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The Bacterial Colonization of Commonly Used Dental Implant Occluding Materials.

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By

Brandon G. Katz

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Title: The Bacterial Colonization of Commonly Used Dental Implant Occluding Materials.
Purpose: To determine the bacterial communities inhabiting commonly used materials intended to block/occlude the screw access channel of dental implant crowns/restorations. To aid in the prevention of peri-implantitis, a material that harbors less pathogenic red and orange complex microbes would be potentially beneficial when microleakage is occurring. A superior material for the purpose of the occlusion of screw access channels in implant-supported prostheses is a material that may provide a surface to which bacteria may not colonize. The choice of an occluding material that is less favorable for bacterial growth, particularly red and orange complex growth, will be more advantageous to use in clinical practice and may assist in the prevention of peri-implant pathology.
Materials/Methods: This was a randomized controlled clinical trial, which included eight subjects. Four commonly used materials (PTFE tape, PVS, Cotton, Foam) were placed as test materials in the dental implant access cylinders giving a total of 32 samples. Materials were sealed and recovered after a minimum of six months. DNA was extracted from the samples and the bacterial community composition was analyzed by high-throughput sequencing of 16S rRNA amplicons. The contribution of particular species, genera, and bacterial complexes to the bacterial community present in each sample was calculated as percent of total sequences. A post-hoc Tukey's HSD test at a significance level of α=0.05 was applied to determine if differences in bacterial community composition among patients or materials were statistically significant.
Results: A total of 286 different genera and 492 different species were recorded in a total of 1,862,900 sequences. On average each sample generated 58,215 sequences. The most abundant microbes, based on genus, were *Lactobacillus*(26.48% of total sequences), *Atopobium*(10.10%), *Streptococcus (*5.22%) and *Selenomonas*(3.61%), none belonging to the classic pathogenic Red and Orange Complexes. The most abundant species was *Atopobium spp.*, also not belonging to the pathogenic microbial complexes. There were a total of six microbial species not found in the colored complexes that were affected by the material used, but no one material seemed to be better than the other three in preventing red and orange microbial colonization. There was no significant

difference in colored complexes amongst materials. This demonstrates that the microbes were not affected by the material used, but rather more influenced by the subject.
Conclusions: 1- There were no statistically significant differences in bacterial colonization amongst the test materials. The choice of an occluding material would therefore be based on clinical judgment. 2- There were a total of six microbial species not present in the "colored" complexes of Socransky and Haffajee that were affected by the occluding materials. These six microbial species were *Atopobium spp., Bilophila wadsworthia, Erysipelothrix spp., Haemophilus parainfluenzae, Rheinheimera spp. and Shuttleworthia satelles*. 3- *Atopobium spp., Selenomonas spp.* and *Fusobacterium spp.,* were amongst the most prevalent microbial species present in the materials tested.

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Introduction

I. Overview

According to the American Academy of Periodontology, a dental implant is an artificial tooth root that is placed into the jaw to hold a replacement tooth. Dental implants may be an option for people who have lost a tooth or teeth due to periodontal disease, an injury, or some other reason.[1]

Dental implants have been used since antiquity. "In approximately 2500 BC, the ancient Egyptians tried to stabilize teeth that were periodontally involved with the use of ligature wire made of gold. The first evidence of dental implants is attributed to the Mayan population roughly around 600 AD where they excelled in utilizing pieces of shells as implants as a replacement for mandibular teeth. Radiographs taken in the1970's of Mayan mandibles show compact bone formation around the implants-bone that amazingly looks very much like that seen around blade implants."[2]Various modifications to the implant design have taken place since the ancient times. The shape of a modern day implant has evolved from spirals, to plates to now root-form and this evolution, including the discovery of osseointegration of titanium surfaces, has led to the common modern day implant. "In 1978, Dr. P. Brånemark presented a two-stage threaded titanium root-form implant; he developed and tested a system using pure titanium screws which he termed fixtures. These were first placed in his patients in 1965and were the first to be well-documented and the most well maintained dental implants thus far." [2]

According, to the American Academy of Implant Dentistry (AAID), implants are increasing as a treatment modality of oral healthcare in American Dental practices. On its website, as of 2015, the numbers from the AAID represent that, "more than 30 million Americans are missing all their teeth in one or both jaws; 15 million people in the U.S. have crown and bridge replacements for missing teeth; 3 million have implants and that number is growing by 500,000 a year; 10 percent of all US dentists place implants but that is increasing; the estimated US and European market for dental implants is expected to reach $4.2 billion by 2022; the success rate of dental implants has been reported in the scientific literature as 98 percent; implants performed by US dentists were 5,505,720 (2006); implants performed by US general dentists 3,103,930 (2006); the global prosthetic supplies market is projected to reach $4 billion by 2018; the dental implant and prosthetic market in the U.S is projected to reach $6.4 billion by 2018".[3]These facts are astonishing and demonstrate the exponential growth and need for dental implants to replace missing teeth in partially edentulous patients.

According to the Glossary of Prosthodontic Terms the word 'edentulism' is defined as the state of being without teeth or lacking teeth.[4]Dental edentulism or partial edentulism is considered, by some, as a condition that is handicapping or debilitating. Dental implants are indicated for replacing missing teeth. "Most patients who are missing one or more teeth can benefit from the application of an implant-retained prosthesis. Edentulous patients who are unable to function with complete dentures and who have adequate bone for the placement of dental implants can be especially good dental implant candidates. More and

more partially edentulous patients are also being treated with dental implant restorations. Many patients, whether they are missing one, several, or all of their teeth, can be predictably restored with implant-retained prostheses".[5]Most patients do not have contraindications to dental implants, but some risks or considerations do exist such as the patient's overall medical status. This includes physical and mental health evaluations as well as patient's behavioral conditions. Examples of risks factors of concern are uncontrolled diabetes mellitus, smoking and other related issues (Table 1).[5] Although a few contraindications exist, when indicated, dental implants can improve a patient's quality of life.

Two of the advantages of dental implants include better ability in chewing and speech. The Academy of Osseointegration (AO) lists the advantages in the FAQ on its website. It states that, "dental implants reduce the load on the remaining oral structures/teeth; dental implants will preserve bone and significantly reduce bone resorption; implant overdentures may allow you to chew your food better and speak more clearly".[6]The AO further discusses a few of the disadvantages of dental implant therapy, "A surgical procedure and a period of healing is necessary, overall dental treatment may entail an increase in cost and mechanical fracture of fixtures, bridges, bridge attaching screws can occur".[6]The benefits from dental implants usually outweigh the negatives even when the disadvantages are considered.

When osseointegrated implants are healed and restored, they usually function without complications. A healthy environment exists around dental implants and their restorations, similar to surrounding teeth, "Following implant installation, a transmucosal passage is formed around the abutment portion of the device. The ridge mucosa at such sites adapts to the new functional demands and a peri-implant mucosa becomes established. The mucosa surrounding implants and the gingiva surrounding teeth have many features in common. Both types of tissues are lined with a keratinized oral epithelium; at clinically healthy sites this is continuous with a thin non-keratinized barrier or junctional epithelium that faces the implant or the tooth surface. In the connective tissue immediately lateral to these thin epithelial linings small infiltrates of inflammatory cells (neutrophils, macrophages, T cells, B cells) are frequentlyseen".[7] When these surrounding tissues become inflamed, a condition known as peri-implant disease occurs.

Peri-implant disease is defined as an "inflammatory processes in the tissues surrounding an implant." As seen in Figure 1, peri-implant disease is categorized into two conditions: "peri-implant mucositis, which is a reversible inflammatory process in the soft tissues surrounding a functioning implant, and, peri-implantitis, an inflammatory process additionally characterized by loss of peri-implant bone".[7] The prevalence of peri-implantitis has been discussed by Heitz-Mayfield, "peri-implantitis—an infectious condition of the tissues around osseointegrated implants with loss of supporting bone and clinical signs of inflammation(bleeding and/or suppuration on probing)—has a prevalence on the order of 10% of implants and 20%of patients 5 to 10 years after implant placement".[8] In the consensus report of the Sixth European workshop on Periodontology, Lindhe states the prevalence to be much higher, "peri-implant mucositis occurs in about 80% of subjects (50% of sites) restored with implants, and peri-implantitis in between 28% and 56% of

subjects (12–40% of sites)".[9] These inflamed sites, as mentioned by Lindhe, have been shown to be associated with certain risk factors/indicators, or predictors of future disease. Heitz-Mayfield further discussed certain indicators for peri-implantitis and concluded that her review identified "strong evidence that poor oral hygiene, a history of periodontitis and cigarette smoking, are risk indicators for peri-implant disease". Future prospective studies are required to confirm these factors as true risk factors.[10] Until then, clinicians need to focus on prevention and management of peri-implant diseases.

The treatment of peri-implant diseases varies depending on the extent and severity. For the most part, treatment is based on non-surgical and surgical approaches. To date there is not one predictable way to treat peri-implant disease and the best treatment for peri-implant disease is prevention. To this end, implants have changed design over the years to try to create the best mechanical and biological product and thus avoid or minimize peri-implant disease.

II. Implant Design

Dental implants are essentially in the shape of a threaded screw that is inserted into a prepared osteotomy in alveolar bone. It is made from titanium or its alloy. The implant serves as a support or "root like" structure for a dental restoration or crown (Figure 2&3). The connection between the implant and the crown is through an intermediary fixation device or abutment. A screw access channel, a cylinder through the top of the dental implant crown, allows the attachment of the crown to the abutment. This screw access channel is blocked with an occluding material (i.e. cotton pellets, foam or silicone.) to protect the screw inside the channel. The crown is joined to the abutment and implant by screws.

After the abutment is placed, a crown is fabricated and screwed or cemented to its abutment. The union of the abutment to the implant is commonly known as the abutment-implant connection. An incomplete seal, or microgap, between these parts always exists. The magnitude of the microgap depends upon the manufacturer and is usually limited to less than 50 μm for commonly used implant systems. Because most of the oral bacteria are usually less than 10 μm in diameter, microbial pathways are present in the space between the implant and its components.[11] These pathways are the conduits of microleakage. Microleakage through a microgap (Figure 4) is defined as the passage of bacteria, fluids, molecules or ions between the abutment-implant connection to and from the surrounding periodontal tissues. Microleakage may be a factor in the etiology of peri-implant disease. If microleakage does occur, then the microbial properties of the occluding material may be a factor to consider when selecting the appropriate material for this function.

There are a number of implant options from which to choose. The options are based on types of implant design. There are one piece systems as seen in Figure 5,which eliminate the microgap. When comparing one-piece versus two-piece designs, Hermann[12] has shown that different implant designs have different effects on tissue response. For example, it has been suggested that one-piece implants, in which there is no microgap, show minimal bone resorption, either because less bacterial colonization takes place or via

the absence of micromovement between the components. A drawback of the one piece system is that there are less options for the abutment selection and the crown in most restorations will have to be cemented. Cement with implant crowns is one of the options for retention of the crown to the abutment.

There are different mechanisms for retaining the crown to the abutment, as well for inserting the abutment into the dental implant that must be considered. The abutment retention can occur with either a screw, or friction leading to a cold-weld, as illustrated in Figure 6. Retention of the final restorations can occur with either a screw or a cement (glue), as illustrated earlier in Figures 2&3. There have been reports of cements leading to peri-implantitis and this has to be taken into consideration when selecting a retention design. If the decision is made to use a design that retains the prosthesis by a screw, two methods are available, and usually determined by the depth, position and angulation in which the implant was placed. The differences between using, and not using, intermediary abutments are shown in Figures 7&8. One major difference that should stand out is the multiple interfaces created within the system between parts. These extra interfaces, or microgaps, as discussed earlier may be conduits for bacteria or their byproducts.

The crown/abutment/implant system is clamped together and the type of screw geometry and coating, as shown in a study by Martin et al.[13], can affect the tightness, or amount of preload. The value of torque, which is a twisting force that tends to cause rotation, should be applied is a valid concern when finalizing selection. The more completely seated, or the more precisely the system fits together, the less micromovements will occur, which may prevent microleakage and mechanical failures.

The abutment needs to be joined to the implant. As stated earlier, the retention could either be with a screw or through a frictional fit creating a cold-weld. In a study by Alves et al.[14] it was concluded that there was no significant difference in *in-vitro* bacterial sealing between implants with abutments tightened by friction without screws and implants with screw-tightened abutments with 30 Ncm of closing. Closing torque, or the amount of torque applied to the screw, altered the *in-vitro* sealing ability of the tested abutments, with a greater contamination for components that received a closing torque of 20 Ncm. [14] This study showed that torque value of the screw is essential.

The amount of torque, or what is called pre-load,(Figure 9), is what leads to the deformation of the screw causing the desired clamping effect. As seen in figure 9 the stretching of the screw causes an elongation that pulls the components. According to Ricomini-Filho et al.[15], mechanical factors, such as the precision of the implant-abutment fit and the abutment screw preload, are involved in the success of implant rehabilitation. The preload loss during the occlusal load with the prosthesis in function favors the misfit of the implant-abutment connection and this can result in stress increase in the implant and connection components, and consequently in the surrounding bone. Basically over time and function, preload is lost and the screw tightness is weakened, leading to more stresses or micromovements.

Additionally, the geometry of the implant connection, or the shape of the connection, is another design option that clinicians are faced with when selecting the appropriate dental implant. Some implants have internal connections versus external connections. An example of an external connection geometry are the external hexagonal (external hex) implants, which have their geometric fit dictated by the shape of the top (platform) of the implant, as seen in Figure 10. Implants also come with Morse taper connections, a type of internal connection, as also seen in Figure 10. Morse taper connections are conical in nature and you can visualize this as two parking cones being placed into each other. Morse taper connections have been proposed as an alternative to external hexagon implants because they may have better stability (reduced micromovements)of the components and less bacterial leakage at the implant-abutment interface. Jaworski[16] compared external-hex and Morse-taper designs with respect to bacterial sealing between implants and abutments and concluded that both systems had bacterial contamination but that Morse-taper configurations did make a tighter seal leading to less contamination. These factors of design in regards to reduction of micromovement are intended to reduce microleakage.

All of these factors regarding type of retention, and amount of torque, are important when trying to select a system that allows the minimum amount of microleakage.

III. Microleakage

Microleakage, as mentioned earlier, in implant dentistry refers to the passage of bacteria, fluids, molecules or ions between the abutment-implant connection (Figure4) to and from the surrounding periodontal tissues. The abutment-implant connection is the area in which the implant and abutment meet. Gross, Abramovich, and Weiss suggest that "The clinical phenomenon of bleeding and malodor characteristic of anaerobic bacteria on the removal of abutments or healing screws may be the result of, in part, the effects of microleakage. Presumably in an *in situ* situation, diffused fluids could also contain bacterial byproducts or nutrients required for bacterial growth".[17]

The phenomenon of malodor and its relationship with gingivitis and periodontitis was discussed by Ratcliff and Johnson in their 1999 review.[18] They stated that "hydrogen sulfide (H_2S) and methyl mercaptan (CH_3SH), are primarily responsible for mouth odor. Although many bacteria produce H_2S, the production of CH_3SH, especially at high levels, is primarily restricted to periodontal pathogens." They conclude that direct contact with hydrogen sulfide and methyl mercaptan can negatively affect fibroblast protein synthesis and stimulate production of cytokines capable of inducing damaging effects to periodontal tissues. This may contribute to the odors explained by Gross, Abramovich and Weiss.[17]

Bacterial microleakage studies, such as the study by Jansen[19], have used several kinds of bacteria, from facultative to obligate anaerobes, varying in size from 1 to 10 μm. Jansen also tested extremely small molecules such as toxins, saliva, and stains, based on the fact that some studies[19] stated that the microgap, or the space, between the implant and the prosthetic components, generally located subgingivally, or below the soft tissue level adjacent to the abutment, is between 1 and 49 μm in length. These microgaps represent,

consequently, an ideal potential site for plaque retention, which would allow the flow of microbial fluid.[20]

To this date there have been many *in vitro* studies completed to prove the occurrence of microleakage. This movement of fluid and byproducts into or out of the screw cylinder has been shown. There are various ways to measure microleakage in implant systems. One method is using colored tracing probes to analyze outward microleakage photometrically. Yet, another method is to analyze the inward or outward migration of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Fusobacterium nucleatum*, bacterial mixtures and endotoxin.[21]

One study by Proff[22] tested the *in vitro* suitability of gutta percha for implant-abutment? gap sealing. As defined by Merriam-Webster, gutta percha is a tough plastic substance from the latex of several Malaysian trees (genera *Payena* and *Palaquium*) of the sapodilla family that resembles rubber but contains more resin and is used especially as insulation and in dentistry in temporary fillings. Proff's group utilized *Porphyromonas gingivalis* and internal connection implants and allowed them to sit in a nutrient solution (thioglycolate bullion with haemin-menadione solution). They sampled the interiors of the implant systems at 24 and 48 hours. They plated the specimens on agar and incubated for 4 days before assessing bacterial growth. They found that microleakage occurred and that *P. gingivalis* survived in the interior of the implant creating a reservoir. The conclusion of this study was, "This *in vitro* trial produced no evidence that sealing with gutta percha is an effective means to prevent secondary bacterial colonization in the implant interior."

Another study by Park[21] analyzed the levels of microleakage in implants whose access holes have been sealed with different materials. This was an *in vitro* study testing cotton pellet, silicone sealing material, vinyl polysiloxane, and gutta-percha as occluding materials. The materials were placed in implants with an internal-hexagonal abutment-implant connection, which were restored with a temporary acrylic resin crown. Cyclic loading, which is the dynamic or variable repetitive loading of stress into a material, with 21 N at 1 Hz was applied 16,000 times to the specimens in 0.5% basic fuchsine solution according to the long axis of the tooth. The absorbance was measured by a spectrophotometer at 540 nm to evaluate the degree of microleakage. The authors found that microleakage was greatest when the cotton pellet was used, then the silicone sealing material, then vinyl polysiloxane, and least with gutta-percha. There was no significant difference in the amount of microleakage between vinyl polysiloxane and gutta-percha. This study demonstrated that the type of access channel occluding material could affect the amount of microleakage in an *in vitro* study environment.[21]

The degree of microleakage between an implant and its prosthetic components depends on variable factors, such as a precise fit between the components, torque, and micromovements between the connected walls during function. Even with precise fits, correct screw tightness, or preload, and decreased/controlled micromovements, microleakage will exist. The concern of microleakage would be the passage of any potential pathogenic substance from the screw channel into or out of the peri-implant tissues. If this channel is occluded with a material that harbors pathogenic microbes, this could have

direct communication with the peri-implant tissues and may lead to potential risks for peri-implant mucositis and peri-implantitis.

IV. Types of Occluding Materials

After understanding and being able to conceptualize the components that make up an implant system, one can understand the need to cover the head of the abutment screw for protection of its integrity. Another important feature is the ability to remove the material to gain access to the screw in the event of an implant or implant prosthesis complication. Materials such as cotton, gutta percha, vinyl polysiloxane, and autopolymerizing acrylic resin have been suggested for sealing the deep part of the screw access channel in screw-retained implant-supported restorations.[23]

Cotton pellets (Figure 11) are utilized frequently due to their low cost. A product by Richmond Dental, which are 100% bleached cotton pellets, come non-sterile but can be sterilized and placed on top of the screw. One of the clinical concerns with cotton pellets is they are organic and break down over time and that they are absorbent and soak up blood and saliva products. In many instances, but not always, clinicians can identify the malodor that is present upon removal when cotton is used. This malodor, as previously discussed, can be attributed to the hydrogen sulfide and methyl mercaptan compounds found within the oral environment.

Other authors such as Adrian[24]have advocated the use of a silicone material as seen in Figure 12. He described his technique in 1991, "A technique is described that isolates the screws, preserves the canal form, and allows for easy access and removal of the screws." Dentsply, a dental product company, makes a family of products called Aquasil (Figure 13). One of the options is a low viscosity polyvinylsiloxane product that can be dispensed (Figure 14) directly into the implant channel. These products usually have fast set times between a minute or two. Benefits of these silicone products in comparison to cotton are that they are synthetic and non-absorbent, allowing for little to no absorption and breakdown.

Synthetic foam is another material commonly used implant dentistry. This material is typically used for holding the files associated with endodontic procedures. The product is called Endoring Foam Inserts and these foam triangles can be cut smaller and sterilized. They are made from open cell polyurethane and can be sterilized before the first use (Figure 15). The benefits of this material are that it is synthetic and will not breakdown but drawbacks include the absorbent nature and sometimes the material shreds on removal.

The use of PTFE (polytetrafluoroethylene) tape, otherwise known as Tef-tape or plumber's tape, has been reported in dentistry since Stean in 1993.[25]He categorized the forms of manufactured PTFE into three: unsintered PTFE, sintered PTFE, and expanded PTFE. Unsintered PTFE has properties that are suitable for dentistry such as it is formed under pressure into tape which is thin, stretchable, and may be very closely adapted to hard or soft tissues, easily removed without tearing or leaving any fragments or residues. It can be formed into sheets of variable thickness and widths, which can be either flexible

or rigid. Stean discussed three different types of uses of PTFE tape: 1-mechanical barrier application, 2-surgical uses, 3-fit-checker.A unique application, which this author empirically believes in, is its use for the management of screw access channels in implant-supported prostheses. The choice to utilize PTFE for this purpose is usually not scientifically supported, as stated, "The choice is dependent on the operator's preference and is influenced by different requirements, such as ease of manipulation, but is seldom scientifically supported".[23]

There is a paucity of studies testing the concept of microleakage *in vivo*. Quirynen et al.[26]utilized the abutment screws as the harboring material for micro-organisms and bacterial counts were evaluated by differential phase-contrast microscopy after 3 months. This study, the only *in vivo* study completed, showed that all screws harbored a significant amount of micro-organisms and that microleakage at the abutment-implant interface is the most probable origin for this contamination.

In a paper by Socransky and Haffajee[27], they discussed microbial ecological communities associated with oral health and disease. They demonstrated that microbes coaggreagate together and are distributed by regions within the periodontal pocket/gingival sulci (Figures 16& 17). They concluded that, "it is clear that periodontal disease status has a major impact on the composition of the subgingival microbiota and that, on average, disease status affects certain species, particularly members of the red and orange complexes, more than others." This study also pointed out that the purple, yellow and green complexes were associated with healthy more supragingival sites. Although this information was related to teeth, other authors such as Persson and Renvert[28] have shown that some of the same bacterial species are found at elevated counts from dental implant sites suffering from peri-implantitis.

This study gives an additional opportunity to examine any association between the microflora found in the inner aspects of a restored implant on the occluding material and the known red and orange pathogenic microbial complexes as defined by Socransky and Haffajee. The hypothesis is that implant abutment channels in which PTFE (polytetrafluoroethylene) tape has been placed will harbor a smaller percentage of pathogenic bacteria belonging to the red and orange complexes. This would possibly be due to the non-stick, non-absorbent property of PTFE. To test this hypothesis, we will need to utilize a technique to examine and identify the microbes we get back on our samples. We desire a technique that will give us the most information from the samples.

V. Techniques to Examine Microbes in Study Samples

Regarding the quantification and identification of bacterial species, there are many techniques that may be employed. Laboratory methods include non-cultural, cultural and immunological techniques. Non-culturing methods include microscopy and gene detection. Cultural methods include solid or liquid media and in some cases animal or human cells for microbial growth. The immunological methods include identifying organisms by detecting antibodies in a patient's bodily fluids, which is useful when the microbe cannot be cultured.

According to a lab manual by Richland College[29] "The two most widely used methods for determining bacterial numbers are the standard, or viable, plate count method and spectrophotometric (turbidimetric) analysis. Although the two methods are somewhat similar in the results they yield, there are distinct differences. For example, the standard plate count method is an indirect measurement of cell density and reveals information related only to live and culturable bacteria. The spectrophotometric analysis is based on turbidity and indirectly measures all bacteria (cell biomass),dead and alive."

Microscopic methods such as the light microscope and molecular methods such as polymerase chain reaction (PCR) are non-cultural. Light microscopy is used commonly with preparation of slides by stained smears. Resolutions of microscopic methods range from magnifications of 10X to the ability with electron microscopy to allow resolution as small as 0.001µm. The limitation of these methods is that most bacteria can not be identified by just strict observation.

Another method, Polymerase Chain Reaction (PCR), as illustrated in Figure 18, is described in the text by Murray, "this technique is a rapid means of amplifying a known sequence of DNA. A sample is mixed with a heat-stable DNA polymerase, excess deoxyribonucleotide triphosphates, and two DNA oligomers (primers), which complement the ends of the target sequence to be amplified. The mixture is heated to denature the DNA and then cooled to allow binding of the primers to the target DNA and extension of the primers by the polymerase. The cycle is repeated."[30] The main advantage of the PCR method is the speed of the technique. Samples can be analyzed in hours as opposed to days by culture methods. "The polymerase chain reaction (PCR) can detect single copies of viral DNA by amplifying the DNA many million-fold. A target sequence can be amplified a million-fold in a few hours using this method." [30]

For this study, PCR was the technique of choice due to the rapid ability to identify all bacteria in our samples. This method is useful regardless if the bacteria were cultivable or not. This is because 'universal' primers can be used that will amplify a fragment of the 16S rRNA gene from almost all known bacteria. PCR, followed by sequencing of the products, gives the ability to identify all species in the sample. The high-throughput sequencing of the PCR products, as discussed by Fabrice,[31] can give tens of thousands of sequences from a single sample.

Aims of the Research

1. To determine the Socransky & Haffajee bacterial complexes harbored on four different occluding materials in a screw-retained implant prosthesis after a period of 6 months in use (at material level). The test materials consist of Teflon tape, cotton pellets, synthetic foam and polyvinylsiloxane. The hypothesis is that Teflon tape will harbor a smaller percentage of "Red and Orange Complex" bacteria when compared to the other test materials.

2. To determine if there is a significant difference in Socransky & Haffajee complexes harbored on the occluding material in a screw-retained implant prosthesis when comparing four commonly used materials in the same individual study subject (at subject level). The hypothesis is that there will be differences in bacterial complexes among the four materials in the same subject.

3. To determine if there is a significant difference in Socransky & Haffajee complexes harbored on the occluding material in a screw-retained implant prosthesis when comparing four commonly used materials in all study subjects (at subject level). The hypothesis is that there will be differences in bacterial complexes among the eight different subjects.

The overall goal of this study is to assess the bacterial community present on blocking materials used to cover the screw in the "screw access channel" in dental implant restorations. Different blocking materials may have different microbiologic environments, which may adversely affect the implant mucosal health.

Materials and Methods

Research Design and Methods: IRB# 409069-1 Approved: 11/21/2013

1. Study population and design

This study was a randomized controlled clinical trial created to evaluate the microbial community colonizing the dental material selected for occluding purposes. This study compared three test materials (PTFE tape, synthetic foam, PVS) and a control material (cotton) and included 7 subjects who have 8 maxillary dental implants supporting a full arch prosthesis. The eighth subject has a different distribution of implants but still satisfied the criteria with having 8 implants between their maxillary and mandibular arches and no teeth to confound the results. Ages of the subjects ranged from 53-73 years old with 6 male and 2 female subjects(Table 2). Subjects were excluded based on the following criteria:

-poor oral hygiene and motivation
-uncontrolled diabetes
-pregnant or lactating
-substance abusers
-current smokers
-psychiatric problems or unrealistic expectations
-acute infection in the area intended for implant sealing
-positive to HIV or hepatitis B or C
-affected by autoimmune diseases such as arthritis rheumatoid, systemic lupus erythematosus, sclerodermia, Sjögren syndrome, or under chronic treatment with steroids or non-steroidal anti-inflammatory drugs.

2. Research site

The studies were conducted at the Dental Care Center's clinical facilities in the School of Dental Medicine (SDM) of Stony Brook University. The dental clinics represent modern patient care facilities serving the needs of Eastern Long Island communities. The periodontal clinic (dental and postdoctoral) examines and treats over 300 patients per month, involving both non-surgical and surgical modes of periodontal therapy. These clinics have provided more than sufficient clinical samples for previous studies carried out by Dr. Carrion. All the microbiological experiments were conducted in Dr. Jackie Collier's laboratory.

3. Screening

Subjects were identified from our existing dental records in electronic databases, strictly based on our inclusion criteria. Subjects were then contacted by phone to determine if they were interested to participate in this study or not. Regardless of their participation in research, patients have to be seen every 6 months for the recall visit and cleaning (standard of care procedures). Pregnant women were excluded from the study due to the length of the appointment (~2 hours). Pregnancy was assessed by asking the potential subject if their pregnancy status.

4. Procedures for sample placement and retrieval

The study consisted of approximately 3 visits over a period of less than 1 year from recruitment:

-Visit 1: Screening/Consent- Patients were invited for a screening appointment to review their medical and dental history and to ensure they understood the study and the importance of compliance with the protocol. The subjects then signed all informed consent documents.

-Visit 2:Cleaningand Occluding Material Placement: consisting of oral hygiene instruction and maxillary debridement by removal of retaining screws and extraoral disinfection of maxillary prosthesis and screws. The maxillary prosthesis was replaced with test materials(Table 3),randomized with 2 samples of each material placed into each subject (8 implants per subject) giving us a total of 8 subjects with 64 material samples (16of each material) and a provisional composite sealing material was placed above the test material to complete the treatment for this visit.

-Visit 3: Material Retrieval (at or after 6 months) with Occlusal Seal Replacement: Samples were retrieved with use of a sterile endodontic K-file; at this point, the two identical material samples of the test materials were placed into one transport tube and then onto an ice bucket. This step left us with four samples per subject. The prosthesis was removed to facilitate collection of periodontal parameters and peri-implant sulcus microbiological samples. Subjects then had the prosthesis re-inserted and cylinders resealed with PTFE and composite resin. The subjects received oral hygiene instruction which included review of cleaning instruments and recommended routine. Subjects will be seen to the completion of the study and then placed on an individualized maintenance schedules.

5. Sample Storage

All samples were stored in a -80C freezer located in Dr. Carrion's lab until further analysis.

6. Sample DNA extraction

The samples were transported in Eppendorf tubes on ice to Dr. Collier's lab where the DNA was extracted utilizing the PowerSoil DNA Isolation Kit, as illustrated in Figure 19.The PowerSoil® DNA Isolation Kit was used due to its ability to remove a variety of PCR inhibitors from DNA. The isolated DNA has a high level of purity allowing for more successful PCR amplification of organisms from the sample. PCR analysis has been performed to detect a variety of organisms including bacteria (e.g. *Bacillus subtilis*, *Bacillus anthracis*), fungi (e.g. yeasts, molds), algae and *Actinomycetes* (e.g. *Streptomyces*)using DNA extracted with this kit.[32]

Transferring samples to the PowerBead tubes was facilitated by washing the Eppendorf tubes that the samples were first stored in with the first solution of the kit. All solution and sample was transferred to the PowerBead tubes and manufacturer protocol was then followed. This first wash was done to ensure that as much DNA was obtained as

possible by limiting the chance of some of the sample left behind in the storage tube..The DNA was stored in a -20 Celsius freezer until being sent out for PCR, sequencing, and analysis.

7. PCR, Sequencing, and Analysis

The 16SrRNA primers selected were based on a study by Klindworth et al.[33](Forward:S-D-Arch-0519-a-S-15 (A519F) CAGCMGCCGCGGTAA; Reverse: S-D-Bact-0785-b-A-18 (802R) TACNVGGGTATCTAATCC). A 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) was run under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. After amplification, PCR products were checked in a 2% agarose gel to determine the success of amplification and the relative intensity of bands. The molecular mass expected was approximately 300b.p. and the V4 region of 16S rRNA was amplified. Multiple samples were pooled together (in this case, sequencing was done on two sets of 37 samples) in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Then the pooled and purified PCR product was used to prepare aDNA library by following the IlluminaTruSeq DNA library preparation protocol. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines. Sequence data were processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, sequences were joined, depleted of barcodes then sequences <150bp removed, and sequences with ambiguous base calls were removed. Sequences were denoised, OTUs generated and chimeras removed. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% identity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from GreenGenes, RDPII and NCBI (www.ncbi.nlm.nih.gov, DeSantis et al 2006, http://rdp.cme.msu.edu).

8. Statistics

Prior to the start of the project, a power analysis was completed by Dr. Stephen Finch, Professor, from Department of Applied Mathematics& Statistics. The primary outcome used to calculate the power analysis was, to see if there was any difference in bacterial populations on 4 test materials. The number of study subjects needed was determined to be 8, and the type of sample randomization was suggested (Table 4).The Latin Square Design was selected due to ability to balance the samples in the various positions in the subjects' oral cavity.

Sixty-four samples were obtained; DNA was isolated and sent out for analysis. From the sixty-four samples, 32 were from the dental materials and were analyzed for the purpose of this study (Table 5).

9. Funding:

This study was funded by the Department of Periodontology of Stony Brook University School of Dental Medicine.

Results

From the eight subjects in the study, 32 material samples were collected and DNA was extracted and amplified with PCR and high-throughput sequencing to generate 1,862,900 sequences representing286 different genera and 492 different species. On average each sample generated58,215sequences. The most abundant taxa, based on genus, were *Lactobacillus spp.* (26.48% of sequences), *Atopobium spp.* (10.10%), *Streptococcus spp.* (5.22%)*and Selenomonas spp.* (3.61%), none belonging to the red or orange complexes. The most abundant species was *Atopobium spp.,* also not belonging to the proposed pathogenic microbial complexes as mentioned by Socransky[27].Chart46 illustrates all data in pie chart format for all of the samples, as well as the averages of the eight subjects and the averages for the four test materials.

With regard to the first specific aim, charts #1-4 illustrate, based on materials, the percentages of bacterial complexes in all the subjects of the study for each material. After an ANOVA was completed, a post-hoc Tukey HSD test at a significance level of $\alpha = 0.05$ was utlized next.There was no significant difference in regard to the proportions of Red and Orange complexes found on any one material. There were a total of six microbial species not found in the colored complexes that were affected by the material used but no one material seemed to be better than the other three. These six bacteria were *Atopobium spp., Bilophila wadsworthia, Erysipelothrix spp., Haemophilus parainfluenzae, Rheinheimera spp.* and *Shuttleworthia satelles.* Some of these microbes associated with halitosis or malodor.

Upon comparison of the materials within the same individual, the second specific aim, charts #13-45 illustrate the percentages of bacterial complexes found on the occluding materials separately in the same subject. For this intra-patient perspective, a t-test at a significance level of 0.01 was used and different subjects had some of the colored complexes at a significantly higher level on some of the specific materials. For example, subject #1 had significantly more Blue Complex species on the cotton sample than all other materials. Other subjects also had significant differences. These included: subject #3,where cotton had significantly more Orange Complex species than the other materials; subject #7, where Teflon produced the significantly lowest Red Complex species and subject #8, where PVS had significantly greater numbers of Green Complex species, but not significantly greater than the cotton sample used in the same subject.

With regard to the subjects in this study, the third specific aim was to determine any differences between subjects. Charts #5-12 illustrate the percentages of bacterial complexes found on all of the occluding materials in any given subject. For this inter-patient perspective, once again a post-hoc Tukey HSD test at a significance level of $\alpha=0.05$ was utilized after an ANOVA was completed. There was no significant difference in colored complexes between the test materials when comparing all subjects. This demonstrates that the material used did not affect the microbes. This illustrates that the differences in microbes were more influenced by the subject. For example, subject #7 had significantly more Red Complex species than the other subjects. Subject #8 had significantly less Green complex species than patient #1. In addition too, Subject #8 had significantly less Yellow

complex species than subjects #1 and 2. Orange, Purple and Blue complexes were not affected by the subject influence.

The Grey Complex, or other species, not belonging to the classic clusters, was analyzed. Table 6illustrates a list of the most prevalent genera in the Grey Complex. *Lactobacillus spp. 26.48%, Atopobium spp. 10.10%, Streptococcus spp. 5.22% and Selenomonas spp. 3.61%*, were the most prevalent.

When comparing the data between the occluding materials and the complexes found in the peri-implant sulci by utilizing the Tukey HSD test at a significance level of $\alpha = 0.05$, the material used did not have any significant effect. When comparing the data sets using a t-test, the proportion of Red and Orange complexes found inside were significantly less than outside. The proportion of Green, Yellow, and Blue complexes found inside were significantly greater than outside. Also, there was no significant difference for the Purple complex on the inside and outside. In conclusion, the difference between subjects seemed to be a greater contributing factor as compared with the difference among samples.

Discussion

In this study we tested four of the most commonly used dental materials for the purpose of occluding the access cylinder to protect the retaining screw. To aid in prevention of peri-implantitis, a material that harbors less microbes would be potentially beneficial when microleakage is occurring. We postulated that a superior material for the purpose of the occlusion of screw access channels in implant supported prostheses, is a material that may provide a surface which bacteria may not favor. The choice of an occluding material that is less favorable for bacterial growth, particularly Red and Orange Complex bacterial growth, will be more advantageous to use in clinical practice and presumably assist in the prevention of peri-implant pathology. Currently, clinicians select materials to occlude the screw channel based on ease of manipulation (Table 7).This study would allow clinicians to make a decision based on a scientific biological foundation rather than anecdotal beliefs.

Although Park et al[21]found a difference between the test materials, this study contradicts their *in vitro* results. There was no effect of the material on the composition of the bacterial community. This clinically confirms a dentist's personal choice for selection of occluding material. Application of these materials can be selected by ease of use as opposed to microbial properties.

This study contributed a body of knowledge regarding implant microbiology. The "Grey Complex" or other species, not grouped by Socransky and Haffajee, were analyzed because the majority of the sequences belonged to this group. Hydrogen sulfide and methyl mercaptan have been shown to be associated with specific microbes. In a paper by Takeshita,[34] is was concluded that distinct bacterial populations in the oral microbiota are involved in the production of hydrogen sulfide and methyl mercaptan. These bacteria included, "The H_2S group showed higher proportions of the genera *Neisseria, Fusobacterium, Porphyromonas* where as the CH_3SH group had higher proportions of the genera *Prevotella, Veillonella, Atopobium, Megasphaera, and Selenomonas*." Since some of these microbes were present in the current study, this may prove to be beneficial for gaining insight into the microbiome of malodor around dental implants.

As seen by the demographics table (Table 2),our average subject was a 58 year old Caucasian male. Although sex and age were not controlled, if there were more subjects included in the study, the subjects may have been more diverse and this may have had an influence on our data sets.

The arrangement of implants in six out of the eight subjects were uniform with subject #6 having seven implants and subject #8 subject having four maxillary and four mandibular implants. Subject #8 did have significantly more Green and Yellow complexes. These complexes are attributable to periodontal health as shown by Socransky and Haffajee. This could be due to ease of hygiene with the four implants having more space between them for less plaque entrapment and access for oral hygiene devices.

Subject#7 did have significantly more Red Complex and upon clinical evaluation, this subject had poor oral hygiene with poor plaque control. This coincides with Heitz-Mayfield[10], who in their review showed that poor oral hygiene is a risk indicator for peri-implant diseases. It was interesting to identify, and should be discussed, that within subject #7, who had the highest percentages of red complex, the PTFE test material had significantly less red complex than the other test materials. Although this was not a uniform finding amongst all the study subjects, this may be a property of PTFE worth future investigation.

Seven out of the eight subjects all had implants that came from the same manufacturer and all had the same surface treatment and platform design. Subjects #2-8 all had implants from Nobel Biocare, with internal connections and subject#1 had their implant from Biomet 3i, with an external hex connection. The two companies, although very similar in design, have different methods of surface texturing to increase surface roughness as seen in Table 8.A review by Abrahamsson and Berglundh[35] concluded that there was no surface treatment or implant system that was superior in marginal bone preservation. When evaluating clinically for our study, there was no observable clinical or statistical differences between the implant systems.

Future studies regarding the occluding material for dental implants can include the same materials applied in this study with a medicament or ointment. Another item of interest can include the material itself being made from a material that is bacteriostatic or -cidal. Findings from these future studies may be clinical techniques and/or materials dental clinicians utilize in patient care.

Conclusions

1- There were no statistically significant differences in bacterial colonization amongst the test materials. The choice of an occluding material would therefore be based on clinical judgment as seen in Table 7.

2- There were a total of six microbial species not present in the "colored" complexes of Socransky and Haffajee that were affected by the occluding materials. These six microbial species were *Atopobium spp., Bilophila wadsworthia, Erysipelothrix spp., Haemophilus parainfluenzae, Rheinheimera spp. and Shuttleworthia satelles*.

3- *Atopobium spp., Selenomonas spp.* and *Fusobacterium spp.,* were amongst the most prevalent microbial genera present in the materials tested. These microbes have been shown to be associated with hydrogen sulfide and methyl mercaptan compounds, causative agent in malodor or halitosis. Although these microbes were not limited to the cotton samples, this can be the etiology for the malodor generally experienced by clinicians when retrieving samples.

FIGURES

Figure 1 – Peri-implant Disease (adapted from reference 7)

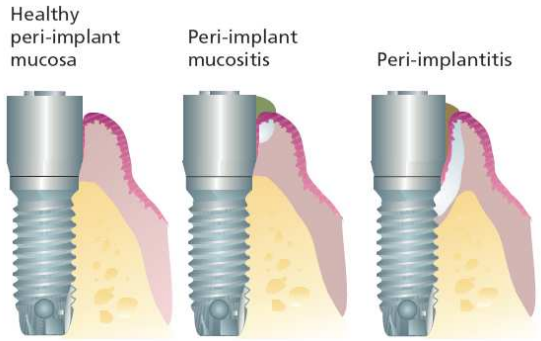


Fig 1:Schematic drawing illustrating healthy peri-implant mucosa, peri-implant mucositis, and peri-implantitis.

Figure 2 - Components of a Screw-retained Implant Crown (adapted from www.dentalimplantlife.com)

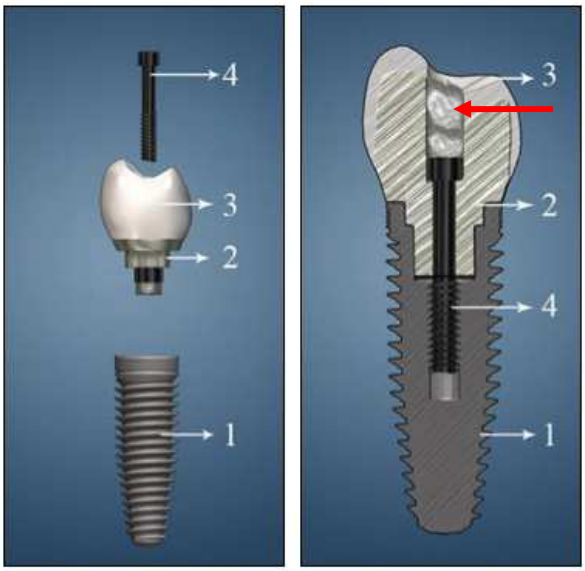


Fig 2:Representation of a disassembled(a) and an assembled(b) implant and screw-retained restoration. The screw (4) secures the crown (2) to the dental implant (1). The white material (as illustrated with red arrow)over the screw is the occluding material and the composite (3) is what seals the access cylinder.

Figure 3 –Components of a Cement-retained Implant Crown (adapted from www.dentalimplantlife.com)

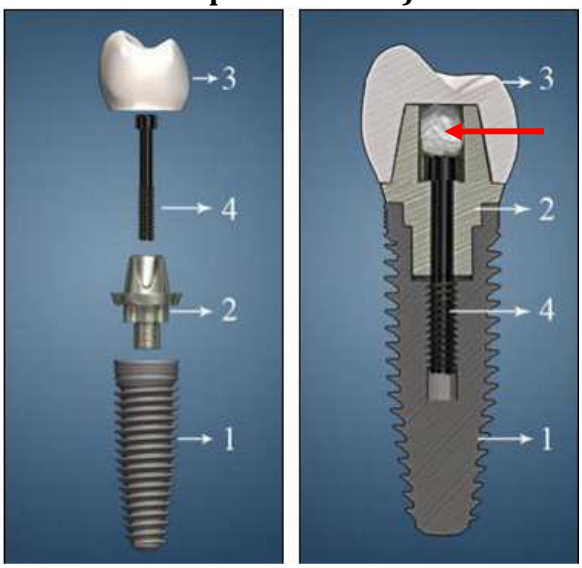


Fig 3: Representation of a disassembled(a) and an assembled(b) implant and cement-retained restoration. The screw (4) secures the abutment (2) to the dental implant (1). The white material (as illustrated with red arrow) over the screw is the occluding material and the crown (3) gets cemented on top and is what seals the system occlusally.

Figure 4 –Microleakage pathway (adapted from www.smile-mag.com)

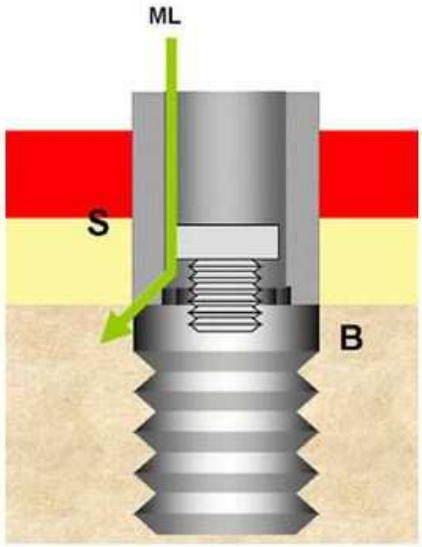


Fig 4: Passage of fluids through the microgap. ML= microleakage, S= soft tissue, B= bone

Figure 5 – Zimmer 1-Piece Dental Implants (adapted from www.hyper-dental.com)

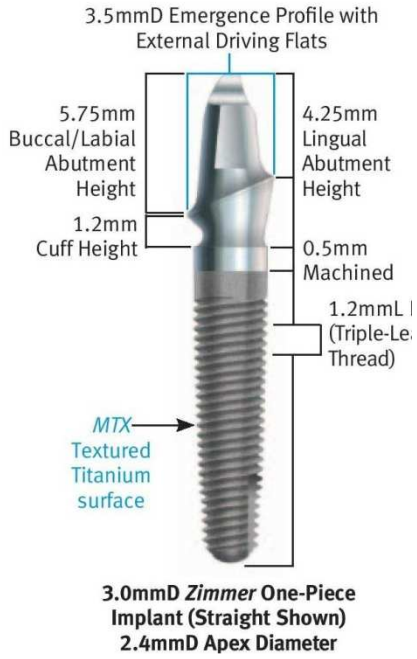


Figure 6 –Screw vs Friction (Cold-weld) (adapted from www.dentalimplantskerala.com)

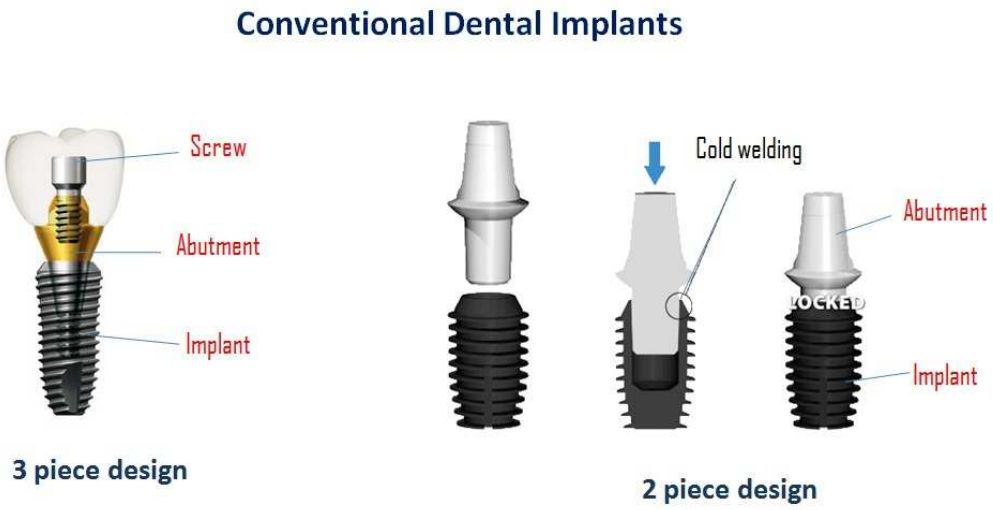


Figure 7 –Screw Retained Prosthesis – Directly to implant (adapted from nobelbiocare.com)

Fig7: Note only 1 screw used with 1 interface

Figure 8 – Screw Retained Prosthesis – With Intermediary Abutment (adapted from www.nobelbiocare.com)

Fig 8: Note 2 screws used with 2 interfaces

Figure 9 – SEM of Clamping Effect/ Preload (adapted J Contemp Dent Pract 2011; 12(5):365-360.)

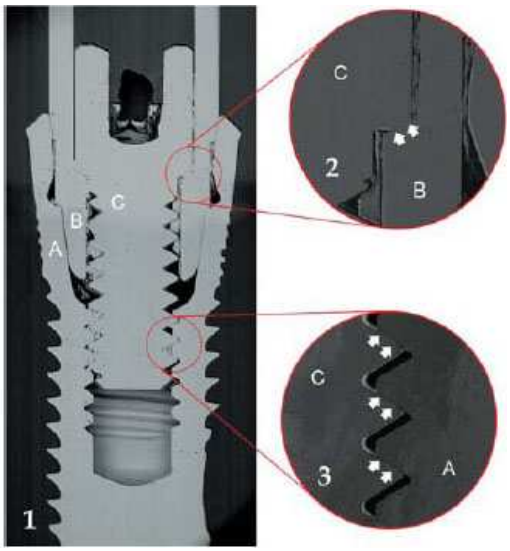


Fig. 9: (1) SEM imaging of the fixture-abutment-screw complex in longitudinal section; A: fixture; B: abutment; C: fixing screw. The mechanical contiguity between the parts is given by the preloading(torque) applied to the screw during the tightening; from a biomechanical point of view, the torque applied provides retention to the system because it produces a superficial plastic deformation on the opposing surfaces. In screw connected implant systems only two areas exist in which the retentive function is expressed, represented by the screw underhead (2) and by the screw spirals in contact with the internal thread of the fixture (3). In fact, in images(2) and (3) the arrows indicate the close contact obtained following the tightening of the connecting screw

Figure 10 – External Hex vs Morse Taper (adapted from reference 7)

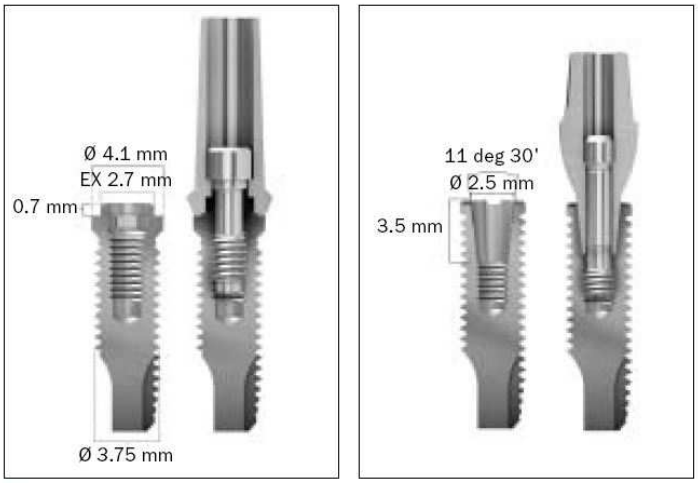


Fig 1 Schematic drawing of the implants with specific dimensions. *(Left)* The external-hexagon implant (Titamax Ti cortical, Neodent). *(Right)* The Morse taper implant (Titamax CM, Neodent).

Fig 10: Schematic drawing of the implants. (Left) External Hex Connection (Right) Internal Morse Connection

Figure 11 –Richmond Cotton Pellets (adapted from www.aluro.co.nz)

Fig 11: Foam pellets used in clinical dentistry

Figure 12 – Cross section of Silicone Plugs (adapted from reference 24)

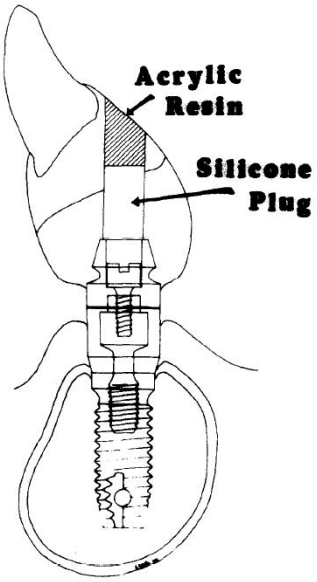


Fig 12: Demonstration of silicone plug with acrylic resin covering

Figure 13 – Polyvinylsiloxane Product Aquasil Family (adapted from www.dentsply.co.uk)

Fig 13: The options of PVS products

Figure 14 – Dispensing of PVS product (adapted from www.i.ytimg.com)

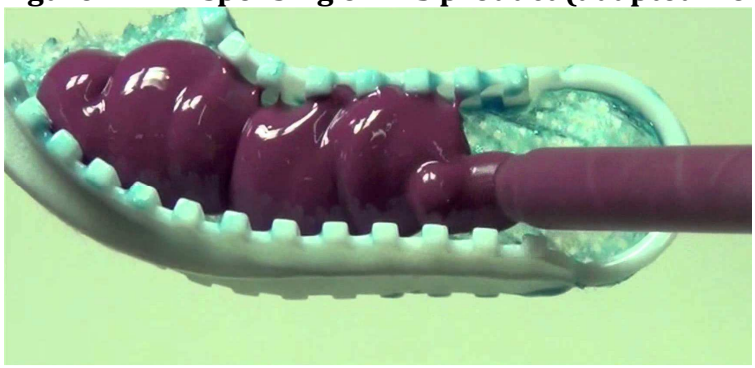


Fig 14: Demonstration of application of PVS prior to final setting

Figure 15 – Jordco Endo Foam Inserts (adapted from www.net32.com)

Fig 15: Synthetic foam product usually used during clinical endodontic procedures

Figure16 -Bacterial Complexes by Socransky &Haffajee (adapted from reference 27)

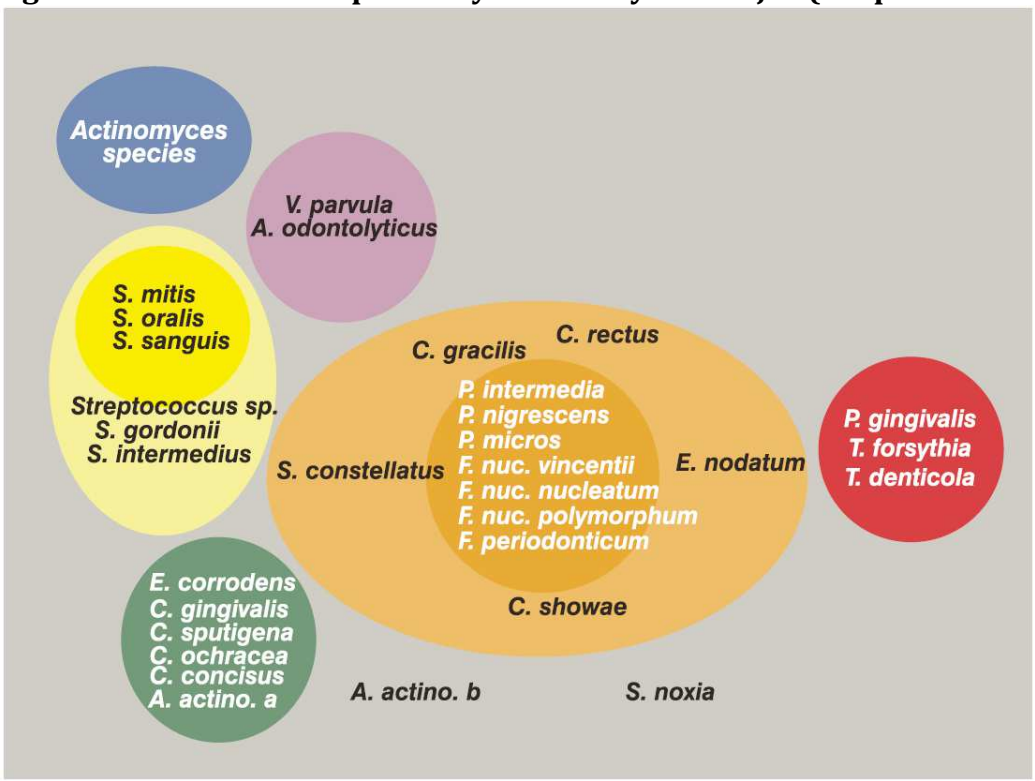


Fig 16: Classic grouping of microbial clusters into colored complexes

Figure17 -Distribution of Bacterial Complexes by Socransky & Haffajee (adapted from reference 27)

Fig 17: Location of microbial clusters in relationship to a tooth

Figure18 -Polymerase Chain Reaction (PCR)(adapted from reference 30)

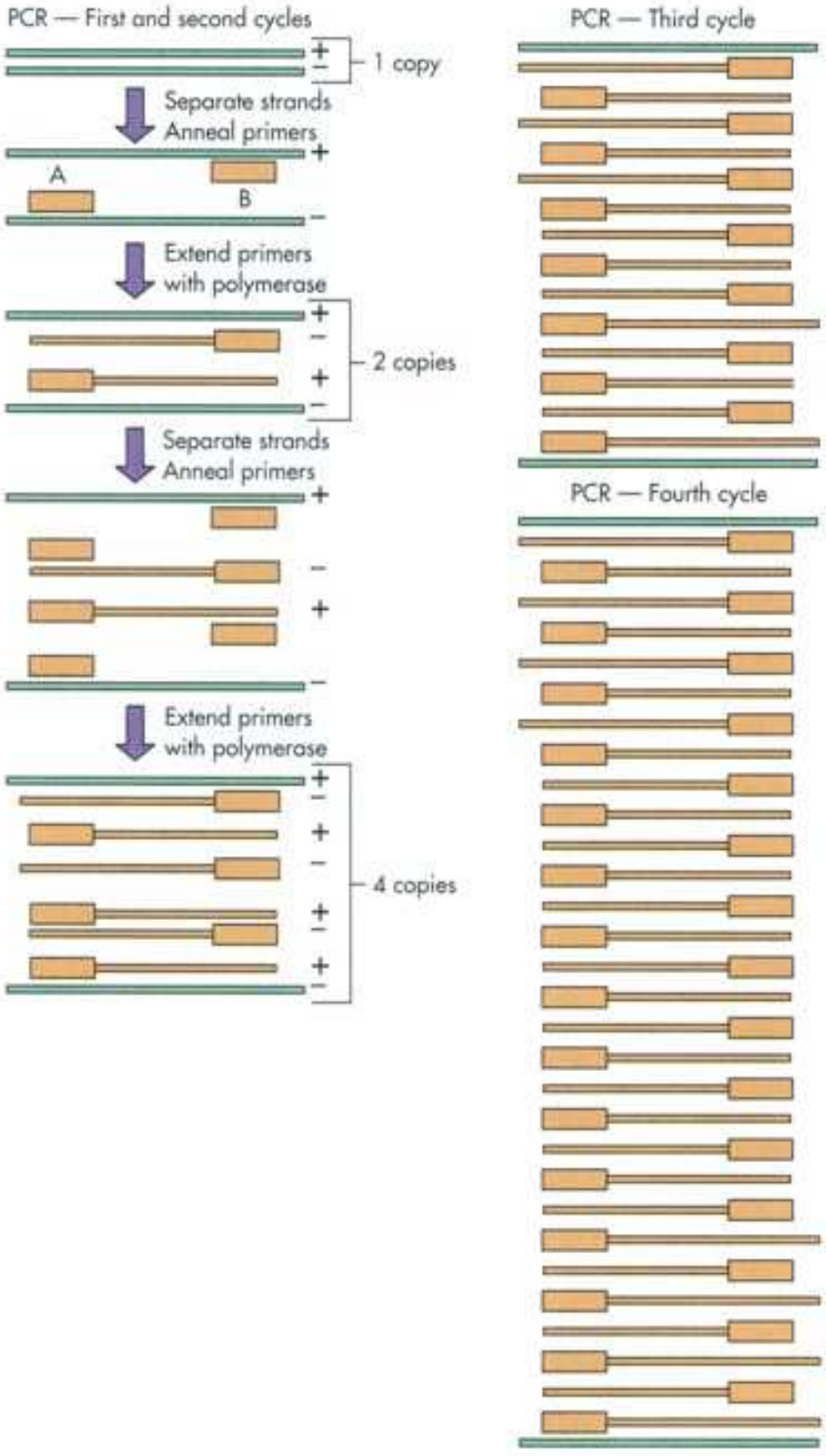


Fig 18: Schematic example of PCR

Fig 19 -Procedures for PowerSoil DNA Extraction Kit (adapted from PowerSoil Instruction Manual)

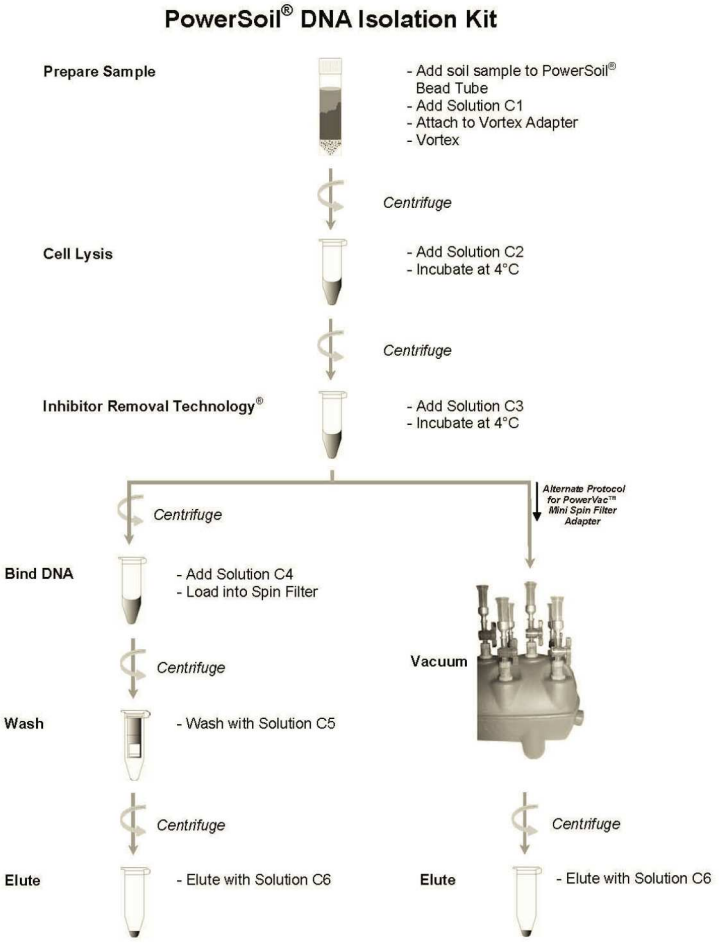


Fig 19: Schematic example of steps used during DNA extraction

TABLES

Table 1. Risk Factors and Contraindications for Implant Therapy (adapted from reference 5)

	Risk Factor	Contraindication
Medical and Systemic Health-Related Issues		
Diabetes (poorly controlled)	?? -Possibly	Relative
Bone metabolic disease (e.g., osteoporosis)	?? -Probably	Relative
Radiation therapy (head and neck)	Yes	Relative/Absolute
Immunosuppressive medication	?? -Probably	Relative
Immunocompromising disease (e.g., HIV, AIDS)	?? -Possibly	Relative
Psychologic and Mental Conditions		
Psychiatric syndromes (e.g., schizophrenia, paranoia)	No	Absolute
Mental instability (e.g., neurotic, hysterical)	No	Absolute
Mentally impaired; uncooperative	No	Absolute
Irrational fears; phobias	No	Absolute
Unrealistic expectations	No	Absolute
Habits and Behavioral Considerations		
Smoking; tobacco use	Yes	Relative
Parafunctional habits	Yes	Relative
Substance abuse (e.g., alcohol, drugs)	?? -Possibly	Absolute
Intraoral Examination Findings		
Atrophic maxilla	Yes	Relative
Current infection (e.g., endodontic)	Yes	Relative
Periodontal disease	?? -Possibly	Relative

HIV, Human immunodeficiency virus; *AIDS*, acquired immunodeficiency syndrome.

Table 2 – Study Population Demographics

Subject Demographics		
Age (Years)	Range	53-73
	Mean	58.14
Sex	Male	6
	Female	2
Race	White	5
	Black	2
	Hispanic	1
Time Study Prosthesis in Function (Years)	Range	1.36-7.94
	Mean	4.43
Time Between T_1 and T_2 (Days)	Range	175-265
	Mean	206.86 (about 6.8 months)

Table 3- Materials Used

Material	Company	Product
Teflon Tape (PTFE)	Merco Co.	Merco M55 Thread Seal Tape
Cotton Pellets	Richmond Dental	Bleached Cotton
Synthetic Foam	Jordco	Endoring Foam Inserts
Polyvinylsiloxane (PVS)	Dentsply	Aquasil
Bonding Agent	Dentsply	PRIME & BOND®NT™
Composite	3M ESPE	Filtek Supreme Ultra Universal Restorative
Pink Composite	GC America Inc.	Gradia Gum

Table 4. Sample Randomization

Subject #	Front Right (FR)	Front Left (FL)	Back Right (BR)	Back Left (BL)
1	Material A	Material B	Material C	Material D
2	Material B	Material A	Material D	Material C
3	Material C	Material D	Material A	Material B
4	Material D	Material C	Material B	Material A
5	Material A	Material B	Material C	Material D
6	Material B	Material C	Material A	Material D
7	Material C	Material D	Material B	Material A
8	Material D	Material A	Material C	Material B

Table 5 –Occluding Material and Control Samples

Subject #	Data Set #	MATERIAL
1	11	Foam
	12	Teflon
	13	Cotton
	14	PVS
2	21	PVS
	22	Cotton
	23	Teflon
	24	Foam
3	31	Teflon
	32	Foam
	33	PVS
	34	Cotton
4	41	Cotton
	42	PVS
	43	Foam
	44	Teflon
5	51	Foam
	52	Teflon
	53	Cotton
	54	PVS
6	61	Teflon
	62	Cotton
	63	Foam
	64	PVS
7	71	Cotton
	72	Foam
	73	PVS
	74	Teflon
8	81	Foam
	82	PVS
	83	Teflon
	84	Cotton

Table 6 – List of Most Prevalent Genera in the "Grey Complex"

A. Over all samples

1)	lactobacillus	26.48%
2)	atopobium	10.10%
3)	streptococcus	5.22%
4)	selenomonas	3.61%
5)	olsenella	3.34%

B. Per Material

I. Foam

1)	lactobacillus	27.99%
2)	atopobium	18.62%
3)	olsenella	5.22%

II. Teflon

1)	lactobacillus	41.62%
2)	selenomonas	9.30%
3)	streptococcus	5.60%

III. Cotton

1)	lactobacillus	23.88%
2)	atopobium	5.98%
3)	streptococcus	5.86%

IV. PVS

1)	lactobacillus	12.38%
2)	atopobium	11.41%
3)	streptococcus	7.49%

C. Per Subject

I. Subject 1

1)	lactobacillus	31.92%
2)	atopobium	28.50%
3)	streptococcus	5.48%

II. Subject 2

1)	selenomonas	16.91%
2)	lactobacillus	10.33%
3)	roseateles.aquatilis	8.11%

III. Subject 3

1)	olsenella	7.05%
2)	lactobacillus	6.12%
3)	roseateles.aquatilis	5.89%

IV. Subject 4

1)	lactobacillus	33.91%
2)	parascardovia.denticolens	7.45%

3)	rothia	6.07%
V.	**Subject 5**	
1)	lactobacillus	59.19%
2)	streptococcus	6.25%
3)	halospirulina.sp.	3.31%
VI.	**Subject 6**	
1)	lactobacillus	42.25%
2)	streptococcus	9.46%
3)	corynebacterium	7.59%
VII.	**Subject 7**	
1)	lactobacillus	23.03%
2)	olsenella	13.05%
3)	rothia	5.17%
VIII.	**Subject 8**	
1)	neisseria	15.95%
2)	corynebacterium	14.76%
3)	streptococcus	8.48%

Table 7 - Clinical Selection of Materials

Material	Absorbent/Non-absorbent	Ease of handling (based on clinician)
Teflon Tape	Non-absorbent +	Easy +
Cotton Pellets	Absorbent -	Not easy -
Synthetic Foam	Absorbent -	Easy +
Polyvinylsiloxane (PVS)	Non-absorbent +	Not Easy -

Table 8 – Surface Treatments for Implants in study (adapted from www.glidewelldental.com)

SURFACE TREATMENT	IMPLANT SYSTEM/SURFACE
Acid-etched Etching with strong acids increases the surface roughness and the surface area of titanium implants.	BIOMET 3i OSSEOTITE® and NanoTite™
Anodized This electrochemical process thickens and roughens the titanium oxide layer on the surface of implants.	Nobel Biocare TiUnite®

CHARTS

Chart 1 – All Teflon Samples with Percentage of Bacterial Complexes

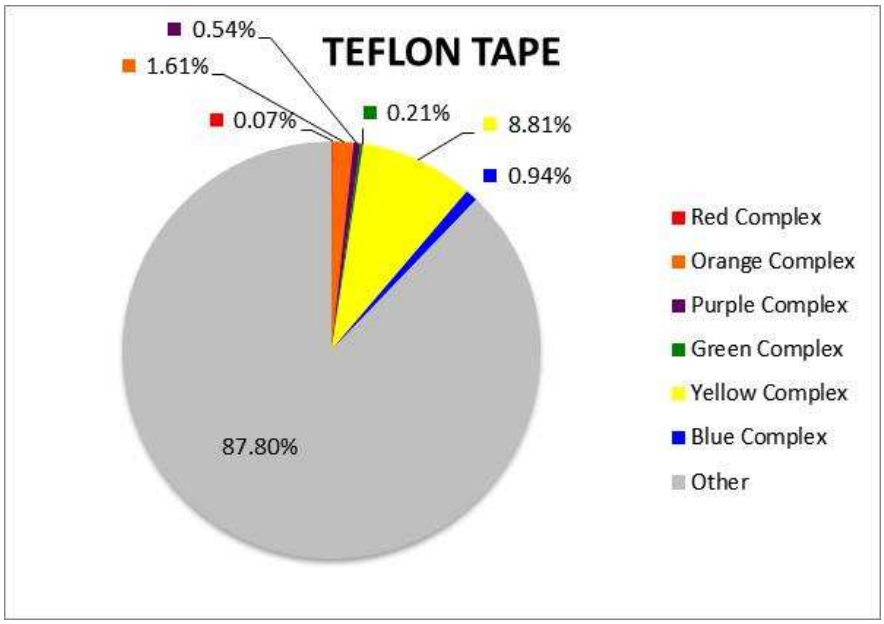


Chart 2 – All Synthetic Foam Samples with Percentage of Bacterial Complexes

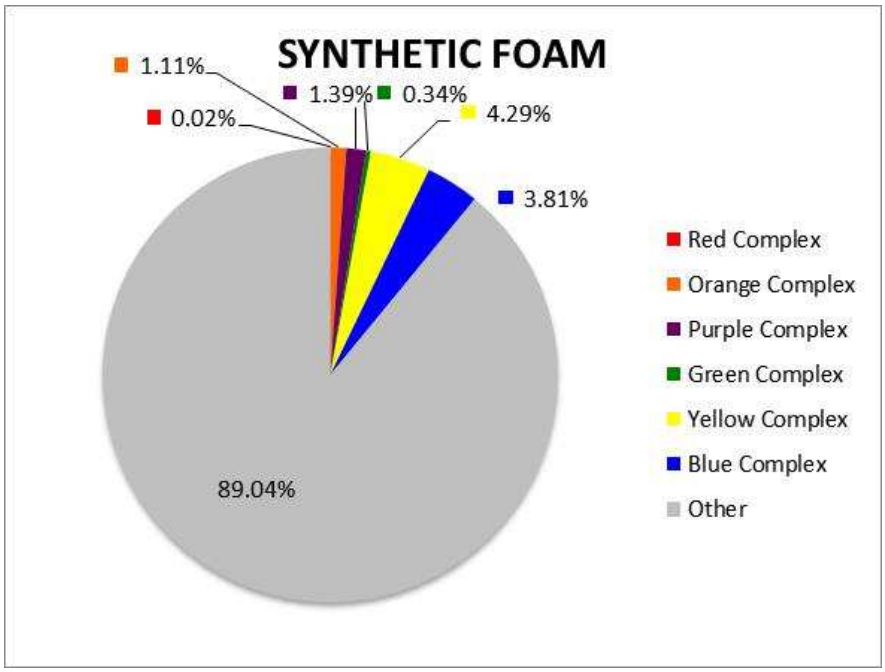


Chart 3 – All Polyvinylsiloxane (PVS) Samples with Percentage of Bacterial Complexes

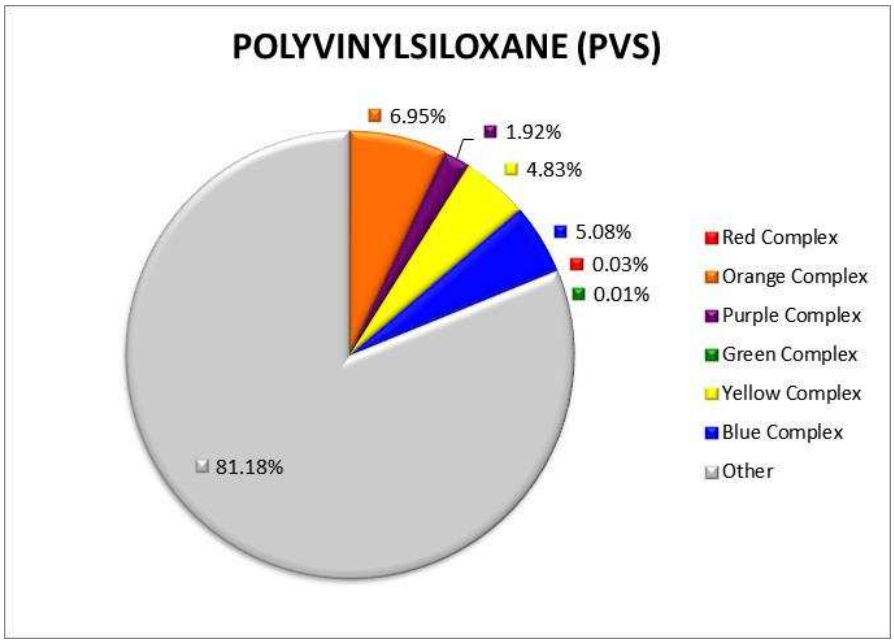


Chart 4 – All Cotton Samples with Percentage of Bacterial Complexes

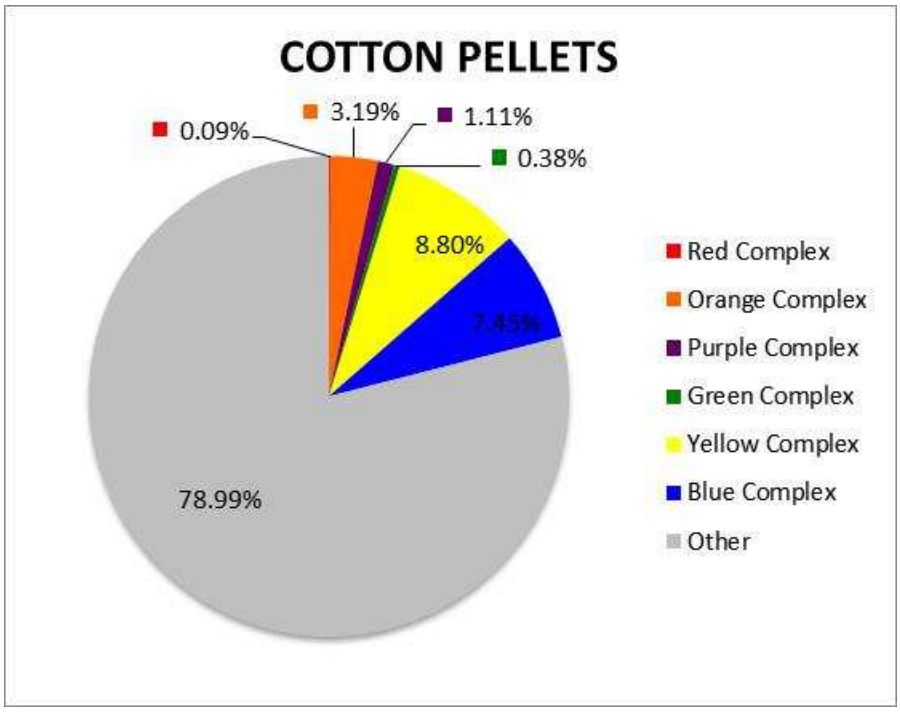


Chart 5 – Subject #1 Percentage of Bacterial Complexes

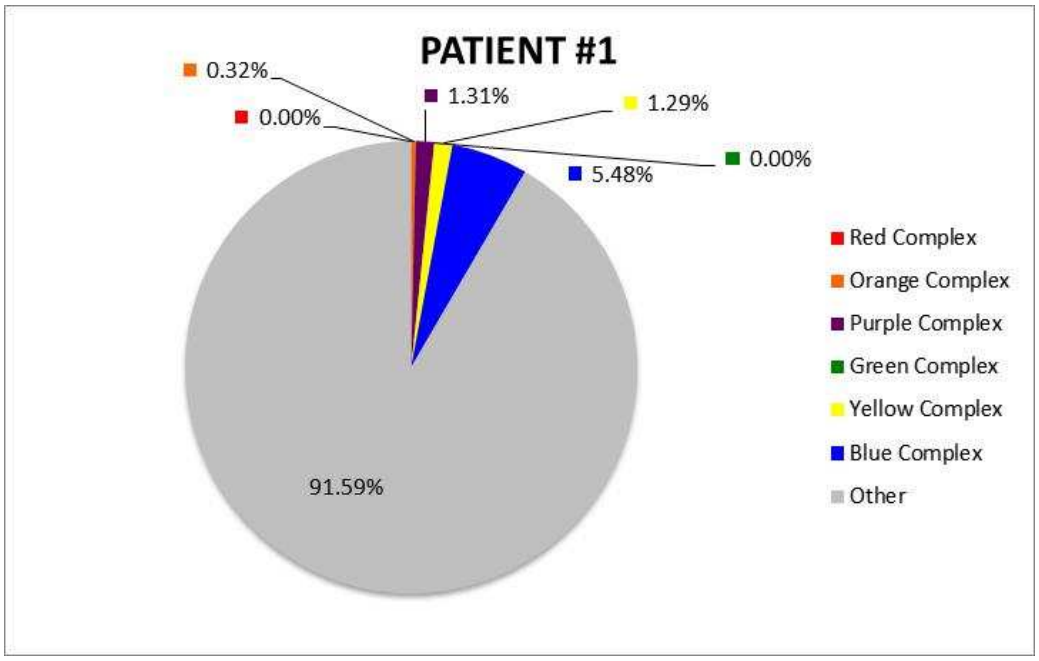


Chart 6 – Subject #2 Percentage of Bacterial Complexes

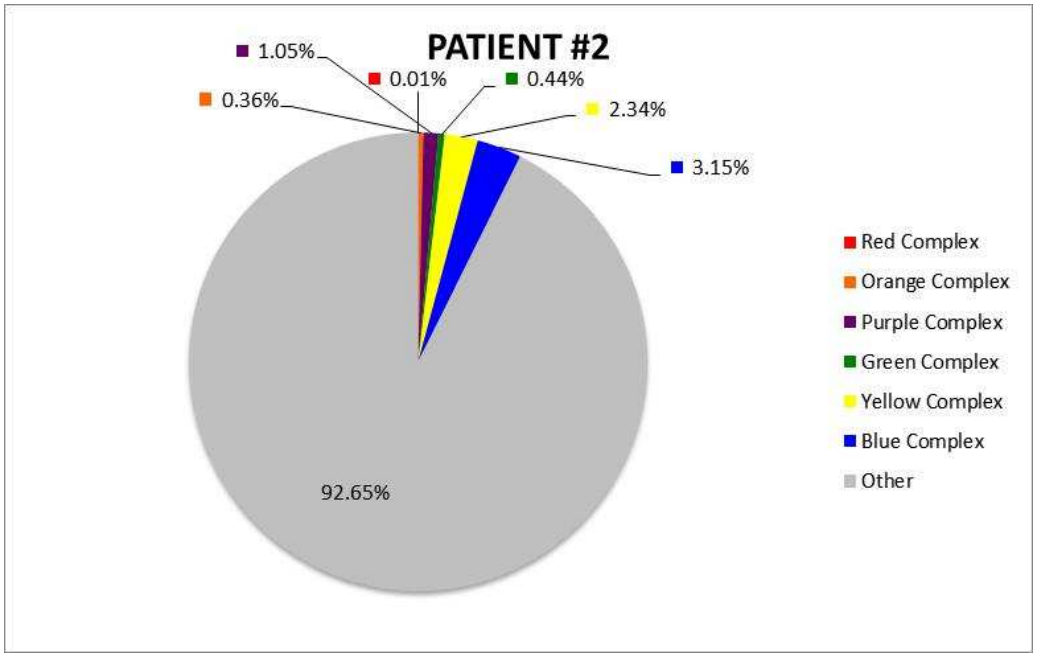


Chart 7 –Subject #3 Percentage of Bacterial Complexes

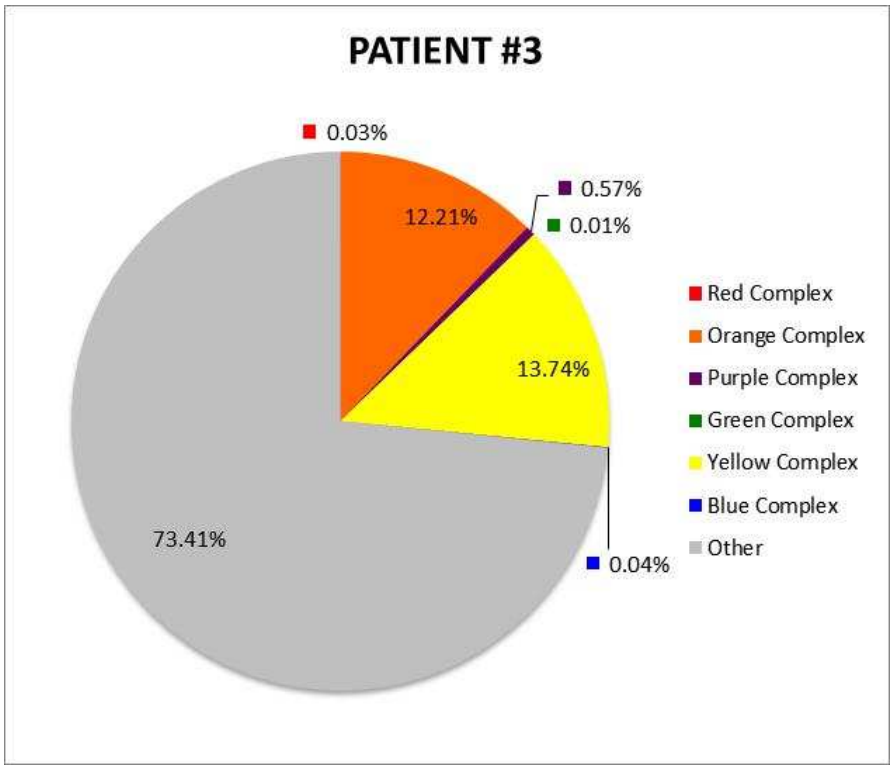


Chart 8 – Subject #4 Percentage of Bacterial Complexes

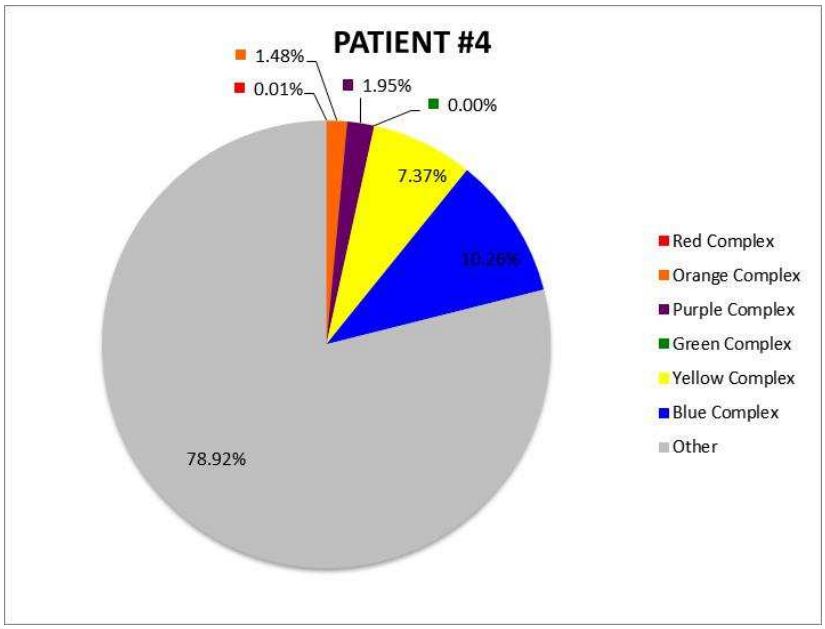


Chart 9– Subject #5 Percentage of Bacterial Complexes

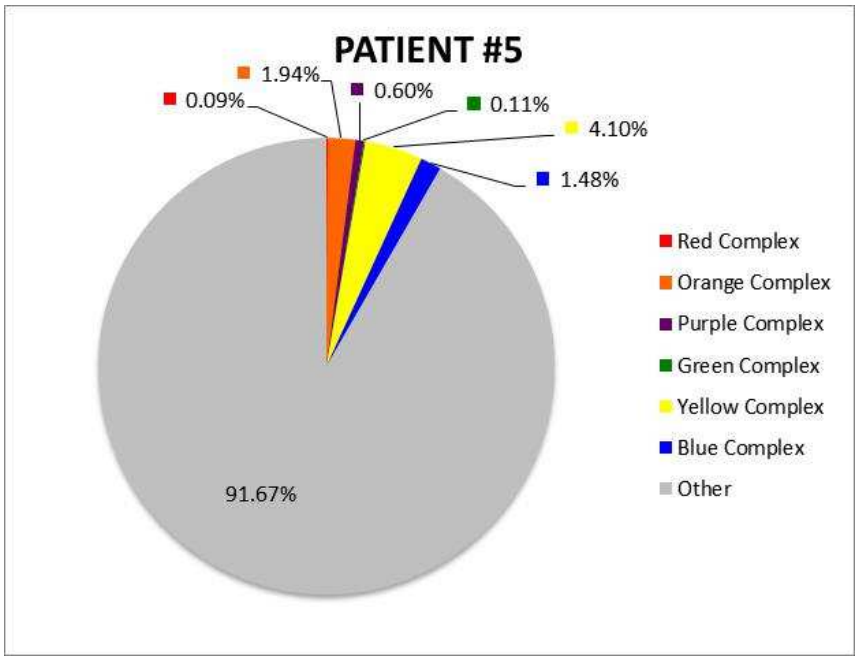


Chart 10 – Subject #6 Percentage of Bacterial Complexes

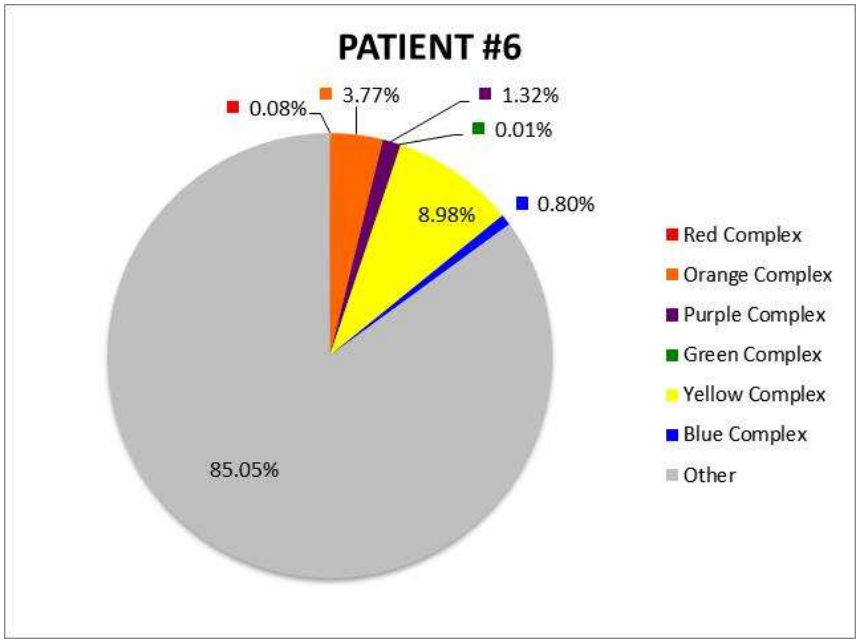


Chart 11 – Subject #7 Percentage of Bacterial Complexes

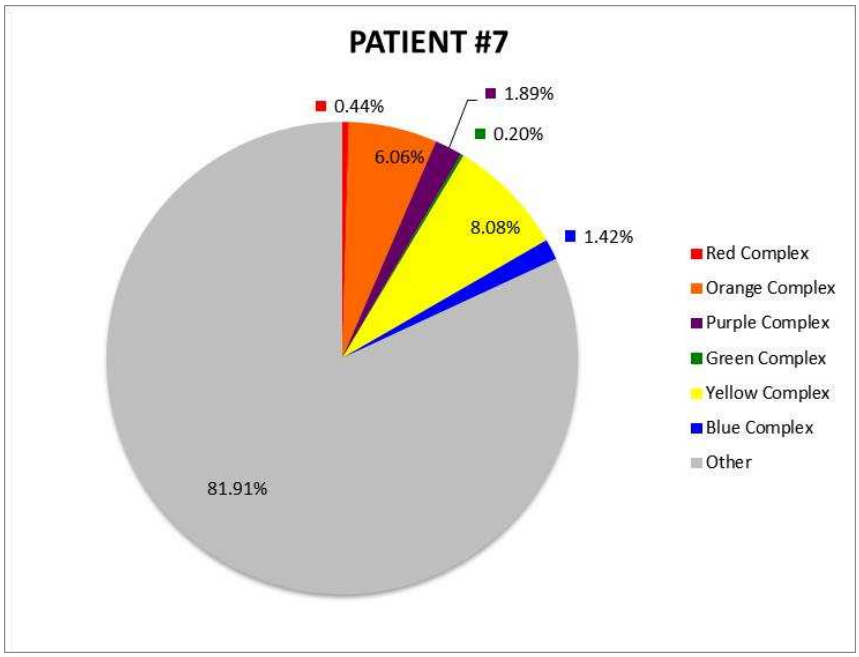
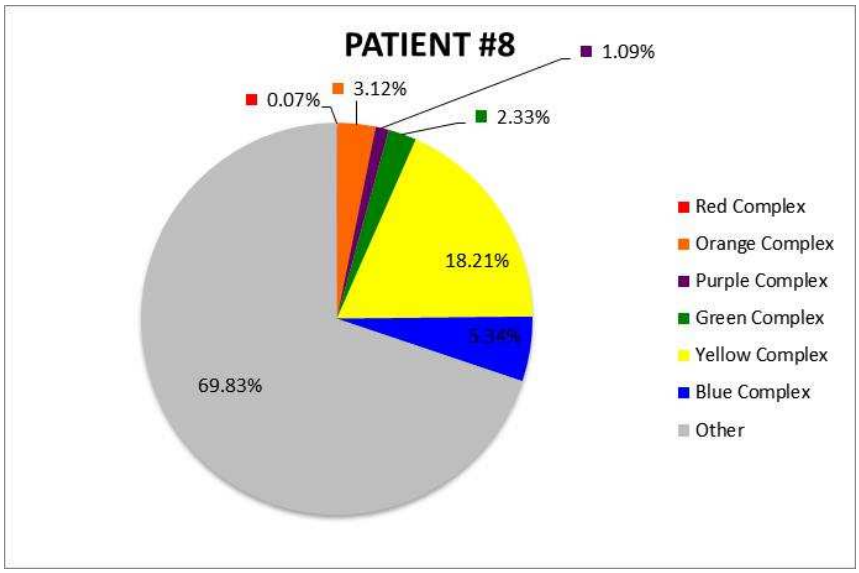
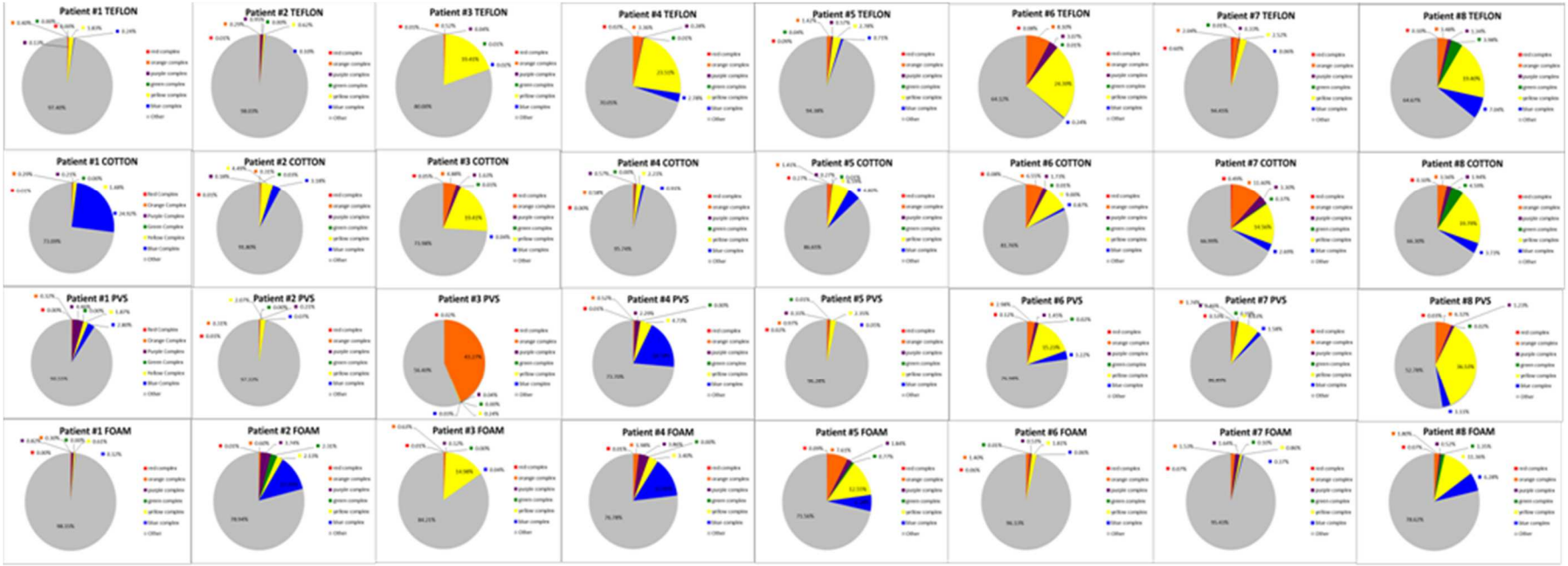


Chart 12 – Subject #8Percentage of Bacterial Complexes



Charts #13-45 Percentage of Bacterial Complexes



Charts #46 - Percentage of Bacterial Complexes from all subjects and materials

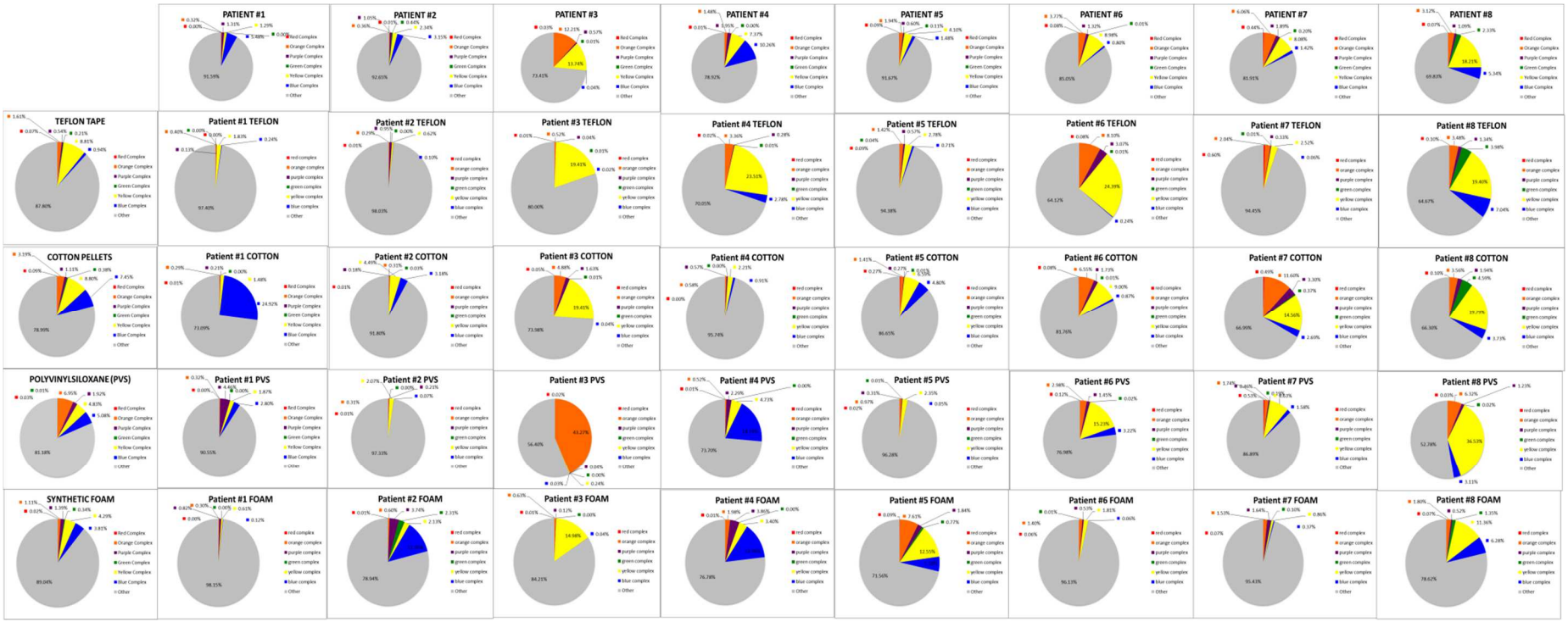


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Figure 1 –Lindhe reference (7)

Figure 2&3 - http://www.dentalimplantlife.com/2012/01/in-office-monitoring-and-maintenance-of-dental-implants

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Figure 6 - http://www.dentalimplantskerala.com/wp-content/uploads/2011/07/Conventional-implant-design.jpg

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Figure 10 -Lindhe reference (7)

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Figure 12 – Adrian reference (24)

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Figure 15 – http://www.net32.com/images/aa/jordco-endoring-ii-foam-inserts-yellow-blue-ERF.jpg

Figure 16&17- Socransky reference (27)

Figure 18 - Murray reference (30)

Figure 19 –PowerSoil DNA Isolation Kit Instruction Manual. Version: 11212013

Table 1 – Carranza reference (5)

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