

**An alginate impression method to detect dental caries in  
patients with crown and bridge restorations**

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Johannesburg

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# **An alginate impression method to detect dental caries in patients with crown and bridge restorations**

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Johannesburg  
2007

## **Declaration**

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**I, Nicolaas Johannes Mentz, declare that this research work is my own work and has not been submitted or incorporated in another dissertation or thesis for another degree. The experimental work was performed in the Department of Oral Microbiology, School of Oral health Sciences, University of the Witwatersrand.**

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**N J MENTZ**

## Dedication

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**This research report is dedicated to my wife JoAnne, and my three children Nicolas, Christine, and Jonathan who supported and encouraged me.**

## Acknowledgements

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**For the tireless efforts of Professor Maeve Coogan whose guidance during the experimental phase and advice in preparing this research report were of immense value.**

## Abstract

**Introduction:** Patients with numerous crowns and bridges are at risk of developing secondary caries on cervical margins of the crowns. This recurrent decay accounts for 60% of the reasons for the replacement of restorations. Detection and treatment of subclinical lesions is important to prevent cavitation. An alginate impression material has been used to locate cariogenic bacteria in established carious lesions. **Aims:** An alginate impression technique was used to assess the level of *Streptococcus mutans* and *Lactobacilli* on the crown margins of the teeth of the restored group and control subjects. Conventional caries activity tests were also used to compare these two groups. **Methods:** Twenty control group subjects with no crowns and a mean DMFS of 16 and 20 the restored group patients with at least 10 crowns and a mean DMFS of 129 were included in the study. Impressions were taken with a hydrocolloid material mixed with culture broth, incubated and examined for the presence of *S. mutans* and *Lactobacilli* on the cervical margins. The results were compared using the two-sample t-test, chi-squared test and a generalized logistic regression analysis. **Results:** More *S. mutans* and *Lactobacilli* were cultured on impressions of the restored group than the control patients ( $p=0.01$  and  $0.002$ ). A higher proportion of patients in the restored group had high concentrations of *S. mutans* and *Lactobacilli* on their teeth than subjects in the control group ( $p < 0.001$ ). In the restored group, sixty six per cent of teeth in the posterior and 48% in the anterior segment were crowns. *S. mutans* and *Lactobacilli* were found in higher concentrations on the posterior than the anterior teeth of the restored group patients than the control patients ( $p = 0.016$  and  $0.047$ ). Furthermore, more *Lactobacilli* were cultured from the saliva of the restored group than control subjects while the buffering capacity of plaque and the ability of saliva to neutralize acids were lower in the restored group than control group subjects. **Conclusion:** The impression technique showed that *S. mutans* and *Lactobacilli* accumulated in the posterior of the mouth and were associated with crowns indicating these particular sites are at risk.

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# Chapter 1

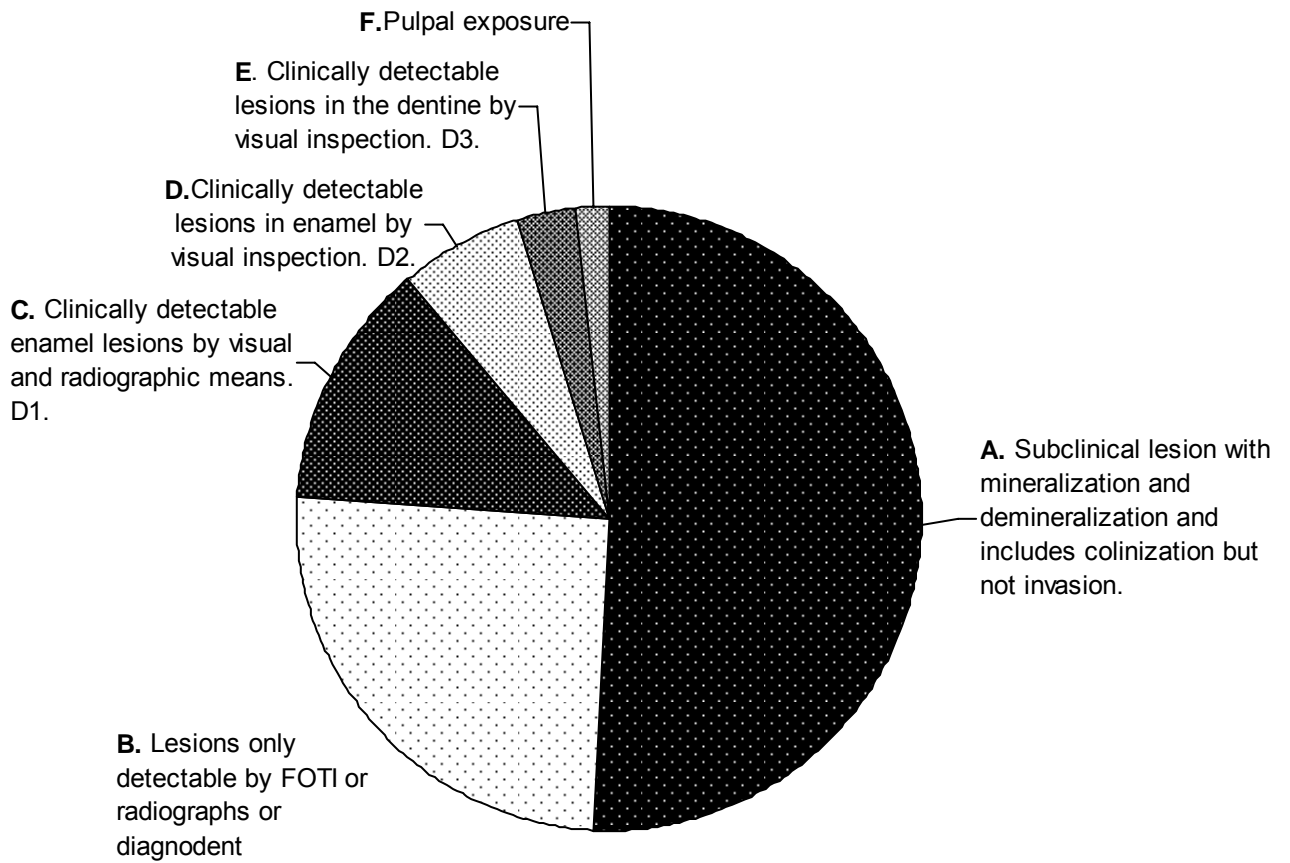
## Introduction

Traditionally dental caries has been defined as the localized destruction of the hard tissues of the tooth by acids produced by bacteria. More recently, this process has been described as an interaction between a dental plaque biofilm and the tooth surface and subsurface. The detectable lesion is a manifestation of one of the stages in the process of remineralization and demineralization. Cavitation occurs if the process of remineralization and demineralization are out of balance (Pitts, 2004).

The disease process can be visualized in terms of a pie chart (Pitts, 1997), with the largest slice being the largest component of subclinical lesions. Lesions are considered by the depth of penetration into the tooth tissue according to a system used by the World Health Organization. Subclinical caries is the earliest change to the dental enamel with subsurface enamel demineralization (Pitts, 2004).

Caries can occur at several clinical levels (Figure 1) and ranges from:

- **A.** Subclinical lesion with mineralization and demineralization and includes colonization but not invasion
- **B.** Lesions only detectable by FOTI or radiographs or Diagnodent®
- **C.** Clinically detectable enamel lesions by visual and radiographic means. D1.
- **D.** Clinically detectable lesions in enamel by visual inspection. D2.
- **E.** Clinically detectable lesions in the dentine by visual inspection. D3
- **F.** Pulpal exposure.



**Fig 1** Proposed model of the stages of development of carious lesions (Pitts, 1997)

## **1.2 The Diagnosis of dental caries**

Primary caries occurs on previously unaffected teeth and is most likely to occur in a younger age group. Secondary caries occurs around old fillings, replacement fillings, crowns and in teeth that already have restorations (Mjör and Toffenetti, 2000). The diagnosis of secondary caries is difficult as demonstrated in a study undertaken by Merrett and Elderton in Scotland in 1984. Several dentists assessed the status of secondary decay on extracted teeth before they were sectioned in the laboratory. The clinical evaluation did not always correspond to the laboratory diagnosis of secondary decay. The reason for operator inconsistency is that the assessment is subjective and variability occurs even amongst the most experienced practitioners, especially in the early stages of the disease. This has prompted the development of alternative methods for the diagnosis of primary and secondary dental caries.

## **1.3 Methods of caries detection**

There are several methods employed in the detection of dental caries. Those most frequently used are visual and radiographic examination.

### **1.3.1 Visual examination**

An explorer, dry teeth, and a good light are considered sufficient for the detection of occlusal lesions (Kidd and Pitts 1990). A sharp explorer is not recommended because diagnoses based on "sticky" fissures are not accurate, and the use of these methods promotes decay (Mitropoulos *et al*, 1990). This method will not detect initial colonization of the tooth surface by bacteria but relies on an existing established demineralised lesion. When this method is used, it is possible to overlook root decay on crown margins especially buccally or palatally during the examination. A further weakness of this method is that it is difficult to detect caries when the light is poor, in the presence of debris and in inaccessible areas. However, the method is accurate when it is employed in the anterior areas of the mouth but poor when used for the posterior areas (Pitts 1991). It may be valuable in patients with crowned teeth because Zoellner *et al*, (2002) detected more caries by visual examination than by radiographs.

### **1.3.2 Radiographic observations**

The literature centers on the detection of approximal lesions by means of radiographs, which play a crucial role in the treatment decisions. The interpretations of radiographs are extremely operator dependent and radiographic caries diagnosis can yield many conflicting interpretations on the same radiograph ranging from over treatment to no treatment (Espelid and Tveit 1986)

Bitewing radiographs are the most frequently performed dental radiographic examination method but have several shortcomings because an established lesion may become arrested, and it is not possible to determine if a cavity is progressing. More than one radiograph is required for a comparative view to detect an increase in demineralization of the tooth over a period (Pitts, 1997). Another limitation is that the method is interpreter sensitive, furthermore there are problems associated with a two dimensional image (Farman 1990, Pitts 1991). However when a lesion on a bitewing x-ray is detected the lesion has progressed into the dentine and remineralization is not likely to occur. An added problem is that radiographs can only detect an established lesion once demineralization has occurred (Kidd and Pitts, 1990). Fluoride treatment may alter the radiographic appearance of the lesion by making it radiopaque with radiolucent areas of active decay (Pitts 1991). The detection of subclinical carious lesions is particularly difficult in patients with crowns and fixed partial dentures, because visual and radiographic examination is only 75% accurate (Zoellner *et al*, 2002).

### **1.3.3 Fibreoptical transillumination examination (FOTI)**

A recent development is the use of a fibreoptic probe to transilluminate the teeth. A study was undertaken to compare this method to visual and radiographic examination. Transillumination revealed three and a half times more dental lesions than visual inspection. This method was more valuable for detecting approximal dentine lesions, but does not readily identify enamel lesions (Pitts, 1997). In addition more lesions can be detected by radiographic examination than transillumination.

This clearly demonstrates that it is not possible to diagnose approximal lesions adequately when only one method is used (Pitts 1991).

#### **1.3.4 Other methods of detection**

Several alternative methods have been used to determine decalcification and cavitation. Seddon (1989) proposed the use of orthodontic elastics to separate the teeth and take an impression, which is viewed under an electron microscope. Rimmer and Pitts (1990) separated the teeth and inspected them visually. They reported an increase in the number of lesions that could be diagnosed using this method compared to radiographs (Pitts 1991). These techniques are of no clinical value because it is impractical.

An electrical conductance method that measures electrical conductivity changes within the tooth caused by demineralization is also not reliable. It has a false-positive rate of 20% and therefore increases the risk of unnecessary operative intervention. The electrical conductance diagnostic tool is used mainly in the diagnosis of occlusal decay. Another method that is used is Laser Fluorescence, which induces fluorescence of the enamel and discriminates between carious and sound enamel. The enamel is less fluorescent in areas of reduced mineral content. The disadvantage of this technique is that wet teeth and plaque films have a negative effect on laser fluorescence (Tam and Mc Comb 2001).

#### **1.4 Factors implicated in dental caries**

Three main etiological factors are associated with dental caries, a cariogenic diet, the action of cariogenic bacteria and a susceptible host.



### 1.4.1 The Influence of diet

A study by Tenuta *et al* (2006) showed that the frequent intake of large amounts of refined carbohydrates has the single most important influence on acid production, microbial growth and the development of decay. Dietary analysis indicates that caries active individuals consumed more sucrose than caries free subjects (Coogan and Motelekar 1996). A definite relationship appears to exist between the consumption of refined sugars and the incidence of dental caries. Bacteria will metabolize the sugar for energy, produce polysaccharides, and create a localized anaerobic condition in plaque that encourages acid production. The result is the formation of organic acids such as lactic, propionic and acetic acid. Extracellular polysaccharides also aid in the adherence of bacteria to tooth structure. In contrast, an increase in dietary fiber intake appears to require more mastication and has an abrasive and cleaning action on the teeth and gums contributing to the maintenance of a healthy condition (Cole and Eastoe 1977).

### 1.4.2 Cariogenic bacteria

There is conclusive evidence to implicate the *Streptococcus* group of bacteria, especially *Streptococcus mutans* in the carious process. *Streptococcus mitis*, *Streptococcus anginosus*, the *S. salivarius*-group, *Enterococcus faecalis*, *Actinomyces naeslundii*, *Actinomyces viscosus* and the *Lactobacilli* can all produce caries under certain conditions. However, the bacteria most often implicated are *S. mutans* and the *Lactobacilli* (Marsh and Martin, 2001). The focus has been on these bacteria because they are acidogenic i.e. able to ferment sugars to acid, and aciduric which is the ability to tolerate high levels of acid. *S. mutans* are moderately aciduric and can grow at a pH below 5.5. Acids particularly lactic acid cause demineralization, and result in the formation of a lesion by the dissolution and removal of calcium and phosphorus ions. The process is characterized by the initial demineralization of the outer enamel, followed by attack on the underlying dentine (Kidd and Joyston-Bechal, 1987a). *S. mutans* can also synthesize extracellular glucans and fructans that aid in the colonization of the tooth surface. *Lactobacilli*

have similar properties but are mainly associated with the progression of caries lesions (Hamilton, 1987).

There is a well-established relationship between the microorganism *S. mutans* and *Lactobacilli* in saliva, and the development of dental caries (Jenkins, 1978). *S. mutans* may be associated with active lesions in the mouth and may also indicate a high sucrose intake because these microorganisms are sugar dependent. However, a relationship exists between decayed and filled surfaces (DFS) and the level of *S. mutans* in the mouth while *Lactobacilli* increase with the caries status of a subject (Fitzgerald *et al*, 1994). The presences of *Lactobacilli* may be an indication of an active lesion, as well as a high sugar intake and the presence of stagnant areas. Stagnant areas are prevalent in patients with fixed orthodontic bands, partial dentures, and erupting third molars (Kleinberg 2002). Smooth surface caries frequently yield a higher proportion of *S. mutans* from white spot lesions and produce a lower and faster rate of pH fall than in adjacent plaque on sound enamel. The presence of these microorganisms may indicate sub clinical dental caries (Marsh and Martin 2001).

In 1985 Ellen *et al* sampled the root surfaces of elderly and infirm patients, with reduced salivary flow for the presence of cariogenic bacteria. Both *S. mutans* and *Lactobacilli* were frequently isolated from surfaces diagnosed as carious while surfaces with neither *S. mutans* nor *Lactobacilli* were found in caries-inactive subjects. *S. mutans* and *Lactobacillus* were isolated more frequently from caries-free surfaces in caries-active than caries-inactive subjects and were recovered from proportionately more surfaces in subjects experiencing new root caries than in those that remained free of new caries. Patients who carried only *S. mutans* had a three to five times higher risk of developing active root caries than those with only *Lactobacilli*. A higher salivary lactobacillus count following root exposure due to periodontal surgery was also noted. They found the best indicator of root caries development is when *S. mutans* and *Lactobacilli* occur together. A shortcoming of this study was that it was unable to predict which surfaces would develop decay.

### **1.4.3 Susceptible host**

A susceptible site is an area that favours plaque accumulation because this promotes stagnation and retention. According to Kidd and Joyston-Bechal (1987a), several sites are particularly susceptible:

- The enamel pits and fissures of molars and premolars;
- Approximal enamel tooth surfaces just cervical to the contact point;
- The enamel of the cervical margin of the tooth just coronal to the gingival margin;
- Exposed root surfaces because of gingival recession;
- The margins of restorations especially if they are overhanging or deficient;
- The tooth surfaces adjacent to bridges or dentures.

Patients with numerous crowns are at risk of developing secondary decay. Those with fixed partial denture abutment teeth have increased secondary decay on the abutment teeth because the interproximal areas are difficult to access for cleaning and are prone to the accumulation of plaque (Zoellner *et al*, 2002). Secondary caries is a frequent cause of failure in fixed prosthodontics (Glantz *et al*, 1993) and accounts for nearly 60% of the reasons for replacement of restorations, most occurring cervically and approximally.

#### **1.4.3.1 Saliva flow**

The effect of salivary flow is important in the regulation of the pH in the mouth. In addition it is supersaturated with calcium and phosphate ions that have the ability to remineralize early lesions. Absence of saliva or diminished flow can lead to an increased incidence of dental caries (Kidd and Joyston-Bechal 1987 b, c). The rate of clearance of carbohydrate after ingestion shows that subjects have either a rapid or a slow clearance irrespective of the type of carbohydrate. Sucrose is cleared rapidly from the saliva, but remains above the fasting level for one hour after a sucrose rinse (Luke *et al*, 1999).

### **1.4.3.2 Plaque pH**

After the consumption of carbohydrates, there is a rapid drop in the pH of plaque that may remain depressed for between 30 and 60 minutes. This pH drop can be plotted against time and is called the Stephan curve after the person who described it in 1944 (Kidd and Joyston-Bechal 1987 a). The acids produced can be either lactic or acetic acid, depending on the amount of carbohydrate consumed. The more carbohydrate consumed, the more lactic acid is formed. This contrasts with acetic acid, which is produced when carbohydrate intake is low and minimal plaque is present. Thus acetic acid is beneficial because it has a buffering effect (Coogan and Motlekar 1996).

### **1.5 The Present study**

Early diagnosis is important in prevention and progression of cavitation considering the implications, of recurrent decay such as revision treatment, endodontic therapy, or the loss of teeth. The recording of non-cavitated lesions is essential (Pitts and Stramm 2004, Pitts 2004). An untreated subclinical lesion forms a cavity that cannot be remineralized and requires a filling (Kidd and Joyston-Bechal, 1987 b). There are limits to the visual, radiographic and tactile evaluation of crowns. More sensitive and specific diagnostic methods are required to detect incipient lesions

Caries activity tests including saliva buffering and flow, the accumulation and buffering capacity of plaque as well as the diet have been successfully employed to evaluate the caries status of patients while *S. mutans* and *Lactobacilli* in saliva can be used as predictors of caries activity (Wilson and Ashley, 1989). Bacteria colonize the tooth surfaces and form a biofilm well before detection of the lesion either by visual or radiographic means (Marsh, 2004; Guggenheim *et al*, 2004). *S. mutans* has been identified in secondary caries lesions by using confocal laser scanning microscopy and immunofluorescent labeling (Gonzalez-Cabezas *et al*, 1995) whereas the *Lactobacilli* are closely associated with active lesions (Kleinberg 2002). Previous studies have shown that high counts of cariogenic microorganisms are associated with the presence and onset of dental decay (Roeters *et al*, 1995). Thus

patients with a high caries experience will have an increased caries risk if bacteria have accumulated in a particular site (Bowden, 1996).

Finding a method that can detect *S. mutans* and *Lactobacilli* and locating them to specific sites on the teeth would be valuable. The presence of these cariogenic bacteria at specific sites could be used for the early diagnosis of carious active lesions not visible on routine examination. An alginate impression material that can locate these cariogenic bacteria in established carious lesions on the teeth has been developed (Coogan, Creaven and Galpin, 1995).

### **1.5.1 Aims**

This study investigated the ability of the impression technique to detect *Lactobacilli* and *S. mutans* on the margins of the teeth of patients with extensive crown restorations and compared these results with impressions of subjects with no visible decay. Thus its use in the early diagnosis of dental caries in patients with extensive restorations would be beneficial. Additional factors that predisposed them to secondary caries may assist with this diagnosis. Therefore the flow rate and buffering capacity of saliva, plaque accumulation, sucrose and fibre in the diet and *S. mutans* and *Lactobacilli* in saliva in the two groups were compared.

## **Chapter 2**

### **Methods and Materials**

#### **2.1 Selection of subjects**

Two groups of 20 individuals each with a minimum of 20 teeth were selected for the study. The restored group included subjects with prosthetic rehabilitation and at least 10 teeth restored with crowns, bridges or implants. The control group had less than two filled surfaces, no erupting teeth, no active carious lesions, no orthodontic bands or removable dentures.

#### **2.2 Clinical evaluation**

Two clinical examinations were undertaken, a radiographic and a visual evaluation.

##### **2.2.1 Radiographic evaluation.**

Two bitewing radiographs were taken to evaluate the posterior occlusion. The radiographs were taken using a Sirona 60 Kv x-ray unit and Kodak type E film and developed in an automatic developer. The x-rays were viewed using a standard viewing box at three times magnification. The anterior teeth were not x-rayed as these teeth could be evaluated by using visual means (Pitts 1991). The radiographs were reexamined after 1 month to check for operator consistency.

##### **2.2.2 Visual examination**

Visual examination was undertaken to establish the presence of decay. The number of decayed missing and filled surfaces (DMFS) was counted. The examination was conducted in a dental chair using a dental light and five times magnification loops. The number of tooth surfaces was recorded as sound, decayed, missing, and filled using the criteria of Kidd and Joyston-Bechal (1987 c). Probing of teeth is not recommended because the visual assessment of the teeth gives the same information as a combined visual-tactile examination (Hildebrandt, 1995).

### 2.3 Plaque index

A plaque index was obtained by painting the teeth with Lorvic® new 2-tone disclosing solution (The Lorvic Corporation, St Louis) using a cotton ball and asking the patient to rinse their mouths with plain water. The number of mesial, distal, palatal and buccal surfaces with plaque was counted and recorded as a percentage of surfaces covered by plaque.

### 2.4 Diet analysis

The patients were asked to fill in a four-day diet sheet, and record everything they ate or drank during the four-days. The daily sucrose and fibre intake was calculated using the tables compiled by Langenhoven *et al* (1991).

### 2.5 Saliva collection

Three samples of saliva were collected in sterile McCartney bottles.

- *Resting saliva sample* was collected while the patients were asked to sit quietly and expectorate into a bottle for 10 minutes.
- *Stimulated saliva produced by chewing*. Patients were asked to chew on a sterile rubber tube while expectorating into a sterile bottle for 10 mins. This saliva samples was used for culturing *Lactobacillus* and *S. mutans*.
- *Saliva stimulated by exposure to acid* was obtained by placing 0.5ml 2% citric acid on the tongue at one-minute intervals for 10 mins while the patients were expectorating into a bottle.

The volume of saliva that was produced in 10 minutes was measured. Thereafter the modified Driesen test was used to test the buffering capacity of the saliva (Driesen *et al*, 1946). Two milliliters of concentrated saliva were placed in a conical flask with two drops of the indicators Bromocresol Purple and Bromocresol Green. The samples were titrated against 0.01N lactic acid to a pH of 4, the end point of Bromocresol Green. The amount of acid that changed the colour from blue to green indicated the buffering capacity of saliva. The results were expressed as mls 0.01N lactic acid.

## **2.6 Buffering capacity of plaque**

The initial pH of plaque on the tongue was determined by placing MERCK Universalindikator pH0-14 (Merck KGaA 64271 Darmstadt Germany) on the tongue and recording the result. Thereafter the patients were asked to rinse their mouths with 25 ml 10% glucose for two minutes. The pH of the plaque on the tongue was recorded after five minutes and thereafter at five minute intervals either for a period of 30 minutes until a pH of 7 or the initial resting pH was reached. The plaque on the tongue was used to measure the pH of plaque because a recent study has shown that the pH of plaque on the tongue is similar to plaque that accumulates on teeth (Ferjaskov *et al*, 1992).

## **2.7 Lactobacilli and Streptococcus mutans counts**

The stimulated saliva produced while chewing was diluted 1:10, a 1:100, and a 1:1000 in sterile phosphate buffered saline with a pH of 7.3. The concentrated and diluted samples were plated on Rogosa Agar (Oxiod, Basingstoke) and Mutans Bacitracin Agar (MBA) consisting of Mitis Salivarius Agar (Difco®) containing 0.00001% Bacitracin. After inoculation the plates were incubated at 37° for 72 hours under CO<sub>2</sub> and the number of colonies was counted.

## **2.8 The Impression technique**

Impressions were taken of the mouth using a modified hydro-colloid impression material mixed with culture broth. (P. G. S. Alginate, Milner Dental. Pty Ltd, Johannesburg). The hydrocolloid was adapted by excluding Bronol, Niasept sodium, peppermint oil excellent F and color 34K48PG green from the alginate. It was sterilized by irradiating with 2 kGy of gamma rays before use (Isotron South Africa Pty. Ltd.).

Two broths were used for the study, Mutans Bacitracin broth (MBB) for the culture of *S. mutans* and Tomato Juice broth (TJB) for culturing *Lactobacilli*. MBB contained 1% peptone, 1% to tryptone, 0.1% glucose, 8.5% sucrose, 0.4% dipotassium phosphate, 0.0075% trypan blue and 0.00008% crystal violet. The broth was made



selective by adding 0.00001% Bacitracin. TJB contained 300 ml filtered whole tomato (Koo®), 5 g yeast extract, 10 g glucose, 15 g agar, 2 ml bromo -cresol purple and 5 ml Salt A (Salts A contains 10 g K<sub>2</sub>HPO<sub>4</sub> and 10 g KH<sub>2</sub>PO<sub>4</sub>) and 1000 ml distilled water. TJB was used because Rogosa broth (Oxoid) that was made up by excluding agar from the recipe inhibited the setting of the alginate.

Patients were seated in a dental chair in a standard position for routine impressions and the impressions were taken under sterile conditions. The operator wore sterile surgical gloves and rinsed the gloves with 70% alcohol between impressions. The sterile impression material was decanted into a sterile mixing bowl, mixed and spatulated for 90 seconds with either sterile MBB or TJB broth to form a smooth impression paste. The paste was placed in a sterile impression tray, inserted in the mouth and allowed to set. Thereafter the impressions were placed in a sterile Petri dish separated by a sterile Perspex tube, transferred to a candle jar and incubated under CO<sub>2</sub> for 48 hours at 37<sup>0</sup>C. Impressions were taken of both the maxilla and the mandible for each subject.

After incubation, the impressions were examined at 10 times magnification under a stereo microscope for the presence of *Streptococcus mutans* and *Lactobacilli* colonies on the margins of the crowns, bridges, implants and teeth of the restored patients and on the gingival margins of the teeth of the control group.

The arches were divided into three sections, the premolars and molars (posterior) on the right and on the left side of the mouth and the canines and incisors in the centre (anterior). The following scoring was used for each section

- no colonies was assigned a score of zero;
- only one colony scored 1
- two colonies scored 2
- three to ten colonies scored 3
- confluent colonies scored 4

The same scoring system was used for the maxilla and mandible

## 2.9 Statistical analysis

The two groups were compared, separately with respect to each of the sets of measurements. The saliva, dental plaque and diet measurements were compared using the two-sample t-tests.

- Since the *S. mutans* and *Lactobacilli* measurements are on a 0 to 4 scale, the two groups of patients were compared using the chi-squared tests. In addition Inter-arch and intra-arch measurements in the different areas of the mouth i.e. the anterior and posterior segments of the maxilla and mandible, on the left and right were compared.
- A generalized logistic regression analysis was performed, which takes into account the ordinal nature of these scores in order to test simultaneously whether the area of the mouth from which the measurements were taken (anterior or posterior, maxilla or mandible) as well as the group from which the patients came (caries or control) affected the *S. mutans* and *Lactobacilli* scores.
- Finally, a total of the *S. mutans* the *Lactobacilli* score was calculated for each patient by summing the individual scores over the different parts of the mouth. A two-sample t-test was used to compare the total score in the two groups.

Results were accepted as statistically significant at the 5% or lower level.

## Chapter 3

### Results

#### 3.1 Saliva buffering and flow

There was no significant difference in the flow rate between the restored and the control groups (Table 1). When the buffering capacity of the saliva of the restored and control patients were compared, the saliva produced by stimulation with acid was significantly lower ( $p=0.020$ ) in the restored than in the control group. There was no significant difference in the buffering between the 2 groups when the resting saliva and the saliva stimulated by chewing were compared (Table 2).

#### 3.2 Buffering capacity of plaque

The buffering capacity of plaque was lower in the restored group than the control group (Table 3). This difference was statistically significant ( $p < 0.001$ ).

#### 3.2 DMFS and plaque index

The mean DMFS of the restored group was 129 and the control group 16.

The plaque index was significantly lower ( $p = 0.018$ ) in the restored than the control group (Table 4). In the control patients, more plaque accumulated on the mandibular teeth of the anterior segment of the mouth than the maxillary teeth and the posterior segments (Table 5) while the distribution of plaque in the control group was similar in all three segments (Table 6).

#### 3.4 Diet

The fiber intake was significantly higher ( $p = 0.029$ ) in the restored than the control group (Table 7). There was no significant difference between the two groups regarding the amount of sucrose consumed and the frequency of intake.

### **3.5 Salivary *S. mutans* and *Lactobacilli***

The *Lactobacillus* count was significantly higher in the restored group than the control subjects ( $p < 0.001$ ). However, the *S. mutans* count was not significantly different between the restored and control subjects (Table 8).

### **3.6 Overall comparison of *S. mutans* and *Lactobacilli* on impressions**

Microorganisms colonized three times more teeth in the restored subjects than controls. Five times more teeth in the restored patients carried both *S. mutans* and *Lactobacilli* and almost double as many *Lactobacilli* than control subjects. The percentage of teeth with *S. mutans* was similar in both groups. In the restored patients, *Lactobacilli* occurred more often than *S. mutans* on implants, teeth, pontics and crowns (Table 9).

### **3.7 The Position of restorations in the restored patients**

Most of the crowns were in the posterior segment of the mandible and the anterior segment of the maxilla while implants were in the posterior segment of mandible (Table 10).

### **3.8 The Distribution of microorganisms on impressions of natural teeth**

Only 7% of teeth in control subjects were colonized by both *S. mutans* and *Lactobacilli* whereas these microorganisms were isolated from 34% of the teeth of the restored subjects (Table 11).

### **3.9 *S. mutans* on impressions**

A generalized logistic regression analysis showed that patients in the restored group had high concentrations of *S. mutans* than the control subjects. This difference was statistically significant ( $p < 0.001$ ). More *S. mutans* occurred on impressions of the natural teeth of the restored patients than the control subjects. This difference was also statistically significant ( $p < 0.010$ ). *S. mutans* was found in significantly higher concentrations on the posterior than the anterior teeth in both the maxilla and the mandible of the restored patients ( $p = 0.016$ ). A comparison of the maxilla of the

restored and control subjects showed there was no difference in the distribution of *S. mutans* (Table 12). In contrast significantly higher concentrations ( $p = 0.031$ ) of *S. mutans* were cultured from the anterior surfaces of the mandible of the restored patients than control subjects (Table 13).

### **3.10 *Lactobacilli* on impressions**

A generalized logistic regression analysis of the data showed that more restored patients had high levels of *Lactobacilli* than control subjects ( $p = 0.001$ ). More *Lactobacilli* were cultured on impressions of the teeth of the restored patients than control subjects ( $p=0.002$ ). Overall *Lactobacilli* were found in significantly higher concentrations on the posterior than the anterior teeth ( $p = 0.047$ ). Higher concentrations of *Lactobacilli* were cultured from the right posterior surfaces of the maxilla ( $p = 0.013$ ) (Table 14) and the left posterior surfaces of the mandible ( $p = 0.005$ ) of the restored patients (Table 15).

Table 1. The flow rate of saliva in the restored and control subjects at rest and stimulated by chewing and acid.

Saliva flow	Subjects	N	Mean	Std. Deviation	t	df	p-value
Resting	Control	20	3.73	1.91	1.229	38	0.227
	Restored	20	3.06	1.51			
Stimulated by chewing	Control	20	8.60	5.87	1.034	38	0.308
	Restored	20	6.99	3.74			
Stimulated by exposure to acid	Control	20	14.07	4.90	1.440	38	0.158
	Restored	20	11.64	5.73			

Table 2. The buffering capacity of resting and stimulated saliva produced by chewing and exposure to citric acid in the restored and control subjects.

Buffering capacity of saliva	Subjects	N	Mean	Std. Deviation	t	df	p-value
Resting	Control	20	5.36	3.50	0.615	38	0.542
	Restored	20	4.77	2.48			
Stimulated by chewing	Control	20	6.06	2.31	1.090	38	0.282
	Restored	20	5.30	2.09			
Stimulated by exposure to acid	Control	20	2.96	2.52	2.434	38	0.020*
	Restored	20	1.43	1.24			

\* Statistically significant

Table 3. Buffering capacity of plaque in the restored and control subjects

Subjects	N	Mean	Std. Deviation	t	df	p-value
Control	20	80.52	5.00	3.633	38	0.001*
Restored	20	69.13	13.09			

\*Statistically significant

Table 4. The plaque index of the restored and control subjects.

Subjects	N	Mean	Std. Deviation	t	df	p-value
Control	20	53.84	16.69	2.471	38	0.018*
Restored	20	37.58	24.25			

\* Statistically significant

Table 5. The percentage of teeth with plaque in the left posterior, right posterior and anterior segments of the mandible and maxilla of the restored subjects.

<b>Mandible</b>		
L posterior	Anterior	R posterior
21.2	48.675	28.35
<b>Maxilla</b>		
L posterior	Anterior	R posterior
28.35	34.65	38.3

Table 6. The percentage of teeth with plaque in the left posterior, right posterior and anterior segments of the mandible and maxilla in the control subjects

<b>Mandible</b>		
L posterior	Anterior	R posterior
55.75	45.03	50.15
<b>Maxilla</b>		
L posterior	Anterior	R posterior
60.5	43.74	63.5 60.5

Table 7. The dietary intake of the restored patients and control subjects

	Subjects	N	Mean	Std. Deviation	t	df	p-value
Sucrose intake in grams /day	Control	20	49.42	30.04	0.093	38	0.926
	Restored	20	48.38	40.06			
Frequency of Sucrose intake	Control	20	2.76	1.23	- 0.331	38	0.742
	Restored	20	2.92	1.81			
Fibre intake in grams /day	Control	20	9.04	2.92	- 2.272	38	0.029*
	Restored	20	13.02	7.28			

\* Statistically significant



Table 8. The logarithm of the number of *S. mutans* and *Lactobacilli* in stimulated saliva produced by chewing.

	Subjects	N	Mean	Std. Deviation	t	df	p-value
<i>S. mutans</i>	Control	20	4.230	0.777	1.223	38	0.229
	Restored	20	3.669	1.898			
<i>Lactobacilli</i>	Control	20	1.803	1.815	-4.435	38	0.000*
	Restored	20	4.264	1.691			

\* Statistically significant

Table 9. Total scores of *S. mutans* and *Lactobacilli* on impressions of the teeth.

	Subjects	N	Mean	Std. Deviation	t	df	p-value
<i>S. mutans</i>	Control	20	6.700	4.835	-2.704	38	0.010*
	Restored	20	11.050	5.326			
<i>Lactobacilli</i>	Control	20	7.600	5.968	-3.265	38	0.002*
	Restored	20	13.550	5.549			

\* Statistically significant

Table 10. The number of teeth, crowns, implants and pontics in the left posterior, right posterior and anterior segment of the mandible and maxilla in the restored patients.

Mandible	Left posterior	Anterior	Right posterior
Teeth	10	90	8
Crowns	41	28	43
Implants	15	2	21
Pontics	5	1	6

Maxilla	Left posterior	Anterior	Right posterior
Teeth	12	21	7
Crowns	50	89	53
Implants	3	6	1
Pontics	5	7	6

Table 11. The percentage of natural teeth, implants, crowns and pontics with *Lactobacilli*, *S. mutans*, *Lactobacilli* and *S. mutans* or no microorganisms in control and the restored subjects.

Subjects	<i>Lactobacilli</i>	<i>S. mutans</i>	<i>S. mutans</i> and <i>Lactobacilli</i>	No microorganisms
<b>Control</b>				
Natural teeth	18%	20%	7%	56%
<b>Restored</b>				
Natural teeth	32%	15%	34%	18%
Implants	45 %	18%	23%	14%
Crowns	28%	18%	30%	24%
Pontics	35%	16%	30%	31%

Table 12. The distribution of *S. mutans* on impressions of the Maxilla teeth of the restored and control subjects. A score of 0 was assigned if no colonies were present in the segment, 1 for one colony, 2 for two colonies, 3 for three to ten colonies and 4 when confluent colonies were present.

**Left Posterior**

	Score					Total	Chi-square	df	p-value
	0	1	2	3	4				
Control	3	7	8	1	1	20	2.686	4	0.612
Restored	1	8	6	3	2	20			

**Maxilla Anterior**

	Score					Total	Chi-square	df	p-value
	0	1	2	3	4				
Control	11	6	2	0	1	20	6.427	4	0.169
Restored	5	5	5	1	4	20			

**Right Posterior**

	Score					Total	Chi-square	df	p-value
	0	1	2	3	4				
Control	6	6	6	1	1	20	3.467	4	0.483
Restored	6	4	4	5	1	20			

Table 13. The distribution of *S. mutans* on impressions of the mandibular teeth of the restored and control subjects. A score of 0 was assigned if no colonies were present in the segment, 1 for one colony, 2 for two colonies, 3 for three to ten colonies and 4 when confluent colonies were present.

**Left Posterior**

	Score					Chi-square	df	p-value
	0	1	2	3	4			
Control	2	9	7	2	0	6.447	4	0.168
Restored	3	5	4	5	3			

**Anterior**

	Score					Chi-square	df	p-value
	0	1	2	3	4			
Control	9	9	1	0	1	10.600	4	0.031*
Restored	3	6	5	4	2			

\* Statistically significant

**Right Posterior**

	Score					Chi-square	df	p-value
	0	1	2	3	4			
Control	9	4	4	3	0	8.123	4	0.087
Restored	4	1	6	6	3			

Table 14. The distribution of *Lactobacilli* on impressions of the maxillary teeth of the restored and control subjects. A score of 0 was assigned if no colonies were present in the segment, 1 for one colony, 2 for two colonies, 3 for three to ten colonies and 4 when confluent colonies were present.

**Left Posterior**

	Maxilla L Post					Chi-squared	p-value
	0	1	2	3	4		
Control	6	4	6	2	2	7.086	0.131
Restored	2	1	6	5	6		

**Anterior**

	Maxilla Ant					Chi-squared	p-value
	0	1	2	3	4		
Control subjects	10	5	4	1	0	5.700	0.223
Restored	5	6	3	4	2		

**Right Posterior**

	Maxilla R Post					Chi-squared	p-value
	0	1	2	3	4		
Control subjects	8	2	7	1	2	12.614	0.013
Restored	3	3	2	1	2		

\* Statistically significant

Table 15. The distribution of *Lactobacilli* on impressions of the mandibular teeth of the restored and control subjects. A score of 0 was assigned if no colonies were present in the segment, 1 for one colony, 2 for two colonies, 3 for three to ten colonies and 4 when confluent colonies were present.

**Left Posterior**

	Score					Chi-squar e	d f	p- valu e
	0	1	2	3	4			
Control	4	11	1	2	2	14.88 8	4	0.00 5
Restored	1	2	6	6	5			

\* Statistically significant

**Anterior**

	Score					Chi-squar e	d f	p- valu e
	0	1	2	3	4			
Control	9	5	3	1	2	7.100	4	0.13 1
Restored	12	8	1 0	2	8			

**Right Posterior**

	Score					Chi-squar e	d f	p- valu e
	0	1	2	3	4			
Control	4	9	3	1	3	9.400	4	0.05 2
Restored	4	1	6	3	6			

## Chapter 4

### Discussion

#### 4.1 Saliva buffering and saliva flow

The salivary tests showed that the buffering capacity of saliva produced by stimulation with acid was significantly lower ( $p=0.020$ ) in the restored than the control group. However, there was no difference between the two groups when the buffering capacity of resting saliva and stimulated saliva produced while chewing, were compared (Tables 1 and 2). The decrease in the ability of the restored patients to buffer the acids may be related to a change in saliva. A recent study has shown that sucking acidic sweets can change whole mouth saliva composition, by exhausting the buffering capacity of the saliva (Jensdottir *et al*, 2005). Even though stimulated saliva is more resistant to changes in pH than resting saliva during hydrochloric acid titration (Moritsuka *et al*, 2006) the ongoing production of organic acids in the mouth by cariogenic microorganisms may eventually exhaust the buffering capacity of saliva.

The difference in saliva buffering capacity may also be related to stress. Twelve of the restored patients were professional or company owners who had demanding life styles and none of the control group belonged to this category. This may have changed the composition of saliva and reduced the flow because Morse *et al*, (1981) have shown that reduction in anxiety increases the flow and pH of saliva. A recent study has also shown there is a significant stress-mediated increase of salivary total protein concentration, alpha-amylase activity, amylase/protein ratio, alpha-amylase output, s-

IgA concentration, and s-IgA output but reduced bacterial aggregation, in saliva in people who are stressed (Bosch *et al*, 1996)

#### **4.2 Buffering capacity of plaque**

An examination of the results of the plaque tests showed that the buffering capacity was significantly lower in the restored ( $p < 0.001$ ) than the control group (Table 3). Furthermore on exposure to a sugar rinse, the plaque pH fell below 5.5 and remained low for a longer period than in control subjects. A prolonged drop in pH below 5.5 indicates that with frequent exposure to sugar the mouth will remain acidic for long periods and cause decalcification of the teeth. The inability to raise the pH probably contributed to caries susceptibility in the restored subjects.

Plaque is unique because it contains acids, proteins, bicarbonates as well as phosphates that resist a change in the pH (Hicks *et al*, 2003). However, the restored patients carried more cariogenic bacteria than the controls (Table 9). These patients would form a plaque biofilm that contains high levels of cariogenic bacteria. When these conditions are combined with a high sucrose intake, the pH will be depressed for a prolonged period leading to subsurface demineralization of the tooth. Furthermore, there is a definite correlation between saliva buffering capacity and plaque buffering capacity (Hicks *et al*, 2003). The situation in the restored subjects would be aggravated by the production of saliva with poor buffering capacity because saliva contains bicarbonates, phosphates and proteins that all contribute to the buffering capacity of the saliva and subsequently plaque (Shellis and Dibdin 1998). Buffering capacity is affected by the diffusion of the saliva through the biofilm of the plaque (Hicks *et al*,



2003). Thus plaque that is able to control changes in acidity would keep the pH stable whereas cariogenic plaque would have the opposite effect.

### **4.3 Plaque index**

The plaque index was significantly lower in the restored group than in the control group ( $p = 0.018$ ) (Table 4). A comparison the distribution of the plaque in the posterior left and right and in the anterior segments of the mandible in the restored patients showed a higher percentage of plaque accumulation on the anterior segment, where the most natural teeth occurred (Tables 5 and 10). In the control patients, most plaque accumulated on the posterior segments of mandible and the maxilla (Table 6).

### **4.4 Diet analysis**

The restored subjects consumed more fibre ( $p = 0.029$ ) than the control subjects (Table 7) while sucrose consumption was similar but relatively high in both groups. The protective effect of a high fiber intake in the restored group is not supported by the findings of Cole and Eastoe (1977) that showed that a high fiber intake is associated with a low DMFS. This supports the idea that caries is a multifactorial disease and that saliva may play an important role in the development and in the control of this infection.

#### **4.5 *S. mutans* and *Lactobacilli* in saliva**

The levels of *Lactobacillus* in saliva was significantly higher (Table 8) in the restored than in control subjects ( $p=0.000$ ). The probable explanation is that the mouths of the restored patients were extensively restored with crowns, bridges and implants that are associated with stagnant areas and the *Lactobacilli* would accumulate readily in these stagnant areas. This is supported by the findings of (Kleinberg 2002) who showed that the presence of restorations encourages the multiplication of these cariogenic microorganisms. The importance of stagnation is emphasized by Kleinberg (2002) who stated that orthodontic bands and brackets create stagnant areas that favour the accumulation of *Lactobacilli* and the development of decay.

Although the counts of *S. mutans* were also high, there was no difference between the controls and the restored group. The high *S. mutans* count may be associated with high sucrose consumption in both groups. The low DMFS in the control group could rather be attributed to the absence of stagnant areas and good saliva flow than the presence or absence of *S. mutans* and the consumption of sucrose.

#### **4.6 *S. mutans* and *Lactobacilli* on impressions**

There were more *S. mutans* on the impressions of the restored ( $p= 0.010$ ) than control subjects (Table 9). When the natural teeth were, assessed *S. mutans* was present in the same proportion in the restored and control subjects (Table 11). The same ratio of un-restored teeth in both groups was colonized by *S. mutans*. This suggests that *S. mutans* may not solely be implicated in the formation of caries. *S. mutans* attaches

readily to the acquired pellicle on teeth even in the absence of sucrose. Once it is attached, it utilizes the acquired pellicle for growth (Marsh & Martin, 2001). *S mutans* will attach to teeth irrespective of the presence of restorations (Table 10). The distribution of *S mutans* in the restored and control subjects in different parts of the maxilla was not significant (Table 12), which is a further indication that the salivary pellicle may be responsible for the attachment of *S mutans*. The growth on impressions suggests that the presence of *S. mutans*, their location and level of the infection does not necessarily indicate that an infected site is at risk.

There were almost twice as many *Lactobacilli* on the impressions of the restored than control subjects (Table 9). This difference was highly significant ( $p=0.002$ ) which suggests that conditions in the mouths of the restored subjects encouraged the colonization of the majority of crowns, pontics, teeth and implants by *Lactobacilli*. These high levels of *Lactobacilli* could be explained by Kleinberg (2002) who found that sites with changes in morphological conditions lead to carbohydrate retention. This will encourage the formation of acidogenic dental plaque and promote the development of carious lesions as well as serving as a source of infection. The high levels of *Lactobacilli* on the impressions was also associated with a significantly higher *Lactobacillus* count in the saliva of the restored patients ( $p=0.000$ ) than control subjects (Table 8). These observations confirm that high levels of salivary *Lactobacilli* are an indication of stagnant sites as well as a high carbohydrate intake (Tenuta *et al*, 2006).

Examination of the sites colonized by both *Lactobacilli* and *S mutans* showed there was a difference between the restored and control subjects. These microorganisms

occurred together on 7% of the teeth in caries-free subjects and almost 30 % of teeth crowns and pontics of the restored subjects (Table 11). This indicates that teeth and crown margins colonized by both these microorganisms are at risk of developing secondary decay. This is supported by Kleinberg (2002) who showed that *S mutans* and *Lactobacillus* must both be present for caries to occur. Furthermore the presence of a combination of *S mutans* and *Lactobacillus* is more efficient in selecting patients at risk than teeth colonized by only *S mutans* or *Lactobacilli* (Stecksen-Blicks, 1985).

These observations support the idea that a lesion is initiated by *S mutans* with the production of acid when plaque accumulates and a diet high in sucrose is consumed. A change in morphology of the tooth or the dentition by the placement of prostheses will favour the associated colonization by *Lactobacilli* and the development of a subclinical or initial lesion. Thus, it appears that the initial stage of decay is associated with an increase in dietary carbohydrate that leads to an alteration in the microorganisms with *S mutans* and *Lactobacilli* becoming more dominant with the acid sensitive species decreasing (Marsh and Martin, 2001). This study has shown that the impression technique is invaluable because it indicates that particular areas are at risk.

#### **4.7 Distribution of *S. mutans* on the maxilla and the mandible**

*S. mutans* occurred in significantly higher proportions in the restored patients than control subjects ( $p = 0.031$ ). They were cultured mainly from the anterior surfaces of the teeth in the mandible (Table 13) and were associated with the accumulation of plaque in this site. The high saliva flow could lead to the deposition of salivary proteins that would encourage plaque accumulation and colonization by *S. mutans*. Even

though plaque and *S. mutans* accumulated in this site the teeth were less prone to decay. The reason could be the washing and buffering effect of saliva and the protective role of immunoglobulins in saliva (Bosch *et al*, 1996).

#### **4.8 Distribution of *Lactobacilli* on the maxilla and the mandible**

*Lactobacilli* were found in significantly higher proportions of patients in the restored than the control group, with high concentrations being cultured on the right posterior surfaces in the maxilla ( $p = 0.013$ ) and on the left posterior surfaces in the mandible ( $p = 0.005$ ) (Table 14 and 15). This difference could not be explained by the available data because there was no relationship between the accumulation of the *Lactobacilli* and the number of implants crowns or pontics. These differences may be attributed to a change in the morphology of the existing prostheses, arch form or differences in tooth brushing habits. They found that right-handed tooth brushers, had an increase in buccal plaque and gingivitis on the contra-lateral side but no differences noted in left-handed brushers. (Addy *et al*, 1987).

#### **4.9 Conclusion**

This study has shown that the buffering capacity of saliva produced by stimulation with acid was significantly lower in the restored than the control group. Furthermore, the poor buffering capacity of the restored patients had a significant impact on their DMFS. The on-going production of acids by cariogenic microorganisms and the consumption of acidic foods and drinks probably exhausted the buffering capacity of saliva. There is an interaction between the buffering capacity of plaque and saliva (Shellis and Dibdin, 1988) with salivary bicarbonate ions replenishing the buffer capacity of plaque and

influencing the formation of organic acids. This had an effect on plaque because the buffering capacity was significantly lower in the restored than the control group. Exposure to fermentable carbohydrates in these patients would lead to a low pH of dental plaque and contribute to caries susceptibility in the restored subjects. The risk is compounded in patients who have poor saliva buffering capacity as well as a high sucrose intake. The buffering capacity of saliva produced by acid stimulation was significantly poorer in the restored patients than in the controls, which suggests that saliva has an important role to play in the regulation of pH and in the control of dental caries.

The initial stage of caries may be the attachment of *S. mutans* whereas the promotion of the lesion is aided by the production of acids by *Lactobacilli*. The presence of restorations will promote the accumulation of carbohydrate that will encourage the multiplication of cariogenic organisms. An advantage of the impression technique was it indicated sites where cariogenic microorganisms accumulated. This was invaluable because it indicated that particular sites were at risk.

This study has shown that it is important to investigate saliva in conjunction with an impression technique to identify the presence and sites colonized by *S. mutans* and *Lactobacilli*. Sites at risk may have developed sub-clinical decay and not detectible by convention methods. The impression technique makes it possible to determine the extent of infection and the location of cariogenic bacteria. This is important because it makes tooth specific treatment possible. This has advantage over visual detection because the early treatment of incipient lesions is possible.

This study indicates that diet, microorganism and host susceptibility determine whether a patient will develop dental caries. Detection of decay at an early stage is reliant on the culturing of specific microorganisms and can significantly alter the future DMFS of the patient. The affected tooth structure can be treated with a regimen of fluoride to promote remineralization and reduce the levels of cariogenic bacteria. In addition a caries promoting diet can be modified and other antimicrobial chemicals can be applied to the teeth. Thus salivary tests, microbial impressions and diet analysis are invaluable for the long-term follow up of the restored patients with extensive restorations. These observations suggest that conventional caries susceptibility tests should be routinely undertaken on patients with a history of decay. These tests combined with the impression technique can be used to identify sites at risk and enable the clinician to treat early lesions and reverse the process of decay. This is important because remineralization at an early stage is possible especially if fluoride is present in the saliva (Kidd and Joyston-Bechal, 1987a).

## Chapter 5

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