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Candida Biofilms on Oral Biomaterials

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1. Introduction

Biological as well as inert surfaces of the oral cavity are exposed to an abundant microflora that is able to initiate the formation of biofilms. Yeasts are frequently involved, such as *Candida* (especially *albicans*), a low-level commensal of oral, gastrointestinal, and genitourinary mucosae in humans. *In vivo* and *in vitro* studies have shown *Candida* incorporation into biofilms covering different biomaterials used in the oral cavity for the manufacturing of dentures, orthodontic appliances, etc. Yeast (*Candida* genus) biofilms can then induce device-related infections mainly in the elderly and in medically-compromised patients with subsequent morbidity and occasional mortality, all bearing high social and financial costs. Generally, scientific literature does not integrate all aspects of material/tissue interfaces: mechanisms of *Candida* biofilm development and biomaterial maintenance, the welfare of patients, and prevention of candidosis. *In vitro* investigations were mainly undertaken with mono-species biofilms whereas *Candida* incorporation into biofilms on oral surfaces tends to correspond with an increase in the yeast/bacteria ratio. This illustrates the need for interdisciplinary insight. This chapter will review 1) the literature data concerning material surfaces in support of *Candida* biofilms in the oral environment, 2) the *in vitro* approaches to understanding the mechanisms of *Candida* biofilm formation on materials, 3) the interfaced manipulations in order to prevent *Candida* biofilm onset, and 4) the precautions when testing new devices *in vivo* in the oral cavity.

2. Materials as a support of *Candida* biofilm in the oral environment

Biomaterials placed in the oral environment offer new surfaces prone to biofilm formation. Rough surfaces allow more biofilms to develop than smooth ones. In contrast with free microorganisms in suspension (defined as planktonic), which are able to grow in liquid, biofilm development is theoretically divided into three stages: 1) attachment to the surface (Figure 1), 2) proliferation into a monolayer of anchoring cells, and 3) growth into several layers of budding cells (blastoconidia) with filamentous structures as hyphae or pseudohyphae (Figure 2).

Numerous studies indicated the presence of *Candida* on oral dentures (Vandenbussche & Swinne, 1984; Abu-Elteen & Abu-Elteen, 1998; Busscher et al., 2010) and other oral devices such as orthodontic appliances (Addy et al., 1982; Hägg et al., 2004). Some authors (Arendorf & Addy, 1985; Jewtuchowicz et al., 2007) demonstrated an effect of *Candida* carriage in the oral environment caused by wearing devices. Indeed, orthodontic appliances

have been shown to increase *Candida* counts in the mouth (Arendorf & Addy, 1985) and in the periodontal pockets of dental device wearers with gingivitis (Jewtuchowicz et al., 2007). Inserted devices act as new reservoirs able to imbalance oral microflora. In a healthy mouth, saliva protects oral mucosa against candidosis; in contrast, dry mouth is associated with increased yeast counts and candidosis risk. *In vitro*, cigarette smoke condensates increased adhesion of *Candida albicans* on orthodontic material surfaces such as bands, brackets, elastics, and acrylic resin (Baboni et al., 2010).

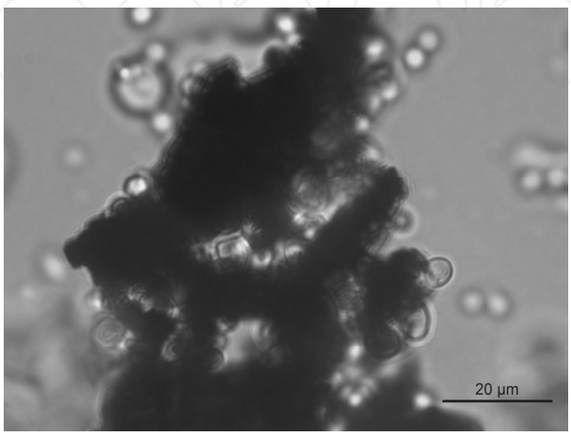

Fig. 1. *Candida albicans* suspension mixed with titanium powder directly observed on microscope in the absence of any stain procedure. Some blastoconidia are already adherent to material (attachment can be attested by MTT test after 3 washings). Magnification: x1000.

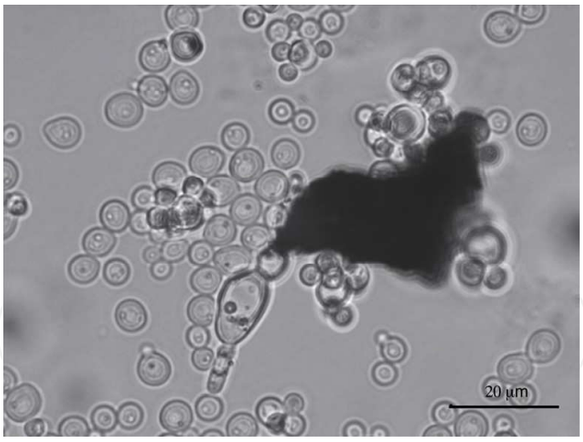

Fig. 2. Titanium grain surrounded by blastoconidia clusters and filamentous structure (pseudohyphae) after a two-week incubation. Magnification: x1000.

2.1 *Candida* on dentures

Candida albicans is often detected on methyl methacrylate polymers or acrylic resins from dentures. Biofilm formation on dentures results from complex interactions among yeast, bacteria, nutrients, and saliva or even serum proteins (Nikawa et al., 1997; Nikawa et al., 2000). *Candida* carriage on acrylic resin has been reported in the literature as varying up to more than 80% of the investigated dentures. For instance, yeasts were found in 14% of

isolates from previously worn dentures in the Northeast and Southwest regions of the United States (Glass, 2010); in their conclusions, the authors pointed out frequent denture use without appropriate disinfection and biofilm formation within the pores of the material.
In a previous study (Vanden Abbeele et al., 2008), authors reported *Candida* contamination of upper prosthesis in 76% of denture wearers hospitalized for long-term care in geriatric units. The most frequently isolated species were *C. albicans* (78%), *C. glabrata* (44%) and *C. tropicalis* (19%). Carriage of more than one yeast species was found in 49% of the contaminated dentures. There was a significant association between denture contamination and palatal mucosa colonization, making *ex vivo* denture decontamination mandatory, together with *in vivo* mucosa disinfection. *Candida* carriage has been observed in different types of dentures, both with and without soft liner fittings (Bulad et al., 2004; Mutluay et al., 2010).
Adhesion of *Candida* to the base materials of the dentures is associated with denture plaque (i.e. denture biofilm) and denture-related stomatitis. Even if many observations support the presence of *Candida albicans* in the biofilms on dentures, insufficient data are available to assess the etiology and to understand the pathogenesis of *Candida*-associated denture stomatitis. Review of the literature (Radford et al., 1999; Pereira-Cenci et al., 2008) does not permit settling specific and non-specific plaque hypotheses. Indeed, denture plaque comprises an ill-defined mixture of bacteria (such as *Streptococcus spp*., *Lactobacillus spp*., *Staphylococcus aureus*, and Gram-negative anaerobic bacteria) with *Candida spp*. also apt to cause mucosa inflammation.

2.2 *Candida* on other materials inserted in the oral cavity

Candida spp. was detected in low proportions at peri-implantitis sites and in failing implants associated with periodontopathogenic bacteria such as *Porphyromonas spp*., *Prevotella spp*. and *Actinobacillus actinomycetemcomitans* (Alcoforado et al., 1991; Leonhardt et al., 1999; Pye et al., 2009), but ecological relationships with their surrounding and eventual pathological roles are yet to be understood. *In vitro*, *Candida albicans* may also adhere to pieces of biodegradable membranes used for periodontal tissue regeneration (Molgatini et al., 1998) and to tissue-conditioning materials for denture relining (Kulak & Kazazoglu, 1998). Additionally, presence of *Candida albicans* has been documented on obturator prostheses (whatever the material may be: silicone, polymethyl methacrylate, or titanium) in patients with maxillary defects (suffering from congenital malformation, tumors, or trauma), and on the mucosa adjacent to the prosthesis (Depprich et al., 2008; Mattos et al., 2009); these patients can present prosthesis-induced stomatitis. Finally, the use of orthodontic appliances leads to an increased carriage rate during the appliance-wearing time, with a significant fall of salivary pH and an increase of *Candida* count observed at different oral sites through various sampling techniques (Hibino et al., 2009).

2.3 *Candida* on devices used outside the oral cavity

Materials inserted in other sites can be colonized by yeasts as well, causing device-related infections (Cauda, 2009): articular prosthesis, cardiac devices (Falcone et al., 2009), catheters, vascular access devices (Brouns et al., 2006), and voice prostheses (Kania et al., 2010). These infections require prolonged antifungal therapy and often device removal.

3. Experimental approach

A better understanding of interface biology and material surface treatments requires experimental models to produce *in vitro* biofilms on supports that are easy to manipulate in

the laboratory (Chandra et al., 2001) and the ability to investigate *in vivo* biofilm growth or drug susceptibility. Different studies have already described such models, mainly addressing procedures that are able to limit yeast adherence and biofilm formation. Some of these technologies were originally proposed as artificial dental plaque biofilm model systems (reviewed by Sissons, 1997), especially for plaque biology studies in caries and periodontitis research. This section will report on the experimental models producing *Candida* biofilms onto biomaterials. Table 1 summarizes some contributions from literature.

design	reference	material
***in vitro* models**		
static culture models		
- material dived in solution	Chandra et al., 2001	acrylic, silicone
- poloxamer gel in Petri dish	Percival et al., 2007	material covered by gel
- 96-well culture microtiter plate	Peeters et al., 2008	polystyrene
- titanium powder	Ahariz & Courtois, 2010	titanium powder
continuous culture models		
- continuous flow culture system	Uppuluri et al., 2009	silicon elastomer
- Modified Robbins Device	Coenye et al., 2008	
- constant depth film fermenter	Lamfon et al., 2003	enamel, dentine, acrylic
***in vivo* models**		
- animal models	Andes et al., 2004 Nett et al., 2010 Ricicová et al., 2010	central venous catheter acrylic denture subcutaneous catheter
- human models	Budtz-Jörgensen et al., 1981	tape or acrylic disk

Table 1. Experimental designs reported in the literature to produce *Candida*-biofilms.

Candida albicans can be grown with or without the addition of saliva on different materials used in dentistry including acrylic resins, denture-lining material, porcelain, composite, amalgam, hydroxyapatite, silicone, and polystyrene.

3.1 *In vitro* models

In vitro models consist of static cultures or continuous cultures.

3.1.1 Static culture models

Mono-species biofilms can be experimentally grown on pieces of materials currently used in dentistry, such as polymethylmethacrylate strips or silicone elastomer disks (Chandra et al., 2001). Some surfaces prepared from mucosa (epithelial cell cultures) or hard tooth tissues (enamel, dentine) were used as well. The materials are immersed in assay tubes or in multi-well culture plates containing a contaminated solution similar to saliva for a predefined incubation period. The polystyrene wall of the container itself has been used as an adherent surface for biofilm formation: 96-well plates allow management of experiments with numerous replications in various conditions (Peeters et al., 2008). Specific studies of the adhesion phase require an intermediate washing step to remove non-adherent yeast cells. Evaluation of the biofilm growth phase is generally based on microbial cell staining (crystal violet, among others), metabolic activities (tetrazolium test), biomass determination (dry weight, incorporation of radioactive tracer such as amino acids), or microscopic examination.

Poloxamer hydrogel, being liquid at low temperatures and solid at cultivation temperatures, has been proposed (Percival et al., 2007) as a culture support in the Petri dish to induce bacteria and yeast biofilm-like aggregates. The thermoreversible gelation makes the preparation and the recovery of biofilm samples easy and reproducible but still requires further confirmation for use in biofilm biology. Powder material, such as titanium powder, provides increasing support surfaces that are similar to cell culture on beads; moreover, it allows the anchored phase to be easily separated from the planktonic phase by simple sedimentation (Ahariz & Courtois, 2010). The titanium surface is not antimicrobial by itself, so it can be used as support for *Candida* biofilm. Titanium is widely employed for implant manufacturing due to its good biocompatibility and mechanical properties, but infection remains as a primary cause for failure, leading to removal. *Candida albicans* biofilms on titanium powder could offer a simple and reliable model for further investigation of new antimicrobial strategies; moreover, the model could be extendable to other microorganisms contaminating implanted materials. Making implant surfaces resistant to microbial colonization should reduce infectious complications; however, such developments need an *in vitro* model that allows the effect of surface modification and coatings on biofilm production to be studied. This aspect will be detailed in the next section.

The approach by means of static cultures simplifies the *in vivo* complexity to interactions between one single species and one single support without considering the numerous salivary compounds and abundant oral microflora in the real oral environment. Microorganisms in biofilms *in vivo* display properties different from those observed under laboratory conditions. The single-species procedures can be extended to a two-membered microbial co-culture or a characterized microbial consortium in order to reconstitute a medium for approaching oral microcosm and containing sterilized or artificial saliva. Multi-species biofilms have already been investigated on various dental materials such as enamel, amalgam, composite, and acrylic to assess the role of surface roughness (foremost in the first steps of biofilm formation) and impact of (pre)conditioning by saliva (Dezelic et al., 2009). Diffusion of drugs through biofilms, including *Candida* biofilms, can be documented by an experimental perfusion system superposing disk, filters, biofilm, and agar containing the drug under evaluation (Samaranayake et al., 2005). Perfusion of drugs in biofilms allows the putative factors that lead to biofilm antimycotic resistance to be evaluated.

3.1.2 Continuous culture models

Contrary to static cultures, continuous culture models (flow cells, Modified Robbins Device, chemostats, artificial mouths, and constant depth film fermenter) take into account oral flow and oral bathing conditions as shear forces and nutrient supplies (Bernhardt et al., 1999; Ramage et al., 2008). Indeed, oral biofilms on prosthetic materials are exposed to salivary fluxes conveying water and nutrients to the aggregated microorganisms in the saliva. For instance, liquid flow has been shown to influence the production of an extracellular matrix by *Candida albicans* biofilms *in vitro* (Hawser et al., 1998). Taking extracellular material produced in static cultures as a basal value, a gentle stirring significantly multiplied this by ~1.25, while a more intense stirring led to complete inhibition. Other data (Biswas & Chaffin, 2005) reported the absence of *Candida albicans* biofilm formation in anaerobiosis even if this yeast can grow in anaerobic environment. Yeast retention against a continuous flow of medium has been used as a marker for yeast adhesion to a surface in the same manner as the retention after liquid washes (Cannon et al., 2010). Cultures under continuous flow conditions facilitated the penetration of *Candida albicans* into silicone elastomers when

compared to static conditions, especially when pure cultures were tested. The presence of *Streptococci* reduced material invasion by the yeast, thereby revealing the importance of testing materials in a biological environment (Rodger et al., 2010).
A continuous flow culture (CFC) aims at cultivating microorganisms in a continuous flow of fresh medium to mimic physiological conditions and to avoid the decline of the culture by nutrient depletion. Such instrumentation is easy to assemble in a microbiology laboratory equipped with an incubator, peristaltic pump, fresh medium flask, tubing, and chamber(s) where the material of interest, on which biofilm was already initialized, is inserted (Uppulari et al., 2009). Serial chambers allow independent samples to be produced under similar conditions (nutrients, flow rate, temperature, and incubation time). *Candida albicans* biofilms have been reported to grow more rapidly under continuous flow than in static culture (Uppulari et al., 2009).
Specific devices have been developed for continuous flow studies. The Modified Robbins Device (MRD) is a small channel-shaped chamber with different openings in which biomaterial discs could be inserted when the instrument is mounted to form the channel wall. These devices are provided with a liquid circulator for low-pressure applications. Microorganisms introduced into the fluid stream can adhere to the plugs and generate a biofilm that is easy to remove for analysis. This instrumentation was used to evaluate some maintenance protocols for oral devices (Coenye et al., 2008), as well as for other purposes.
The constant depth film fermenter (CDFF) is an instrument that is able to generate standardized biofilms on any materials that can thereafter be removed for subsequent investigations; the advantages of such a device includes controlled experimental conditions for growth that mimic the oral environment. Some authors (Lamfon et al., 2003) used the CDFF to produce *Candida* biofilms in the presence of artificial saliva on enamel, dentine, and denture acrylic disks. Their data demonstrated that the roughness of the material influences formation and development of *Candida* biofilms.

3.2. *In vivo* models

The rat catheter biofilm infection model (Andes et al., 2004; Lazzell et al., 2009; Ricicová et al., 2010) allows evaluation *in vivo* of the comportment of one microorganism that is exposed to host proteins and immune factors. Other animal models have been utilized to monitor materials and devices that are placed in the bathing conditions of the oral cavity and to mimic denture stomatitis with fungal invasion and neutrophil infiltration of the adjacent mucosa. The rodent acrylic denture model was developed for such purposes (Nett et al., 2010). In humans, abrased pieces of self-adhesive (Budtz-Jörgensen et al., 1981) or acrylic resin disk (Avon et al., 2007) were fixed on dentures to gain some information *in vivo* concerning biofilm formation in the oral cavity.

4. Surface treatment to control *Candida* biofilms

Candida in biofilms on prosthetic materials is difficult to remove. If it is absurd to eradicate a commensal of the oral environment, it is important to consider the prosthesis and the patient simultaneously because the prosthesis is a nest for *Candida* growth and a possible source of infection for the oral mucosa. Daily brushing should be encouraged in all denture-wearers; in denture stomatitis, decontamination of the denture becomes a mandatory part of treatment. Often, the family or professional caregivers must compensate for the difficulties that the elderly face due to loss of independence, dexterity, or memory. Thus, antifungals

(azoles, nystatin) should be reserved for patient treatment and are less active against biofilms on dentures (see *infra*); moreover, they may cause the emergence of resistant strains. Use of antiphlogistic solutions has been highly successful; as many of them are fungicidal. However, there are no comparative studies that examine all aspects of the problem.

Rarely, if ever, do study authors take into account the views of all professionals involved in denture care, not only the opinions of the dentist who treats the patient, but also those of the prosthetist who sees the potential deleterious effects of some decontaminating procedures and the microbiologist who isolates and studies yeast *in vitro*. Microbiologists are able to determine the minimum inhibitory concentrations of antifungals on *Candida* growth in suspensions, or better (but less often), on *Candida* biofilms produced in the laboratory. Dental technicians are involved in the relationship with the materials they provide, biofilms, and the decontamination procedures. Clinicians' decisions cannot rely on evidence-based studies alone since they lack data from large-scale clinical trials (i.e., *in vivo* studies).

4.1 Anti-biofilm agents

Many molecules that are embedded in antiseptic mouthwash or in effervescent tablets are candidacidal. Sodium hypochlorite, a major component of bleach that is also produced *in vivo* by myeloperoxidase from the neutrophils, has an anti-*Candida* effect. Ozonated water with or without ultrasound reduces yeasts' adherence to the resin. The use of ultrasound reduces the concentration required for effectiveness of most antiseptics or fungicide antimycotics. The use of a microwave oven is not recommended because the conditions that suppress the yeasts are too close to those that damage some prosthesis materials. When misused, some products can damage the materials: the repeated use of chlorhexidine colors resins brown; hypochlorite at a high dose bleaches them. Hydrogen peroxide is active only at a very high concentration that is close to the mucosal toxicity level; moreover, in the presence of hydrogen peroxide, *Candida* over-expresses catalase and glutathione oxidase, which in turn reduces the concentration of hydrogen peroxide and protects the yeast cells against oxidation.

An alternative to prevent biofilm formation could involve a reduction of microorganism adherence to materials by anti-adhesive/anti-microbial coatings, with or without drug release. Indeed, the anchoring of microorganisms to surfaces such as mucosa, teeth, or biomaterial is a pivotal step in initiating biofilms into the oral cavity. Adhesion can be quantified by measurement of the microorganisms' retention after fixed incubation periods and washings or by the microorganisms' retention against a continuous flow of medium (Cannon et al., 2010). Many protocols have been proposed to limit biofilm formation on various materials used in dentistry (recently reviewed by Busscher et al., 2010): antibiotic and peptide coatings, silver and polymer-brush coatings, and quaternary ammonium couplings. *In vitro*, titanium dioxide coating inhibits *Candida* adhesion to the denture's base in acrylic resin (Arai et al., 2009). Surface protection from bacteria and yeast by chitosan coating is also worthy of further pharmacologic and clinical studies (Carlson et al., 2008). Surface treatment must solve numerous challenges before clinical implementation: the amount of bioavailable drug on the material's surface, kinetic and safety of the released compounds, interferences with the oral environment, and the quest for multifunctional effects such as biofilm control, tissue integration, and/or tissue regeneration.

4.2 New strategies based on research in *Candida*-biofilm biology

The decreased susceptibility of yeast biofilms to classical antifungal drugs encouraged scientists to explore other means to inhibit *Candida* and to limit the deleterious effects of its biofilm. Knowledge of interactions between *Candida* and oral tissues and between *Candida* and oral bacteria should present new perspectives for therapy. Microorganisms in biofilms (yeast included) are less sensitive to antimicrobial agents than free microorganisms in suspension. Authors (Thurnheer et al., 2003) have shown a decreased drug diffusion rate through polyspecies biofilms, containing *Candida* among others, proportional to the cubic root of the drug's marker molecular weight, suggesting the deviousness of the diffusion route through biofilm depth as the cause of delay in molecule penetration. Nevertheless, drug resistance could also be attributed to metabolic properties and gene expression induced by microorganisms living in the community and to the production of an extracellular matrix.

Other *in vitro* studies (reviewed by Nobile et al., 2006) suggest several molecular factors that explain biofilm development and biofilm drug resistance, such as specific biofilm phenotypes (Finkel & Mitchell, 2011), adhesins, cell to cell communication, and quorum sensing (Deveau & Hogan, 2011). The link between hyphae production and *Candida* biofilm development *in vitro* and between hyphae production and pathological conditions *in vivo* led to the investigation of the genetic regulation of hyphal morphogenesis. The rapid initiation of biofilm in the presence of new surfaces available for anchoring oriented the genetic analysis towards a gene expression distinct from that found in the planktonic state.

Quorum sensing pathways that allow microorganism colonies (including yeast) to sense their cell density involve small molecules such as farnesol and tyrosol. The former is known to promote resistance against oxidative stress and inhibit hyphal morphogenesis and biofilm formation, whereas the latter is a putative biofilm facilitator. The (over)expression and polysaccharidic matrix production of adhesins is also linked to biofilm formation. All of these biological characteristics contribute to make *Candida* biofilms "a well-designed protected environment" (Mukherjee P. et al., 2005). A better knowledge of molecular events in *Candida* biofilm formation could present new strategies to prevent oral candidiasis contracted from biomaterials inserted in the oral cavity.

4.3 New strategies based on research in exocrine biology

Antimicrobial molecules/systems derived from exocrine secretions are interesting topics of research. Studies *in vitro* have already shown the benefits of lysozyme, lactoferrin, histatin (Pusateri et al., 2009), and peroxidase systems with thiocyanate, chloride, and especially iodide. However, transferring such data to *in vivo* studies hasn't yet provided the expected results because of the immense complexity of the oral environment. Again, the publication of large clinical studies is still being awaited.

Research in peroxidase biology is an illustrative example of the multiple facets of knowledge transfer from fundamental sciences to clinical applications. In the presence of hydrogen peroxide, for example, peroxidases in exocrine secretions are able to catalyze the production of hypohalous compounds that carry an antimicrobial effect: hypoiodite *in vitro* and hypothiocyanite in saliva. Previous studies have shown that a 30-minute exposure to hypoiodite was sufficient to inhibit planktonic growth *in vitro* (Majerus & Courtois, 1992). Moreover, the development of *Candida* biofilm on material surfaces could be reduced or even suppressed by lactoperoxidase-generated hypoiodite and hypothiocyanite. This was

the case not only when peroxidase and substrates system were dissolved in the liquid phase into which the material was immersed, but also when peroxidase precoated on material was activated by simple addition of the substrates to the liquid surrounding this material. Those data also demonstrated the efficiency of peroxidase systems against a *Candida* strain during a three-week incubation period and concomitantly suggested a possible interest in coating the material with peroxidase.

Other investigations demonstrated 1) that lactoperoxidase activity was not modified by coating onto titanium, 2) that lactoperoxidase incorporated in oral gel maintained its activity for at least one year, and 3) that the substrate exhaust (namely hydrogen peroxide and iodide) was the true limiting factor (Bosch et al., 2000; Ahariz et al., 2000). Previous investigations indicated an antibacterial effect on Gram-positive and Gram-negative bacteria, which suggests a non-specific inhibitory effect of hypoiodite on microbial metabolism and growth (Courtois et al., 1995). The ability to transfer this knowledge from bench to clinic is questionable. Indeed, the immunogenicity of a material surface coated with lactoperoxidase should restrict the applications of this system to *ex vivo* conditions. Besides the toxicity of oxidant products on host cells, the competition between iodide and thiocyanate is another limiting factor for *in vivo* use. Thiocyanate is not only present in several exocrine secretions (e.g., human saliva) but is also the preferential substrate of lactoperoxidase. Simultaneous incorporation of iodide and thiocyanate in the same gel decreased the beneficial effect of 2 mM iodide in the presence of increasing concentrations of thiocyanate ranging from 0.25 to 4 mM, which correspond to the normal range of this ion in saliva.

5. Precautions for testing new devices *in vivo*

Finally, the investigators must be aware of the biases frequently encountered in clinical trials that evaluate microbial contamination and colonization of oral devices and prosthesis. Recommendations and guidelines to evaluate the benefits of prophylactic anti-*Candida* procedures are similar to these advocated for any oral care product. Two important biases that must be taken into account are the influence of investigators on patients' hygiene behavior (Grimoud et al., 2005) and the galenic formulation of products lacking the active molecule. Evaluation of dentifrice efficiency for denture hygiene also needs other controls: one testing the product without brushing and one testing the mechanical brushing alone. The abrasive effect of the product must be evaluated, and the abrasiveness of saliva itself is another concern to be considered.

Quantification of the *Candida* biomass that is adherent to the device is difficult in practice. Yeast samples from the oral environment can be collected by rinsing, imprinting, or swabbing. Swabs and imprints are more suitable for gathering yeasts attached to surfaces, and swabbing is easier for clinical studies on a larger scale. Procedures to quantify yeast biomass *in vitro* are not applicable *in vivo* for epidemiological studies and hygiene purposes, particularly since denture-wearers are not always compliant. Dentures can be rinsed in saline and brushed in standard condition to harvest microbial cells. The suspension is thereafter serially diluted for counting (Panzeri et al., 2009).

Another study (Vanden Abbeele et al., 2008) documented the reliability of oral swabbing to investigate yeast carriage on denture. Sampling dentures for *Candida* is more than just a diagnostic tool: it could present an opportunity to verify the patient's compliance with hygiene advice as well as the efficiency of new topical antifungals. Yeast counts after swab

culture reflect the biomass present on the oral surfaces, but this is not the number of yeast cells included in oral biofilms. Colony forming units (CFU) counted on the agar medium represented only a small part of the cells harvested by the cotton, as assessed by three successive spreadings of the same cotton that provided similar data (in the range interval of 0.1 logarithmic units). Furthermore, two successive swabs of the same oral surface yielded similar quantities of yeast cells (in the range interval of 0.5 logarithmic units).
Finally, investigators themselves can influence the hygiene behavior of the subjects under study. The study previously quoted (Vanden Abbeele et al., 2008) also analyzes the effect of the oral care program. In the absence of any hygiene advice, a second denture swabbing taken in 46 patients after an interval of one week demonstrated only minor variations, thus minimizing the hygiene-stimulation effect produced by pursuing the collection of samples. Repeated sampling (at one-week intervals) of 46 different healthy denture-wearers demonstrated yeast counts remaining in the same range (±1 logarithmic unit) for more than 85% of the denture swabs and mucosa samples. Values below the lower limit (-1 logarithmic unit) occurred in less than 15% of denture and mucosa swabs. This was attributed to behavioral changes in hygiene practice following the investigators' first visit. By contrast, a hygiene program including a placebo oral gel (tested to be inactive *in vitro*) led to a decrease of yeast carriage after two weeks.

6. Conclusion

Yeasts belonging to the *Candida* genus usually colonize the human oral cavity. *In vivo* and *in vitro* studies have shown *Candida* incorporation into biofilms covering different biomaterials and devices such as dentures. These biofilms may indicate an increased risk factor for invasive candidosis when the host immune system is compromised. Daily denture brushing is recommended to all denture (and other device) wearers in order to prevent the development of *Candida* biofilm. Family members and healthcare workers must assume this task when there is a deficiency in dexterity and/or a loss of autonomy, especially in elderly and disabled persons. In case of candidosis in denture-wearers, decontamination of dentures is mandatory. Antimycotics such as azoles or nystatin must be reserved for curative treatment of infected patients; they are less active against *Candida* biofilms on dentures and could lead to emergent resistance if applied daily to dentures in order to prevent yeast colonization. Nevertheless, few studies, if any, integrate all aspects of denture care: welfare of denture-wearers, prevention of candidosis, biomaterial defects after decontamination processing, and possible *Candida* biofilm development. Daily brushing of dentures remains the key recommendation. A better understanding of *Candida* biology in the oral environment will provide new tools to control *Candida* biofilms, the possible development of more appropriate biomaterials for dentistry (or surface improvements), and better management of biomaterial use in the oral cavity. Further investigations in this field will require cooperation among dentists, biologists, and engineers.

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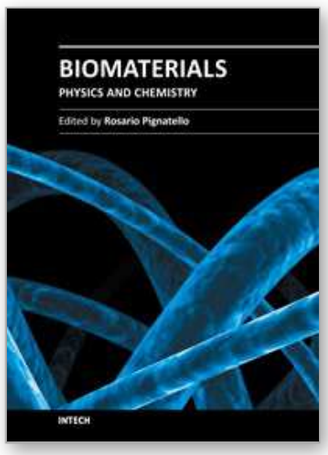

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