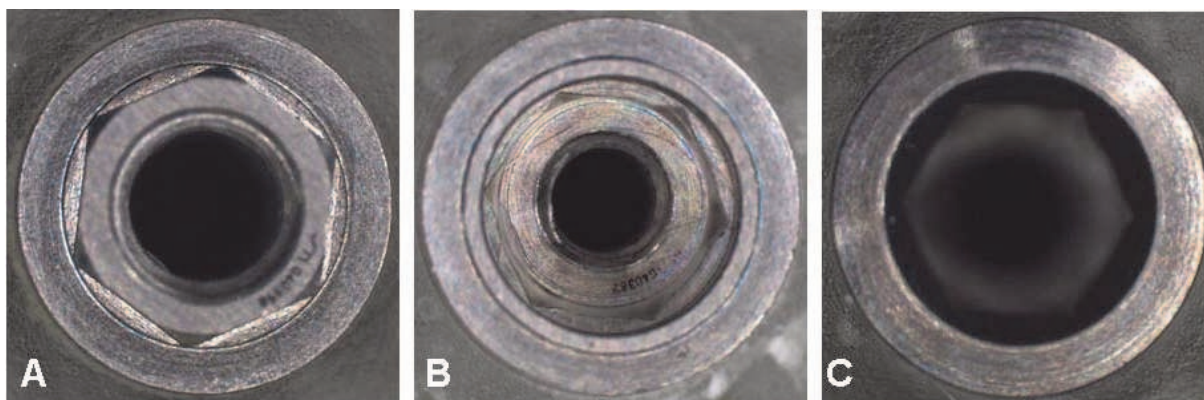

Fig. 3. Microscopy picture showing the External Hexagonal (A), Internal Hexagonal (B) and Morse Cone (C) platform.

2.3 Abutment materials

Peri-implant soft tissues act as a protection barrier between the oral cavity environment and the peri-implant bone (Welander et al., 2008). The type of material used in the abutment fabrication seems to be crucial in determining the final quality of the mucosa in contact with theabutment surface (Abrahamsson et al., 1998). It has been observed that when titanium abutments are used, the surrounding mucosais mainly composed of epithelial and connective healthy tissues. Healthy soft tissues should promote an effective barrier against the passage of microorganisms and/or their products, preserving the adjacent bone from being damaged (Welander et al., 2008).

Precious and basic metals, as well as ceramic materials, are also used in the manufacture of abutments. Ceramic materials, such as zirconium dioxide, or simply zirconia, are popular materials, increasingly used in prosthetic abutments. They are similar in color to dental structures and have potential advantages over metallic materials (Brodbeck, 2003; Watkin & Kerstein, 2008) besides other properties such as higher translucency, (Denry & Kelly, 2008), good tissue adhesion (Pessková et al., 2007), less tissue discoloring effect (Bressan et al., 2010), lower bacterial adhesion and growth (Scarano et al., 2004) and lower toxicity (Uo et al., 2003).

Zirconia has excellent mechanical properties besides being material of choice for esthetic and biological reasons. It has high resistance against fractures (Anusavice et al., 2007; Manicone et al., 2007; Denry & Kelly, 2008) which was demonstrated in short term (3-4years) *in vivo* studies with satisfactory clinical results (Glauser et al., 2004; Canullo et al., 2007; Ekfeldt et al., 2011).

Although abutments with esthetic properties are continually sought, conclusive clinical evidence of their ability to maintain healthier periimplant tissues is still lacking in the current literature (Linkevivius & Apce, 2008). Few studies have compared bacterial adhesion in metallic and non-metallic components. While some authors claim that bacterial adhesion is lower in zirconia components (Scarano et al., 2004), others show that there is no difference between zirconia and titanium components (Salihoglu et al., 2010). Results from a study in animals (Abrahamssom et al., 1998) and a recent one in humans (Van Brakel et al., 2010), indicate that zirconia and titanium abutments may exert a similar healthier effect on peri-implant tissues.

On the other hand, the use of zirconia abutments in esthetic components can be limited in cases of cemented prosthesis, due to the low adhesion of the cement to the ceramic material, although the use of recently developed primers may minimize the problem. Another recent proposal to improve the treatment outcome with implants by taking advantage of the best properties of different materials is the use of hybrid connection systems, composed of titanium abutments surrounded by a zirconia-overlaid collar. Future studies are expected to evaluate the biomechanical behavior of these systems.

2.4 Effects of surface characteristics (chemistry, free energy and roughness)

The surface physico-chemical properties of implants and abutments, like roughness, chemical treatments and free energy have an important role in the formation and maintenance of bacterial biofilms (Grossner -Screiber et al., 2001).

Bacterial adhesion to hard surfaces in the oral cavity is highly influenced by rugosity, which in turn is related to the number of colonies formed (Quirynen et al., 1996b). Scanning electron microscopy analysis of bacterial behavior on different materials used in implant and prosthetic components, indicated that rough surfaces have higher indexes of bacterial adhesion (Grossner-Schreiber et al., 2001; Amoroso et al., 2006). Implants and components showing an average roughness lower than 0.1 μm partially inhibited bacterial biofilm formation and growth after 24 hours (Rimondini et al., 1997). High surface rugosity and consequent high hydrophobic properties (low wettability) tend to favor biofilm formation and bacterial adhesion (Drake et al., 1999). Steam-autoclave sterilization of titanium increases the oxide layer thickness, accumulation of impurities on the metal surface, and hydrophobicity, as shown by Drake et al. (1999). The authors demonstrated that surfaces that were steam-autoclaved 10 times showed significantly greater CFU/mm2, specially of *S. sanguis*, than other methods of sterilization, regardless of surface roughness. .

Surface treatment of components has a relevant impact on bacterial adhesion and colonization. Titanium implants treated with titanium nitrate (TiN) or zirconia nitrate (ZrN) followed by thermal oxidation or laser irradiation produced significant reduction of biofilm formation and bacterial adhesion when compared to surfaces of polished titanium (Grosner-Schreiber et al., 2001). Other surface treatments proposed to inhibit microorganism adhesion such as, antibacterial coverings (Ge et al., 2010), electro-chemical treatments (Visai et al., 2008), and new metallic alloys (Pautke et al., 2009) still need further evaluations to warrant applicability.

Biofilm formation is directly, but less intensely, related to component surface free energy (SFE) (Burgers et al., 2010). SFE results from the interaction of cohesion and adhesion forces, responsible for the wettability property of surfaces. SFE facilitates accumulation of bacterial biofilm, by promoting firm adhesion to contact surfaces besides selecting specific bacterial species, a property intimately related to the acid-basic characteristics of bacterial cell walls. In a literature review, Quirynen & Bollen (1995) conclude that smooth surfaces with low SFE can potentially reduce the occurrence of caries and periodontitis. In contrast, rough surfaces promote plaque formation and maturation, and high-energy surfaces are known to collect more plaque and to select specific bacteria.

3. Culture and culture-independent methods focusing on oral microbiota of dental implants

Identification of microorganisms inhabiting peri-implant crevices and the internal parts of implants has been of relevant importance in respect to the outcome of the treatment with dental implants, since several studies showed a correlation between bacterial species of the oral cavity, especially those involved in periodontal diseases, and the occurrence of failure in the treatment with implants (Ong et al. 1992; Shibli et al., 2007). Periodontal pathogenic bacteria in peri-implant crevices and teeth with periodontitis close to dental implants are considered risk factors for the success of dental implants (Gouvoussis et al., 1997; Saito et al., 1997). To date, a large number of microbial species related to periodontal and peri-implant diseases have been identified and can be quantified by different methods.

3.1 Conventional cultures

Bacterial culture is a well-known method historically used to characterize the oral cavity microbiota, and considered a classical reference method in microbiology. Traditionally, culture-dependent methodologies are used to isolate, enumerate and detect probiotic organisms, especially from mixed cultures (Charteris et al., 1997). Several variables in culture technology, especially an appropriate sample collection technique and media selection, have been recognised as having a significant impact on the sensitivity and specificity of the test, mainly on the organism recovery rates and time for reporting results (Riedel & Carroll, 2010). This method constitutes an important epidemiological tool, with results that serve as a base for building an empirical therapeutic strategy. Also, this methodology is essential in the initial phase of several culture-independent techniques, where bacterial growth and isolation is necessary to DNA probes confection.

These methods are essentially designed around the recovery and (or) enumeration of viable bacteria in the contaminant media. Detection of viable bacteria is traditionally performed by implementing a means of culturing growth of individual species. The use of non-selective media such as trypticase soy agar or standard methods agar, known as the aerobic or standard plate count, is routinely applied in this methodology. In addition, in specific conditions, the increased sensitivity of these standard media has been achieved using a selective agar overlay approach designed to recover a larger proportion of bacteria from contaminant media (Specket et al. 1975; Harrigan, 1998).

Most studies describing the microbial leakage through the implant-abutment interface are based on results with conventional culture method (Jansen et al., 1997; Piatelli et al., 1999; Steinebrunner et al., 2005; Pautke et al., 2009; Aloise et al., 2010). However, an inherent limitation of microbiological cultures is that the difficulties to identify strict anaerobes,

frequently associated with periodontal and peri-implant diseases, as well as fastidious species (Barbosa et al., 2009; Roças et al., 2010). It is estimated that 50% of the oral microbiota is not cultured by conventional methods and several of these species are directly related to infectious processes in the oral cavity (Arank et al., 1969; Paster et al., 2001; Parahitiyawa et al., 2010). Furthermore, non-viable cells, still able to produce aggressive compounds against peri-implant tissues are not detected by culture methods.

Despite of the efforts to optimise broth composition, enhance the growth of microorganisms and prevent contamination during procedures, the methodology is time-consuming and the microbial viability is essential to confirm the presence pathogens. Cell killing and degradation by bacteriocin as well as degradation of DNA by proteolytic enzymes and endonucleases has been demonstrated in several studies (Loyola-Rodriguez et al. 1992, Cowman & Baron 1993, Cascales et al. 2007). These substances may cause deleterious effects on the peri-implant support tissues. Therefore, despite the advantages of these familiar culture methods in detecting viable bacteria, such as ease of use and low cost, assay sensitivity is still relatively low compared with alternative methods (such as molecular-based approaches).

Quantitative bacterial measurements are widely used in microbiology. Many years of research studies using quantitative microbiology on solid media have demonstrated that such measurements provide clinically valuable information. For example, bacterial load is predictive for the occurrence of complications (Yagupsky & Nolte, 1990). However, the bacteria quantitation in conventional culture method is difficult to achieve and is rarely practised in clinical laboratories because it requires subsequent plating on solid media rather than incubation in liquid media. The time required for liquid culture bottles to become positive provides some suggestion of bacterial load, but is a weak quantitative measure and varies with the microorganisms present. Also, each bacterial must be individually evaluated with a specific media.

3.2 Culture- independent methods

In the last two decades great advances in molecular diagnostic methods were achieved, which have been extensively used in the detection and identification of microbial species inhabiting the oral cavity (Sakamoto et al., 2005; Haffajee et al., 2009; Costa et al., 2010). These techniques are more rapid, sensitive and specific when compared to the conventional culture methods. Species showing diverse phenotypic behavior may be identified by their genomic characteristics, which are not dependent on cell viability, a great advantage in studies evaluating anaerobic infections, when cell death may occur during sample collection or transportation (Whelen & Persing, 1996; Pitt & Saundres, 2000). These techniques have also promoted advances in the knowledge of the microbiota in other parts of the human body (Eckburg et al., 2005; Dethlefsen et al., 2007; Grice et al.,2008; Oakley et al., 2008) revealing a great quantity of bacterial species not cultured, whether associated or not to infectious processes (Turnbaugh et al., 2007; Mallard, 2008)

3.2.1 Checkerboard DNA- DNA hybridization

Methods based on cellular DNA characterization, used for microbial detection and quantitation have been documented in the current literature. The Checkerboard DNA hybridization technique utilizes genomic DNA probes to identify and quantify several bacterial species simultaneously in a great number of samples from the oral cavity, and has been largely employed in studies on the oral microbiota (Socransky et al., 1994). Several

areas in dentistry have recently been using the technique to evaluate the composition of bacterial biofilms in health or disease conditions (Aberg et al., 2009; Teles et al., 2010; Kim et al., 2010, Vettore et al., 2010), and in studies on the association of local and systemic factors that can affect biofilm formation (Borges et al., 2009; Demmer st al., 2010). The technique is also employed to evaluate bacteria associated with endodontic lesions (Roças & Siqueira, 2010) and to verify changes in biofilm composition as a result of periodontal treatments (Haffajee et al., 2009).

More recently the DNA hybridization technique has been used to identify and quantify multiple bacterial species associated to dental implants. *In vitro* studies show that several bacterial species colonizing the internal surfaces of implants may be consistently identified and quantified (do Nascimento et al., 2009; Barbosa et al., 2009). The DNA Checkerboard method is significantly more sensitive in the bacterial detection than conventional cultures according to Loesche et al. (1992). Barbosa et al., (2009) compared the two methods in an *in vitro* study involving the investigation of *F. nucleatum* in the interior of implants with an external hexagonal connection. The microorganism counts were significantly higher with the DNA –Checkerboard method leading to the conclusion that it is more sensitive than conventional cultures and allows identification of bacteria that are viable or not. Considering that bacterial cell structure and its degradation products may act as nutrients to other opportunistic species, the identification of non viable species may be relevant to risk determination associated to peri-implant tissues.

3.2.2 PCR-based techniques

Other molecular diagnostic methods utilize amplified genetic material by the Polymerase Chain reaction (PCR). These methods are more specific and sensitive and may be useful to complete results obtained by DNA hybridization. PCR-based techniques are very sensitive and specific allowing the identification of species in the interior of implants even when they are present in very small quantities (Haffajee et al., 2009). Susceptibility of contamination due the amplification procedure is the main limitation of this method.

PCR methods involve the enzymatic synthesis of a specific DNA segment of the target species by DNA polymerase. The reaction develops the annealing and enzymatic extension of an oligonucleotide pair utilized as initiators, the so-called primers, which delimit the DNA sequence of the double strand targeted by the amplification. Three variants of the technique can be employed in dentistry to identify bacterial species in the oral cavity: PCR (conventional or qualitative), RT_PCR (Real time PCR or Quantitative PCR) and 16SrDNA-based PCR.

The high specificity of amplification by the qualitative PCR technique discriminates numerous species of microorganisms in the oral cavity, including pathogens not easily detected by other methods. However, detection is limited to the plateau reaction and it is unable to determine the precise number of bacteria present in a determined site (Higushi et al., 1992; Jervoe-Storm et al., 2005). Real-Time PCR precisely quantifies the species in the study. The number of product molecules synthesized in this technique depends directly on the number of molecules used as standards. Quantification data are collected in the exponential phase of PCR, producing a precise quantification of the number of target DNA copies when internal and external standard are used. Real Time PCR has been used as a complement to conventional cultures or DNA Checkerboard to quantify main periodontal pathogens, as for instance, *P. gingivalis, A. actinomycetemcomitans, T. denticola* and *T. forsythia.*

The sequencing of the DNA ribosome gene (rDNA) 16S, found in the bacteria genome, has recently been used to evaluate the microbial diversity of the oral cavity (Gu et al., 2009), esophagus (Macfarlane et al., 2007), stomach (Li et al., 2009), intestine (Hill et al., 2010, colon (Mäkivuokko et al., 2010) and vagina (Oakley et al., 2008). In contrast with molecular methods, which employ DNA probes, like the DNA Checkerboard, this methodology identifies large populations of non-cultured species, many times associated to the disease conditions. Through these methods it was possible to characterize the subgengival microbiota of the periodontal pouch as being composed of more than 700 diverse species isolated from different individuals. The great majority does not have a DNA totally identified and sequenced and, thus, cannot be investigated by methods using DNA probes (Roças et al., 2010).

4. Conclusions

Implant materials, connection systems, and surface properties have all relevant impact on the initial formation of biofilm and on the characteristics of the peri- and intra- implant microbiota. These factors can influence bacterial adhesion to implants and prosthetic abutments, with varied impact on the peri-implant tissues, and may interfere on the success with this type of treatment.

More stable connections, showing smaller and less frequent free spaces after component adjustments, have shown to be more efficient in containing bacterial leakage through the interfaces. Modifications of the titanium surfaces, or the surfaces of other materials used in the manufacture of implants systems, may reduce biofilm formation and consequent outcomes. Diagnostic techniques currently used to evaluate bacterial contamination of implant related structures have specific characteristics and applications, and thus, should be considered as complementary.

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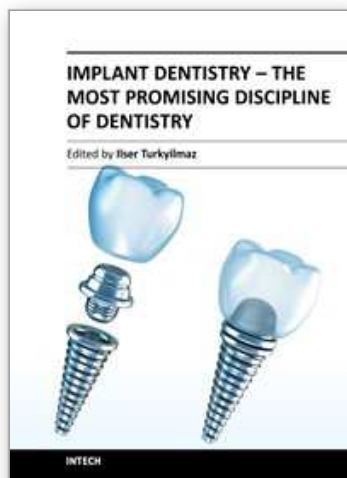
Implant Dentistry - The Most Promising Discipline of Dentistry

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