Novel approach to treat root caries: A pilot study to investigate Chlorhexidine-modified Glass Ionomer Cement applied using ART

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Abstract

**Background:** Root caries is common amongst the older population. The risk of caries increases with irregular attenders and special need groups. A simple, reliable and cost-effective therapeutic method is required to deal with this issue.

**Objectives:** To investigate the clinical effectiveness, patient acceptability and potential to reduce cariogenic microorganisms of a novel restorative material to treat root caries placed using the atraumatic restorative treatment (ART).

**Methods:** Two clinically-visible root surface carious lesions per patient (n=9) were restored using ART. One root carious lesion was restored with a conventional glass ionomer cement (GIC) and the other with a GIC cement modified with 5% chlorhexidine digluconate (GIC-CHX). Patient acceptability and survival rates of the restorations were evaluated using questionnaires and the modified Ryge criteria (Ryge 1980) at baseline, and after 1, 3 and 6 months. Plaque and saliva samples were collected around both restorations and microbiological analysis for bacterial viability were completed at baseline, 1, 3 and 6 months.

**Results:** Eighteen restorations were placed using GIC and GIC-CHX in nine patients. After reviewing the restorations at 1, 3 and 6 months following placement, most of the participants (n=8, 88.9%) indicated that they were satisfied with the appearance of the ART restorations, felt no pain during the procedure and experienced no change in taste over time. The set time for GIC-CHX was quicker or the same as GIC (77.8%). At the 6-month examination, 77.8% (n=7) of the GIC-CHX restorations were continuous with the tooth anatomy, whereas 44.4% (n=4) of the GIC restorations were discontinuous. The survival rates of GIC-CHX and control GIC restorations were 88.9% and 66.7% respectively, the main reason for failure was gross marginal defects; however, this result was not statistically significant using the Chi square test (p>0.05). There was no statistically significant reduction in the mean count of the tested microorganisms in plaque samples for both types of restorations at 1, 3 and 6 months although the mean count of *Candida* and *Streptococcus mutans* in saliva suspension tended to be lower.
**Conclusion:** Restoration of carious root surfaces using the ART with GIC-CHX resulted in higher survival rates compared to a control GIC, however, a larger number of patients is needed to confirm the validity of this finding. GIC-CHX restorations applied using ART may be a viable approach for use in outreach dental services to restore root surface carious lesions in the older adults and other special needs groups.
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<tr>
<td>ART</td>
<td>Atraumatic Restorative Treatment</td>
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<tr>
<td>CHX</td>
<td>Chlorhexidine</td>
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<tr>
<td>CFSS-SF</td>
<td>Short Form of the Dental Subscale of the Children’s Fear Survey Schedule</td>
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<td>DAS</td>
<td>Corah’s Dental Anxiety Scale</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>DMADDM</td>
<td>Dimethylaminododecyl methacrylate</td>
</tr>
<tr>
<td>DMFT</td>
<td>Decayed Missing Filled Teeth</td>
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<tr>
<td>DMFS</td>
<td>Decayed Missing Filled Surfaces</td>
</tr>
<tr>
<td>Er:YAG</td>
<td>Erbium-doped:Yttrium-Aluminum Garnet</td>
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<tr>
<td>GIC</td>
<td>Glass Ionomer Cement</td>
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<tr>
<td>GIC-CHX</td>
<td>Glass Ionomer Cement modified with 5% Chlorhexidine</td>
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<tr>
<td>HT</td>
<td>Hall Technique</td>
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<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
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<tr>
<td>MID</td>
<td>Minimal Intervention Dentistry</td>
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<tr>
<td>NCDs</td>
<td>Non-Communicable Diseases</td>
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<tr>
<td>RMGIC</td>
<td>Resin Modified Glass Ionomer</td>
</tr>
<tr>
<td>SLS</td>
<td>Sodium Lauryl Sulphate</td>
</tr>
<tr>
<td>SSCs</td>
<td>Stainless Steel Crowns</td>
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<tr>
<td>UN</td>
<td>United Nations</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Chapter 1
1.1 Introduction

Dental caries remains the most common chronic disease worldwide (Philip et al., 2018). According to recent global estimates, 621 million children had untreated cavities in dentine in primary teeth and 2.4 billion people had untreated cavities in dentine in permanent teeth and the burden of dental caries disease is expected to rise due to population growth and decrease in total tooth loss (Kassebaum et al., 2015). In New Zealand at least one in three older adults had untreated root caries lesions (Smith et al., 2015). Minimal Intervention Dentistry (MID) has been promoted for over 20 years involving a combination minimal caries removal and comprehensive patient management including diet, oral hygiene and caries assessment (Walsh and Brostek, 2013). Some of the examples of MID techniques are the Hall technique (HT) mainly for primary dentition (Innes et al., 2007) and Atraumatic Restorative Treatment (ART) for both deciduous and permanent dentitions (Frencken and Holmgren, 1999).

1.2 Hypotheses

The first hypothesis of this study was that root caries lesions restored with glass ionomer cement (GIC) modified with chlorhexidine (CHX) will have a reduced load of microorganisms in plaque samples when compared to teeth restored with conventional GIC. The second hypothesis was that the microbial count of unstimulated saliva will be reduced after application of GIC modified with chlorhexidine.

1.3 Aims and objectives

The main aim of this study was to investigate the clinical effectiveness of ART when using glass ionomer cement (GIC) modified with 5% chlorhexidine as a restorative material in the treatment of root caries.

Other objectives of this research were:

- To assess the clinical effectiveness of ART using chlorhexidine-modified GIC in delaying or halting the progression of root surface caries over a period of 6 months;
• To measure the changes in the mean count of cariogenic bacteria in plaque and saliva samples following ART.
• Assess the participant’s acceptability of the ART
• Assess the operator’s acceptability of the ART
• Compare the survival of GIC-CHX in relation to GIC restorations over 6 months.

1.4 Thesis format

This thesis is divided into 5 chapters; chapter 1 provides general background and introduction to the topic followed by the project hypotheses, aims and objectives. Chapter 2 is the literature review (pages 5-24). Chapter 3 describes the clinical evaluation of glass ionomer cement modified with 5% chlorhexidine (GIC-CHX) (pages 26-47). Chapter 4 introduces the microbiological aspect of the research project (pages 50-73). The thesis concludes with Chapter 5 which summarises the findings of the previous chapters and proposes future directions (pages 75-78).
Chapter 2


2 Literature Review

2.1 Introduction

Dental caries is one of the most prevalent chronic diseases worldwide which affects all ages and those with teeth remain susceptible to this disease throughout life (Bagramian et al., 2009). It is a complex disease that occurs as a result of acid produced by microorganisms found in dental plaque and is modified by many factors such as saliva, diet and host factors. Root caries is a condition that commonly affects older adults due to increased gingival recession and poor oral hygiene. Some medications may have a hyposalivatory effect thereby reducing the protective action of saliva, increasing the risk of developing root caries. Older patients are often overburdened with complex medical problems such as Parkinson’s disease, dementia decreasing manual dexterity and increasing plaque retention. Furthermore, some patients have difficulty for reaching dental professionals and therefore receive little care (Saunders and Handelman, 1992). The Atraumatic Restorative Treatment (ART) is a simple and effective method in treating such a cohort. Another way to improve the outcome for such individuals is to enhance the preventive properties of restorative materials. This can be achieved by utilising existing restorative materials as a vehicle for antimicrobial agents. This literature review will explore these matters in more detail including some of the treatment concepts utilised in the treatment of root caries in older adults.

2.2 Dental caries

Dental caries is defined as the localised destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates. It is a slow chronic disease that can affect the enamel, dentine and/or cementum (Selwitz et al., 2007). Dental caries is an entire disease process (Featherstone, 2008) with the signs of carious demineralisation seen on the hard dental structure. However, the disease process is initiated in the bacterial biofilm. Dental plaque is a complex environment mainly composed of bacterial microcolonies encapsulated in an organic matrix of polysaccharides, proteins, and DNA (Selwitz et al., 2007). This provides protection from
desiccation, host defences, and more importantly, provides certain resistance to antimicrobial agents (Selwitz et al., 2007).

In recent decades, the disease process of dental caries has been much better understood from a number of aspects including microbiology, saliva composition and flow rates, tooth mineral composition, tooth ultrastructure, diffusion processes, and the kinetics of demineralisation and remineralisation (Featherstone, 2008). The very early carious changes in enamel are usually not detected clinically or radiographically, and late stages usually involve dentine tissue resulting in cavities to a varying degree depending on the stage of the disease process (Selwitz et al., 2007).

Dental caries is not caused by microbial pathogens but rather by microorganisms belonging to the resistant oral microflora which are normally harboured by most individuals (Marsh, 1994). Microbial fermentation of carbohydrate substrates causes the production of acid. Exposure to low pH will gradually lead to growth inhibition of acid-sensitive species and the selection of organisms with an aciduric physiology such as mutans streptococci and lactobacilli. These two groups of the so-called lactic acid bacteria are not only able to survive in an acidic environment but are also able to produce more acid by means of metabolism, thus lowering the pH even further. Ultimately, this will cause a breakdown of microbial homeostasis and cause a major shift in the composition of microflora favouring acidogenic and aciduric bacteria such as Streptococcus mutans and lactobacilli (Marsh, 1994).

Caries commonly begins with the loss of calcium ions from the surface apatite crystals which forms the majority of the calcified tissues. When there is balance under normal conditions, the process of losing calcium ions (demineralisation) is compensated by the uptake of calcium ions (remineralisation) from the tooth’s surrounding environment. This dynamic process is continuously occurring under normal conditions (Banting, 2001). However, when the balance is tipped towards demineralisation, the loss of calcium and phosphate ions initiates the caries process (Banting, 2001).

Carious dentine can be subdivided into two distinctive histopathological zones; the outer zone or the caries-infected zone and the inner zone or the caries-affected zone (Banerjee, 2013). The peripheral infected zone usually lies close to the enamel dentine
junction and it is irreversibly damaged as a result of a combination of demineralisation of the inorganic component followed by denaturing of collagen matrix that make up most of the organic dentine as a result of long-lasting bacterial invasion and contamination. This creates a soft leathery heavily infected tissue that is usually stained and can be easily excavated (Banerjee, 2013). As for the caries-affected layer which lies deeper to the first layer is characterised by the fact that it is reversibly damaged by the caries process. This occurs because the collagen matrix is not damaged or denatured and therefore under the right conditions, caries can be successfully arrested and reversed and the affected zone could be repaired (Banerjee, 2013).

Dental caries is a global disease affecting all different ages and sectors of the population (Ferreira Zandona et al., 2012). Despite the advancement in early detection and treatment, it remains the most common chronic disease in New Zealand (Foster Page and Thomson, 2012). Previous New Zealand studies have examined caries levels in adolescents (15-year-olds), and their findings show a trend in declining caries experience in the mid-1990s, from an observed mean decayed missing filled teeth (DMFT) of 17.3 in 1968 to 3.7 in 1995 (Beck, 1968; Kanagaratnam, 1997). Although a recent study confirmed this decline in the DMFT scores, it also showed that there was no significant improvement in the last 10 years (Foster Page and Thomson, 2012).

The Dunedin Multidisciplinary Health and Development Study in New Zealand is the only known dental study that has followed a group of individuals from birth to adulthood (Broadbent et al., 2008). Group-based trajectory analysis was used to explore developmental trajectories of dental caries over the life-course. The rate of increase in decayed missing filled surfaces (DMFS) with increasing age appeared to be linear (Broadbent et al., 2008). The findings of the Dunedin study showed that the caries rate increases with age. This is particularly true with root caries, as reported by Du et al. (2009) with 2160 Chinese older (65-74 years) participants, in which the prevalence of root surface caries was 43.9%, and in the middle age (35-44) group was 31.1% (Du et al., 2009).

### 2.2.1 Risk factors of dental caries
Dental caries is a complex disease caused by the interaction of biological, behavioural and social factors (MacEntee et al., 1993). Risk factors for dental caries are not stationary and change over time (Selwitz et al., 2007). Physical and biological risk factors for both crown and root caries include inadequate saliva composition and salivary flow, high numbers of cariogenic bacteria, insufficient fluoride exposure, gingival recession, immunological components, and genetic factors (Selwitz et al., 2007). Biological and behavioural factors have been well-studied and documented in children and adolescents, but social factors have received little attention in the literature. Social factors such as physical dependency and place of residency can contribute to the complexity of the dental caries process, especially in relation to older adults (MacEntee et al., 1993). Physically-dependent individuals usually rely on their carers to maintain good oral hygiene, especially those who reside in nursing homes. This can be quite challenging as shown in the dental study of nursing homes in Adelaide, Australia (Chalmers et al., 2002). In that study, although carers reported daily oral hygiene habits were practised, many residents had poor oral and denture hygiene. Since oral hygiene is one of the most influential factors in the development of oral diseases in older adults, the lack of proper oral hygiene had led to high incidence of coronal caries (64%) and root caries (49%) over the one-year observational period (Chalmers et al., 2002).

The risk factors for caries development depends largely on the lifestyle and behavioural factors of a particular individual. For example, the frequency and nature of oral hygiene practices and dietary habits can affect the risk of caries development (Selwitz et al., 2007). High consumption of carbohydrates is one of the main drivers of caries (Papas et al., 1995). Several factors have been identified to be associated with caries incidence and consumption of fermentable carbohydrates. Some of these factors include the amount of carbohydrates consumed, sugar concentration of food items, the physical form of the carbohydrate, oral retentiveness or length of time which teeth are exposed to low plaque pH, frequency of eating meals and snacks, length of interval between eating events and eating close to bedtime (Papas et al., 1995). Therefore, increasing the frequency of sugar intake increases the odds of developing root caries. This has been shown by Papas et al. (1995) when a group of individuals with restricted sugar intake due to diabetes were compared to a control group in terms of age, teeth and gingival recession, caries prevalence was found to be lower in the group with restricted sugar intake (Papas et al., 1995).
Root caries is found more regularly at the cementum-enamel junction, although it can also be confined entirely to the root surface. Banting et al. (1985) observed that the majority of root caries occurred within 2 mm of the gingival crest, which is the area where plaque was usually found (Banting et al., 1985). Acid present in root surface dental plaque as a by-product from carbohydrate fermentation, causes demineralisation of cementum exposing the collagen fibrils which are gradually broken-down by bacterially-derived enzymes on the root surface (Featherstone, 2004). Root caries starts on the root surface, with primary root caries occurring in the absence of restorations and secondary root caries being related to caries occurring near an existing restoration (Banting, 2001).

2.2.2 Microorganisms of root surface caries

Microorganisms in the oral cavity, especially bacteria, are dynamic and susceptible to changes in the oral environment. This concept of bacterial population shift was described by Ritz and co-workers more than 50 years ago, when they noticed a change in the bacterial population from aerobic and facultative to predominately anaerobic as dental plaque matured over nine days (Ritz, 1967). Distinctive microflora live on root surfaces during the development stages of root caries. The transition between these phases is very complex and may not be easily recognised clinically (Brailsford et al., 2001). The initial transition of plaque on an exposed root surface in subjects with no detected caries, to plaque on a root surface of individuals at risk of developing caries, is not the same. However, this transition usually involves undisturbed accumulation of plaque as a result of inadequate oral hygiene habits, but not necessarily combined with an increase in the frequency of consumption of fermentable carbohydrates. If these processes continue, the environment increases the risk of forming root-caries lesions. The factors involved in this transition are much less understood, but may simply involve the prolonged undisturbed accumulation of plaque on an exposed root surface, with the result that the dentine is demineralised, subjected to proteolysis and bacterial invasion (Brailsford et al., 2001). The environmental factors driving microbiological changes are not apparent, especially in the first transition; however, a persistent accumulation of aciduric bacteria is more apparent in the flora of active root caries lesions when compared to similar sites with no caries lesions. These transitions may not be constant with respect to time, since factors such as oral hygiene and diet may change over a very short period,
with severe effects on the microflora and on the underlying dentine (Brailsford et al., 2001).

Similarly, the composition of cariogenic microorganisms changes as the condition or the state of the carious lesion changes (Bowden, 1990). A good example is the increase in the proportion of *Streptococcus mutans* and lactobacilli in individuals with root caries (Preza et al., 2008). The presence of these two groups of microorganisms in great number in a root caries lesion may indicate an active lesion, in addition, it may also indicate the high caries risk of the individual (Bowden, 1990). Likewise, changes in systemic factors may also cause bacterial population changes. This change has been linked to an impaired immune system and colonization of non-oral bacterial species such as staphylococci and enterobacteria (Preza et al., 2009).

Because of the complexity of the oral microflora, which contains several hundred species of bacteria and millions of cells growing on a single tooth surface, no single bacterial species can be used to predict caries development in a particular person. However, in a study conducted in Vancouver to identify the predictors of dental caries in older adults, high numbers of lactobacilli together with high sugar intake and poor oral hygiene were found to be associated with increased risk of caries (MacEntee et al., 1993). Moreover, colonisation by mutans streptococci and other cariogenic bacteria could be a key risk factor for caries development. However, the role of mutans streptococci as the main cause of caries has not been proven (Selwitz et al., 2007).

Root caries lesions are most often initiated at the gingival margin in association with the accumulation of dental plaque, which must harbour microorganisms able to produce acid from carbohydrates and capable of growth in a low pH environment. Bacteria with these characteristics include non-mutans streptococci, *Actinomyces* spp., lactobacilli, and bifidobacteria, each of which have been reported to possess at least one of these two traits (Brailsford et al., 2001).

### 2.3 Oral health in older adults

Increase in life expectancy has led to an increase in the number of older adults in the overall population (Frencken, 2014). Older people experience an increased risk in
chronic diseases such as cardiovascular disease, hypertension, cancer, diabetes and Parkinson’s disease (Petersen and Yamamoto, 2005). The issue of a growing aging population and the concurrent increase in chronic diseases has led the World Health Organization (WHO) to highlight the need for strong health promotion strategies amongst older adults (Petersen and Yamamoto, 2005). Knowing and minimising risk factors of chronic diseases will lead to a healthier lifestyle. Ultimately, this will reduce the number and severity of chronic diseases. Engagement of older adults in productive lifestyles and participating in social activities will improve their general wellbeing and will make them productive members of the society (Petersen and Yamamoto, 2005).

Non-communicable diseases (NCDs) such as cardiovascular disease, lung disease, cancer and diabetes are the leading causes of human fatality worldwide (Jin, 2013). The United Nations (UN) has recently acknowledged that oral disease, as one of the common NCDs, is among the major global health burdens, which shares a number of common risk factors with other major NCDs. Some of these factors include increases in sugar intake, alcohol and tobacco use (Jin, 2013).

The WHO Global Oral Health Programme emphasises that oral health is integral and essential to general health and it is a major determinant factor for quality of life (Petersen, 2003), (Petersen and Yamamoto, 2005). Globally, poor oral health amongst older people has been particularly evident resulting in high levels of tooth loss and dental caries experience, as well as a high prevalence of periodontal disease.

Due to the growing aging population, improvements in oral health mean that now people are retaining their natural teeth for longer (Du et al., 2009). However, the majority of older people have systemic chronic diseases that ultimately lead to a decline in health. These diseases, and social and environmental impairments such as such as dementia, Alzheimer’s disease, physical disability, xerostomia and loneliness may have negative effects on oral health. These individuals often require dental care but they are not necessarily able to source dental treatment (Frenckken, 2014).

Older adults are at higher risk of developing root caries due to increased gingival recession resulting in exposure of root surfaces that are more prone to caries (Teich and Gilboa, 2011). This is exacerbated by reduced manual dexterity (Nevalainen et al., 1997)
and mental capacity leading to inadequate oral hygiene (Vilstrup et al., 2007). A reduced salivary flow rate can impair plaque removal and reduce the buffering capacity of saliva (Imazato et al., 2006a). In addition, some medications are known to cause a reduction in salivary flow rates (Saunders and Handelman, 1992). A study that evaluated the effects of hyposalivatory medications on saliva flow rates and dental caries in adults aged 65 and older found reduced salivary flow rates and a higher incidence of root caries in individuals taking medication such as antidepressants, anti-asthmatics and diuretics (Saunders and Handelman, 1992).

Some medications modify the causal factors of dental caries in two ways. First, they may act to reduce salivary flow to a level where the normal salivary buffering mechanisms are impaired because of their diminished volume. These mechanisms tip the pH balance towards demineralisation leading to an increase in caries development (Thomson et al., 2002). The second way is through the development of xerostomic symptoms as a result of changes in saliva quality, the perception of reduced salivary flow, or changes the characteristics of the mucosa itself (for example, by reducing minor salivary gland flow). This may lead individuals to seek certain means of relieving these symptoms such as chewing hard sugary sweets. This will eventually lead to acid production by aciduric bacteria causing demineralisation of the dentition. These two different pathways may occur separately or together (Thomson et al., 2002). In a study published in 2002, Thomson et al. reported that medication was not a risk factor for the occurrence of coronal root caries (Thomson et al., 2002). A similar result was found in another study which evaluated salivary flow rates, daily medication and root caries in older adults (Narhi et al., 1998). This study also found that men were less affected by reduced salivary flow rates than women, although the incidence of root caries was higher in men. Narhi et al. (1998) also concluded that adequate oral hygiene as well as preventive programmes can modify caries risk, which makes the assessment of risk of root caries difficult. However, they found that microbial tests seem to be a useful tool in monitoring the risk of root caries in aging individuals under varying medical conditions (Narhi et al., 1998).

2.3.1 Barriers to oral health services in older age
The need for dental care is higher among disadvantaged and vulnerable groups in developed countries (Petersen and Yamamoto, 2005). Unequal utilisation of preventive measures can be one of the reasons for this phenomenon, since those with a high socio-economic status are more likely to access preventive services more regularly while those with high needs mainly receive symptomatic restorative treatment (Schwendicke et al., 2015). Several global reports have shown that the use of professional dental health services is low among older people, particularly among individuals with a low socio-economic status (Petersen and Holst, 1995).

There are still many barriers to accessing oral health services in older people, such as impaired mobility (Petersen and Yamamoto, 2005). Given that some older people may experience financial hardship following retirement, the cost or perceived cost of dental treatment, together with lack of dental care habits and negative attitudes to oral health, may prevent them from visiting a dentist. Moreover, older people living in rural areas were more likely to have poor oral health status and inadequate utilisation of dental care (Vargas et al., 2003). All these factors make older adults more vulnerable to oral diseases.

In New Zealand, ethnicity and socioeconomic status are likely to influence whether someone visits a dentist. This has been shown in the last annual report from the Ministry of Health, when only 37% of people living in the most socio-economic deprived areas had visited a dental health professional within the last 12 months, compared to 59% of adults in the least deprived areas (Ministry of Health, 2015). In addition, 70% of Māori only see the dentist when they have a problem, and in the 12 months preceding the annual report, 10% of teeth extracted in this group were as a result of caries. These figures are even higher among Pacific Island adults (80% symptomatic attenders, 12.7% extractions due to caries) compared to adults of all other ethnicities (54% symptomatic attenders, 7.5% extractions due to caries) (Ministry of Health, 2015). This suggests the need to implement measures to address this problem of lack of regular attendance to dental care. There are different methods to reduce such inequalities. One is to be able to provide a cost-effective simple treatment that is beneficial, at the same time as being acceptable to patients. In particular, older adults are more in need of such methods because of their higher risk of developing oral diseases, particularly root caries. One such method that has shown good promise is the atraumatic restorative treatment.
2.4 **Atraumatic Restorative Treatment (ART)**

If preventive measures are not implemented properly, the risk of developing root caries in older adults increases. Utilisation of preventive measures such as fluoride application reduces caries incidence (Nyvad and Fejerskov, 1986). Once a cavity has developed, a restoration must be placed to remove the bacteria and prevent further destruction of the tooth, even though it does not deal with the disease in the rest of the mouth (Featherstone, 2008). Minimal intervention dentistry has gained popularity in recent years. The “Hall Technique” and the “Atraumatic Restorative Treatment” (ART) are two examples of minimally-invasive dentistry for caries treatment that have had good results.

The Hall Technique is a method for managing carious lesions in primary molars by cementing stainless steel crowns (SSCs) using glass ionomer cement with no tooth preparation and no local anaesthesia (Innes et al., 2015). In a randomised controlled trial, this technique was tested against conventional methods involving conventional tooth preparation and restorations (Innes et al., 2007). The results showed that the Hall Technique caused significantly less discomfort compared to conventional methods and this method was also preferred not only by the children, but also by their carers and dental professionals. Moreover, SSCs placed using the Hall Technique suffered only 2% major failures after 23 months when compared to control restorations with a 15% major failure rate (Innes et al., 2007). This supports the concept that if dentinal caries is well sealed off, caries progression can be greatly slowed and even arrested. This has been confirmed with a more recent follow-up study, where the Hall Technique was shown to be an effective caries management technique for carious primary molars (Innes et al., 2015).

As for primary root caries there are different types of atraumatic treatments available. One such treatment is chemo-mechanical caries removal. The pioneering work regarding this treatment started in the early to mid seventies when the use of chemical agent GK-101 (N-Chloroglycine) was shown to be superior to saline in removing carious dentine (Schutzbank et al., 1975). A clinical trial was set out to test the efficacy of chemo-mechanical removal of primary root caries using Carisolv™. Thirty eight participants were included in that study with a total of 60 primary root caries lesions. Group one had 34 root caries lesions and they were treated using chemo-mechanical method and the
other group was treated using conventional drilling method. Participants acceptance of the treatment was high with few requiring local anaesthesia. The restorations were followed for 1 year and there was no difference in success between the two groups. The authors concluded that chemo-mechanical removal of caries is an effective alternative method of treating root caries (Fure et al., 2000). Later development in the chemo-mechanical technology included the development of pepsin-based gels which can be used in combination with nylon brushes to abrade away the carious tissues before restoration (Banerjee, 2013)

Laser has been used in medicine and dentistry since the sixties. Reducing pain and discomfort during cavity preparation was the driving force for the research into this field with the result of the first commercial system, the Kay Laser 1, beams introduced by Kavo in 1992 (Bader and Krejci, 2006). Effective ablation of carious root lesions using erbium-doped:yttrium-aluminum garnet or Er:YAG laser has been shown to be effective when removing caries dentine. In a study that was conducted to evaluate the efficacy of using laser to remove caries in vitro compared to conventional drilling, the authors found that Er:YAG removed caries equally as well as conventional methods although longer time was required to completely remove all the carious dentine. Less vibration was noted and no significant heat was produced during the procedure (Aoki et al., 1998).

Air abrasion is another technique that has been used to remove caries with minimal tooth preparation. This technique has been used in dentistry for over 70 years. Aluminium oxide particles are blasted at the carious tissue with controlled pressure and the result is non-selective removal of tooth tissue. Because of the lack of tactile feedback that clinicians are used to and the lack of selectivity, this technology has been reserved for enamel preparation for bonding, removal of staining or to gain access to cavities. However ease of use is changing with advancement in powder technology and better education of clinicians (Banerjee, 2013).

All these techniques, however, require either sophisticated equipment, a power source to be able to operate the different tools, some of which may not be mobile so may be difficult to move around if domiciliary visits are required for example. Moreover, high level of training are required and the equipment is not usually readily available for every
clinician. Therefore, an atraumatic treatment method that is simple, cost-effective and readily available was needed, especially for those in rural areas who may not have access to high end dentistry.

The Atraumatic Restorative Treatment (ART) is one example of minimally invasive dentistry that can be applied in both deciduous and permanent dentitions. ART has evolved in the last 30 years to include not only relatively large cavities but also small dentine cavities. This has led to expanding the application of the unconventional preventive and restorative care concept that became known in the early 1990s as the ART approach (Frencken et al., 2012). In comparison to the Hall Technique, ART involves removal of carious dentine using only hand instruments. The development of wear-resistant glass ionomer cements in the mid-1990s replaced the originally used medium viscosity glass ionomers and until today these two are the material of choice when using ART (Frencken et al., 2012). Although the development of ART was mainly intended for underprivileged children in developing countries (Frencken et al., 2012), it has gained popularity in treating frail older adults who are home bound and also institutionalised patients. It can be conducted without sophisticated instruments, as only hand instruments are used, so neither electricity nor local anaesthesia are needed which makes it an even more versatile technique and appealing to many patients (Frencken, 2014).

Restoring teeth with ART was found to be more effective in the management of dentally-anxious patients especially for the management of younger patients under the age of 6. Hu et al. (2005) compared conventional restorative techniques with ART in patients suffering from xerostomia as a result of radiation therapy. It was found that ART had similar efficacy to conventional restorative techniques (Hu et al., 2005). Previous studies have shown some promising results in treating root caries lesions using ART (Honkala and Honkala, 2002; Da Mata et al., 2015).

In addition, ART was found to be more effective and easier to perform than using rotary instruments in older patients (Frencken et al., 2012). Several studies have shown that the ART approach is less time-consuming compared to conventional restorations, since it does not require local anaesthesia or polishing after the material is set (Da Mata et al., 2014). Furthermore, the ART is quite a simple procedure when compared to more conventional methods. This is advantageous since it could reduce the cost of treatment.
because a big portion of the cost is related to labour. Dentists have higher pay rates so there is a possible cost-reducing effect by possibly employing dental therapists for such procedures (Da Mata et al., 2014). However, this is currently not possible in a New Zealand population due to the specified scope of practice set out by the Dental Council (Dental Council of New Zealand, 2011). Originally, ART was used under field conditions without access to sophisticated equipment or electricity usually. For this reason, an adhesive restorative material that required only hand-mixing and is chemically-cured would be desired (Frencken et al., 1996). Glass ionomer cement had these properties with the extra advantages of chemical bonding to enamel and dentine, and the added benefit of fluoride release (Frencken and Holmgren, 1999).

The success of restorations placed using the ART approach has been reported in the literature to be in the range of 80%-95% for Class I and Class V single-surface restorations after 1 year, and about 90% after 2-3 years. However, large and multi-surface restorations are less successful especially when exposed to occlusal forces due to the inherent weakness of the material under tensile and compressive stresses (Mickenautsch et al., 2010).

2.5 Glass ionomer cements

Glass ionomer cements (GIC) are restorative materials that were first introduced to dentistry in 1971 (Wilson and Kent, 1971). Glass ionomers are known scientifically as glass-polyalkenoate cements. They are true acid-base materials where the base is a fluoroaluminosilicate glass with a high fluoride content. This component interacts with a polyalkenoic acid resulting in a cement consisting of glass particles surrounded and supported by a matrix arising from the dissolution of the surface of the glass particles in the acid (Mount, 2002). Calcium polyacrylate chains are formed after mixing the two components and these chains form the initial matrix that holds the particles together. Aluminium ions form aluminium polyacrylate chains once calcium ions are involved, and since these are less soluble and stronger, the final matrix formation takes place. During this process, fluoride is released from the glass in the form of micro-droplets that lie free within the matrix, but play no part in its physical make-up (Mount, 2002). This fluoride is either retained in the matrix or bound to aluminium and it can leach out or get taken back into the matrix during ion exchange reactions without affecting the physical
properties of the set restoration. Following mixing and setting, fluoride makes up about 20% of the final glass powder which becomes more readily available from the matrix than from the original glass particles (Mount, 2002). These fluoride ions might also contribute to caries prevention (Causton, 1981). Fluoride release profile while initially high, within a day or so of the setting reaction initially, falls below the therapeutic levels.

GIC has the ability to adsorb permanently to the hydrophilic surfaces of hard oral tissues, thus offering the possibility of sealing margins at the tooth-restoration interface (Lin et al., 1992). The basic component of the glass is a calcium aluminosilicate which contains fluoride ions. The acid is a polyelectrolyte, which is a homopolymer or copolymer of unsaturated carboxylic acids. The glass ionomer cement sets as a result of a reaction between the acid and base. The end product of the reaction forms as a hydrogel salt which acts as a binding matrix (Lin et al., 1992).

Fresh glass ionomer releases more fluoride than maturated glass ionomer material. The release of fluoride ions is highly concentrated in the confined space between the restoration and the tooth. This high concentration of fluoride not only hardens soft dentine but also has an effect on the remaining bacteria in the dentine region (Forsten, 1991). However, this effect of fluoride release has been shown to diminish after a period of time (Causton, 1981), but glass ionomers can uptake fluoride from the surrounding environment. This has the effect of recharging GIC with fluoride and releases it gradually back into the surrounding environment (Forsten, 1991).

Once set, GIC has a water content between 11%-24% that can be divided into loosely bound water and tightly bound water. The loosely bound water can be easily removed with dehydration and tightly bound water cannot be removed and remains as an important part of the set cement (Mount, 2002). For this reason, it is important to protect the GIC during the setting process from water loss or water uptake (Mount, 2002). It has been shown that protection from water loss or uptake is important to prevent loss of aluminium ions required for cross-linking in the set cement. This cross-linking increases with time so the longer the GIC is protected, the greater the cross-linking which ultimately leads to stronger GIC restorations (Causton, 1981).
Nano-filled surface coating of glass ionomers reduced the initial burst of fluoride release and allowed for sustained release of fluoride when compared to non-coated specimens. This was due to the reduced solubility of coated glass ionomer cements and reduced leaching of fluoride by up to 60% when compared to non-coated specimens (Tiwari and Nandlal, 2012).

A number of surface coating materials have been tested and their effect on GIC setting has been evaluated (Brito et al., 2010). Some studies have shown that the best material is nail varnish. Nail varnish has been shown to efficiently maintain the hardness of glass ionomer cement. However, this material contains toluene which can be toxic to the nervous system by causing fatigue, mental confusion, loss of memory, nausea, loss of appetite and loss of vision. These harmful effects have prevented the use of nail varnish routinely in dentistry. On the other hand, petroleum jelly is found to be a good alternative to nail varnish because it provides adequate protection to GIC with no ill effects on health (Brito et al., 2010). Currently, many GIC manufacturers produce cavity varnishes for protection of GIC during setting (Shintome et al., 2009). Surface protection of GIC is an important feature because the acid-base reaction in the GIC continues slowly over many hours and the microhardness of the material increases with time but it is dependent on the surface protection (Shintome et al., 2009).

Originally, calcium ions (Ca$^{2+}$) were the main constituent of the powder in GICs, however it has been replaced by strontium (Sr$^{2+}$) ions (Ngo, 2010). The main reason for this was to make the GIC restorations radiopaque. Both Ca$^{2+}$ and Sr$^{2+}$ are very similar in their polarity and atomic size, this in turn made both elements interchangeable in the composition of GIC as well as hydroxyapatite. Furthermore, Sr$^{2+}$ can replace Ca$^{2+}$ in the hydroxyapatite lattice without causing any detrimental effect (Ngo, 2010). There is also some evidence that Sr$^{2+}$ can have anticariogenic properties, especially when combined with optimal fluoride levels (Curzon et al., 1978).

Initial setting of GIC after mixing involves cross-linking of the polyacid chains by either the Ca$^{2+}$ or Sr$^{2+}$ ions. This cross-linking is not stable and is susceptible to water uptake or loss. The second phase of GIC setting involves exchange of ions between GIC and the external environment. GICs ability to uptake and release ions makes it a rich reservoir of apatite forming ions such as fluoride (F$^-$), Ca$^{2+}$, Sr$^{2+}$ and phosphate (Ngo,
This leads to hardening of the restoration over time with the surface hardness increased by up to 39% after 40 days storage in saliva as a result of diffusion of ions such as Ca\(^{2+}\) and PO\(_4^{3-}\) into the hydrogel matrix (Okada et al., 2001). When the GIC is placed in direct contact with affected dentine, the migration of apatite forming elements (F\(^-\) and Sr\(^{2+}\)) from the GIC to the carious dentine can be extensive (Ngo, 2010).

The F\(^-\) and Sr\(^{2+}\) ions contained in the GIC were both found to cross the interface into the partially demineralised dentine adjacent to the restorative material and they were able to penetrate deep into the lesion with a depth of 1.5 mm on average for both elements (Ngo et al., 2006). This may contribute to the remineralisation of the demineralised dentine. In order for this to happen, the restoration needs to be totally sealed off from the external environment and there must be an intimate contact between the GIC and the partly-demineralised dentine (Ngo et al., 2006).

Originally, medium viscosity GICs were the only available materials to be used for ART. However, with the introduction of high viscosity GICs in the mid-1990s, it has become the most widely used material for ART (Frencken et al., 2012). Modification of high viscosity GICs with antibacterial agents have been introduced when used in ART to enhance such restorations. It is known that the ART approach does not remove all dentinal caries and it depends on the establishment of a good seal to prevent the progression of caries. However, some researchers have questioned the properties of recently developed GICs for ART, in particular their fluoride release and sealing ability (Turkun et al., 2008). Therefore, improving the antibacterial properties of such materials will help in eliminating the risk of progression of dentinal caries and will likely improve the overall success of the ART (Turkun et al., 2008).
2.5.1 Modification of dental restorations to improve antibacterial properties

There are a number of antimicrobial agents available, but only a few have been able to be incorporated into restorations without negative effects on the mechanical and physical properties of restorative materials. Increasing the antimicrobial properties of restorations is important to reduce dental biofilm building on dental surfaces. Dental biofilm constitutes an ecosystem of bacteria that exhibits a number of physiological characteristics such as acid production as a result of fermentation of carbohydrates leading to demineralisation of tooth surfaces (Takahashi and Nyvad, 2011). Some examples of antimicrobial agents which have been incorporated into restorative materials include silver nanoparticles (Ahn et al., 2009), quaternary ammonium monomer (dimethylaminododecyl methacrylate; DMADDM) (Wang et al., 2016) and chlorhexidine, both in its powder form such as chlorhexidine acetate (Palmer et al., 2004), and liquid form chlorhexidine gluconate (Marti et al., 2014). Chlorhexidine has been the most researched and documented antimicrobial agent to be incorporated into restorative materials.

Glass ionomer containing (3% and 5%, W/W) TiO$_2$ nanoparticles showed improved fracture toughness, flexural strength and compressive strength compared to the unmodified glass ionomer. However, a decrease in mechanical properties was found for glass ionomer-containing (7%, W/W) TiO$_2$ nanoparticles. Glass ionomer-containing (5% and 7%, W/W) TiO$_2$ nanoparticles also had a compromised surface micro-hardness, although the setting time of glass ionomer-containing TiO$_2$ nanoparticles met the requirement of water-based cements. The bond strength to dentine and the fluoride release of the glass ionomer were not compromised. Glass ionomer-containing TiO$_2$ nanoparticles had greater antibacterial activity against S. mutans compared to the unmodified GIC (Elsaka et al., 2011).

Silver nanoparticles have also been added to restorative materials. Silver is known for its antimicrobial properties, and it has been added successfully to dental materials such as glass ionomer and composite (Ahn et al., 2009; Magalhães et al., 2012). A study conducted to evaluate the long-term antibacterial and mechanical properties of
experimental nano silver containing GICs, found that the inhibition zones in both conventional light cure GIC and nano silver modified GIC were not statistically different to each other. As the material aged no growth inhibition was noted after 2 days. The authors suggested that silver and fluoride ions were responsible for the initial inhibition but as time passes these ions leach out and their effect reduces with time (Fujun et al., 2013). It is worthwhile noting that most of the research regarding TiO$_2$ and silver nano particles was done in *vitro* and no clinical trials were done to confirm their validity in the treatment of root caries in the oral environment.

### 2.5.1.1 Chlorhexidine

Increasing the antimicrobial properties of restorative materials is an important aspect of the long-term success of restorations. If any remaining bacteria are present in the cavity, the antimicrobial activity of the restorative material may eradicate these viable bacteria and reduce the risk of recurrent caries (Papas et al., 1995).

A number of studies have been conducted to evaluate the effect of incorporating antimicrobial agents into GICs (Jedrychowski et al., 1983; Palmer et al., 2004; Millett et al., 2005; Takahashi et al., 2006; Wyatt et al., 2007; Frencken et al., 2007; Turkun et al., 2008; Farret et al., 2011). Having a restorative material that possesses antimicrobial properties will provide many benefits to patients. Some of these benefits involve the elimination of recurrent caries around the margins of restorations, the inhibition of plaque accumulation near restorations and reduction of the number of microorganisms in the salivary fluids and the oral cavity (Jedrychowski et al., 1983).

Chlorhexidine (CHX) is one of the antimicrobial agents that can be added to glass ionomer cements. Its chemical structure is shown in (Figure 2-1).
Chlorhexidine is a cationic bisbiguanide and has a broad spectrum antibacterial activity against gram-positive and gram-negative bacteria, *Candida* spp and lipophilic bacteria (Hennessey, 1973; Harbison and Hammer, 1989; Salim et al., 2013). In addition, chlorhexidine has a low mammalian toxicity with a strong binding affinity to skin and mucous membranes (Jones, 1997). Different concentrations of chlorhexidine have different effects. At low concentrations it is bacteriostatic, whereas it is bactericidal at high concentrations (Jones, 1997). These effects are different on different microorganisms. For example, the mean minimum inhibitory concentration (MIC) of chlorhexidine for *Streptococcus mutans* was found to be 0.0002%, while the MIC for *Pseudomonas aeruginosa* was greater than 0.07% (Hennessey, 1973). A chlorhexidine concentration of 0.02% resulted in 99.99% elimination of gram-positive and gram-negative bacteria tested; however, this effect was reduced dramatically in the presence of biological fluids. For example, addition of serum required a four fold increase in chlorhexidine concentration to produce the same effect. Similarly, in the presence of 5% sucrose, *Streptococcus mutans* required significantly higher concentrations, and this was attributed to the binding of chlorhexidine to polysaccharides thus reducing the availability of free chlorhexidine (Hennessey, 1973).

Chlorhexidine has been shown to have great substantivity on human dentine. Both the gel and solution forms were shown to have up to 90 days retention in dentine (Souza et al., 2012). This has made it a particularly useful product for the irrigation of root canals (Souza et al., 2012).
2.5.1.2 Modification of GIC with chlorhexidine

Ribeiro and Ericson (1991) and Hoszek and Erickson (2008) have investigated the effect of adding chlorhexidine to GIC restorative materials and luting cements in two different forms: chlorhexidine digluconate and chlorhexidine diacetate. It was found that addition of two variants of chlorhexidine had an inhibitory effect on *Streptococcus mutans* and this effect was dose-dependent (Ribeiro and Ericson, 1991; Hoszek and Ericson, 2008).

Jedrychowski et al (1983) tested the addition of two antibacterial compounds, chlorhexidine gluconate and chlorhexidine dihydrochloride to composite resin and glass ionomer restorative materials in different concentrations. The antibacterial effect was tested on microorganisms commonly found in the oral cavity such as *Streptococcus mutans* and *Lactobacillus acidophilus* (Jedrychowski et al., 1983). It was found that chlorhexidine gluconate demonstrated significantly more bacterial inhibition than chlorhexidine dihydrochloride for each microorganism. The study also tested the effect of the addition of chlorhexidine on the mechanical properties of GICs. It was found that addition of 5% chlorhexidine gluconate altered the adhesive shear strength values but there was a significant increase in compressive strength values (Jedrychowski et al., 1983). A more recent *in vitro* study found that the addition of 0.5% chlorhexidine digluconate to GIC resulted in increased antimicrobial properties with no significant effect on the mechanical properties or setting time. However, higher concentrations of chlorhexidine digluconate (1%, 2%) increased the setting time and decreased the mechanical properties of the GIC (Marti et al., 2014).

Chlorhexidine diacetate in a powder form had been used to modify GIC in order to improve the antimicrobial properties of the restorative material. Takahashi et al (2006) tested different concentrations and found that the addition of 1% chlorhexidine diacetate improved the antimicrobial properties of GIC without significantly affecting the mechanical properties, bonding abilities or setting time. Higher concentrations of chlorhexidine diacetate (more than 2%) had a significant detrimental effect on the compressive strength of the GIC (Takahashi et al., 2006).
The increased antimicrobial activity of GIC modified with chlorhexidine has been tested not only *in vitro* but also *in vivo*. A study in a mobile dental clinic in the University of Johannesburg, South Africa, investigated whether a 1% chlorhexidine diacetate modified glass ionomer was more effective than conventional glass ionomer in inhibiting the growth of microorganisms left in infected and affected dentine under a restoration (Frencken et al., 2007). A statistically significant difference in the reduction of microorganisms such as *Streptococcus mutans* and lactobacilli in infected and affected dentine samples was observed with chlorhexidine-containing GICs compared to the conventional GIC over a 7 day period (Frencken et al., 2007). In another *in vivo* study, comparable results were found with regard to microleakage when modified GIC with 1% CHX diacetate was evaluated in the primary dentition. The authors concluded that GIC modified with CHX could be a useful alternative in clinical use in particular when ART is used (Mathew et al., 2013).

Changes in mechanical properties of chlorhexidine-modified GIC had been investigated in band cementation of orthodontic appliances. It was found that addition of 10% chlorhexidine digluconate did not have a significant effect on retentive strength or survival time of bands cemented with modified GIC when compared to conventional GIC (Millett et al., 2005). This has also been confirmed in a more recent study investigating the antibacterial and micromechanical properties of GIC following the addition of 10% and 18% chlorhexidine digluconate (Farret et al., 2011). It was found that both concentrations had increased antimicrobial properties against the tested strains of *Streptococcus mutans* and the zone of inhibition was larger with higher concentrations. On the other hand, both concentrations had little influence on the diametral tensile, compressive or shear bond strengths of GIC (Farret et al., 2011).

Similar results have been obtained with resin-modified GICs by Sanders et al. (2002) who tested the diametral tensile strength of these materials after being modified with 5% chlorhexidine diacetate (Sanders et al., 2002). In this study, they found that the mechanical properties of the test resin-modified GIC were not greatly affected by the addition of chlorhexidine, whereas antimicrobial properties improved significantly against *Streptococcus mutans* and peaked about 3-4 weeks after which the antimicrobial effect decreased (Sanders et al., 2002). The authors suggested the decrease in antimicrobial effect could have occurred because of the loss of chlorhexidine due to
elution or as a result of the formation of insoluble salts with the glass ionomer (Ribeiro and Ericson, 1991). Although the concentration of chlorhexidine decreased with time, it may be sufficient in the microenvironment of the cavity to induce a bactericidal effect and therefore, prevent recurrent caries for a longer period of time (Sanders et al., 2002; Turkun et al., 2008). To investigate this in an in vivo study, De Castilho et al (2013) added 1.25% chlorhexidine digluconate to resin-modified GIC liner. Samples were taken from carious dentine at baseline and 3 months and it was found that the CHX-modified resin-modified GIC resulted in complete elimination of Streptococcus mutans, whereas the conventional resin-modified GIC did not have any significant reduction in the number of Streptococcus mutans (De Castilho et al., 2013).

From the literature, it is clear that chlorhexidine digluconate had greater inhibition zones specially for S. mutans and lactobacilli and this effect was concentration-dependent (Farret et al., 2011). This means that as the concentration of the chlorhexidine digluconate increases the inhibition zone increases as well. This was clearly shown by a number of authors (Hoszek and Ericson, 2008; De Castilho et al., 2013). On the other hand, the inhibition zones of chlorhexidine diacetate (which is a powder form) is not concentration-dependent (Takahashi et al., 2006). It also appears that chlorhexidine digluconate had a lesser effect on the mechanical properties of high viscosity glass ionomer cements when compared to other forms. Again this has been reported by several authors (Millett et al., 2005; Farret et al., 2011), although others have found greater concentrations could affect the mechanical properties quite significantly (Jedrychowski et al., 1983). Jedrychowski and colleagues 1983 study was conducted before the introduction of high viscosity glass ionomer in the mid-1990s, and therefore their findings may not be translatable to these later generations GICs. Another study has shown that the addition of low concentrations of chlorhexidine digluconate to GIC had minimal effect on the mechanical properties. Nevertheless, concentrations of chlorhexidine digluconate exceeding 2.5% had significantly lower hardness values when compared to the control, while diametral tensile strength, compressive strength and biaxial flexural strength showed insignificant decreases (Turkun et al., 2008).

To date, only one in vivo study in the UK has investigated the use of chlorhexidine digluconate modified GIC as a restorative material using ART. Many laboratory tests had been performed in the Leeds Dental Institute to determine the best formulation of
CHX and GIC, and it was determined that a 5% chlorhexidine digluconate modified GIC was an appropriate material of choice for a clinical trial to investigate its effectiveness as a restorative material using ART (Devine et al., 2011).

2.6 Summary

In summary, dental caries is a very complex chronic disease that is considered to be the most prevalent disease worldwide (Selwitz et al., 2007). Many risks and modifying factors contribute to the caries process and therefore determine the individual’s risk. Older adults are considered at risk of developing root caries and this risk increases as a result of factors such as poor oral hygiene and impaired general wellbeing. The most vulnerable individuals are generally most in need of dental care but are not necessarily able to get access to it due to mobility issues or lack of support. Simplifying dental treatment and improving antimicrobial properties of restorations are some of the innovations that can be used to help such people. ART is a technique that only uses hand instruments in most cases, so it may be used to provide treatment in the place of residence as opposed to a dental office, allowing access to treatment for those who may not otherwise be able to access dental care.

The first hypothesis of this study is that root caries lesions restored with glass ionomer cement (GIC) modified with chlorhexidine (CHX) will have a reduced load of microorganisms in plaque samples when compared to teeth restored with conventional GIC. The second hypothesis is that the microbial count of unstimulated saliva will be reduced after application of GIC modified with chlorhexidine. The main aim of this study was to investigate the clinical effectiveness of ART when using glass ionomer cement (GIC) modified with 5% chlorhexidine as a restorative material in the treatment of root caries. Other objectives of this study are to investigate the participant’s and operator’s acceptability of the ART and to compare the survivability of the GIC-CHX to GIC only restorations.
Chapter 3
3 Clinical performance and patient acceptability of glass ionomer cement modified with 5% chlorhexidine (GIC-CHX) compared to conventional glass ionomer cement (GIC) for the treatment of root caries

3.1 Introduction

With the increase in life expectancy, adults are retaining more of their natural teeth. The aging population experience a range of medical conditions, and other physical, intellectual and cognitive disabilities that can hamper their oral health, general health and wellbeing. This places an additional burden on the oral health care system (Petersen and Yamamoto, 2005).

Dental caries is a global disease affecting different ages and sectors of the population. It is a multifactorial disease that occurs as a result of interactions between the host environment and microorganisms. Active caries usually requires a susceptible host with suitable environmental factors such as a high carbohydrate diet intake and/or poor oral hygiene practices (Hellyer and Lynch, 1990; Papas et al., 1995; Fontana and Zero, 2006).

Older individuals have a high chance of developing root caries. This can be attributed to many factors, such as a reduced salivary flow. Reduced salivary flow is caused by multiple factors including polypharmacy, and radiotherapy involving the salivary glands (Saunders and Handelman, 1992). Saliva provides lubrication and protection to the mouth from bacterial infections, particularly dental caries. The risk of dental caries increases due to inadequate saliva flow and this is evident in individuals who undergo radiation therapy (Hu et al., 2005). Also, at risk are older individuals who may be fragile, and/or living in rest homes and who may have poor oral hygiene practices. This risk is increased for those with motor neuron diseases such as Parkinson’s and dementia (Brailsford et al., 2002; Frencken, 2014). Therefore, such a cohort requires a simple, fast method to deliver cost-effective treatment that is pain- and stress-free. If preventative measures such as fluoride application are not implemented properly, the risk of developing root caries in older adults increases considerably (Nyvad and Fejerskov, 1986). Once a cavity has developed, a restoration must be placed to replace the carious
tissue, ideally also removing any remaining microorganisms and preventing further
destruction of the tooth (Featherstone, 2008). This is because cavities create niches for
bacteria that the patient is unable to access and clean.

Minimal intervention dentistry has gained popularity in recent years. The “Hall
Technique” and “Atraumatic Restorative Treatment” (ART) are two examples of
minimally invasive dentistry that have shown promising results (Smales and Yip, 2002;
Innes et al., 2015). Atraumatic Restorative Treatment (ART) can be applied in both
deciduous and permanent dentitions and has significant advantages compared to
conventional restorative treatments. These include being pain-free, not using electricity
or anaesthetics, requiring minimal intervention and minimal cavity preparation, high
restoration survival rates and low cost (Frencken and Holmgren, 1999). Although the
development of ART was mainly intended for underprivileged children in developing
countries (Frencken et al., 2012), it has gained popularity in treating older and frail
patients. ART can be performed with no sophisticated instruments, as only hand
instruments are used. Most importantly, studies have shown that the ART treatment is
cost-effective and has high restoration survival rates which makes it a versatile treatment
modality (Frencken et al., 2007; Mickenautsch et al., 2010; Da Mata et al., 2014).

The development of wear-resistant glass ionomer cements in the mid-1990s
replaced the originally used medium viscosity glass ionomers and until today, these are
the material of choice for ART (Frencken et al., 2012). The aim of this study was to
evaluate the clinical performance, survival rate and acceptability of a modified glass
ionomer cement with added chlorhexidine (GIC-CHX) when applied using ART,
compared to control GIC restorations. The hypothesis was that GIC-CHX would have
better survival rates compared to control GIC, and ART is an acceptable treatment
modality.
### 3.2 Experimental Approach and Methods

#### 3.2.1 Study design

Ethical approval for this research was obtained from the Health and Disability Ethics committee (approval number 16/CEN/174). The study was designed as a randomised control trial with a split mouth design. One root caries lesion on one side of the mouth was restored with chlorhexidine-modified GIC (test), and on the contralateral side another lesion was restored using conventional GIC (control). If the participant had only one root caries lesion, a healthy tooth from the contralateral side was chosen and sampled as the control.

Participants with root surface carious lesions that required operative intervention were recruited from the Faculty of Dentistry, University of Otago. Participants who were aged 50 years or more and had one or more teeth with root caries were included in the study. Exclusion criteria included:

- Participants with full dentures;
- Those undergoing current antibiotic therapy, or who had radiotherapy of the head and neck region in the last 12 months;
- Participants using or who had used chlorhexidine-containing oral products in the last four weeks or who were allergic to chlorhexidine.

Participants with significant dental problems such as acute infections or significant discomfort were referred for appropriate dental management prior to the start of the study. Once participants were informed of the study, an information sheet and consent form were given to them. Only those participants who provided informed consent and were willing to follow the research schedule were included in the study.

A power calculation done prior to the start of the study suggested that the inclusion of 30 participants was sufficient to detect trends or statistically significant changes with 80% power and a Type 1 error rate of 5%. To allow for dropouts or loss to follow-up during the trial period, the original aim was to recruit 34 participants (i.e. 4 extra).
3.3 Methodology

3.3.1 Clinical procedure

Participants were fully-informed of the procedures involved and were given instructions not to use antimicrobial mouthwashes for the duration of the study. Participants received a full oral health assessment at baseline and their medical history was recorded. Participants received a full oral health assessment and detailed oral instructions were given. They were instructed to brush their teeth twice daily with the supplied toothpaste (Colgate Total ®, Colgate Palmolive, New York, NY, USA) using a soft tooth brush and to avoid using mouthwashes. One root caries lesion was restored with a GIC (ChemFil® Superior, DENTSPLY, Konstanz, Germany) modified with 5% chlorohexidine digluconate (Lab Express International, Fairfield, USA) (GIC-CHX) and another carious lesion on the contralateral side was restored using conventional GIC, which acted as the control sample. The test and control teeth were randomly determined for each participant. A healthy unrestored tooth was sampled for plaque if no other carious lesion was found. Plaque samples were obtained from the control and the test teeth before application of ART and at 1, 3 and 6 months after application. Unstimulated saliva samples were also collected at the same time points. Plaque samples were collected from interproximal sites and around the root caries lesion using small size interdental brushes (TePe™ brushes, Malmö, Sweden). This sample was split in half and one half was used for microbiological analysis, while the other half was stored for future genomic analysis. Carious tissue excavated from the caries lesion was also stored. At the final visit (6 months after placing the restorations), another full examination was performed by a blinded operator to evaluate and record the survival of restorations. This assessment used the modified Ryge’s criteria (Table 1) to evaluate factors such as marginal defects, wear, and the need to replace or repair the restorations (Ryge, 1980).

The preparation of the root surface carious lesions and application of ART followed the WHO ART guidelines (Frencken et al., 1996). Modified GIC was applied to clinically visible root surface caries lesions Figure 3-1, identified using visual and surface texture criteria (Banting et al., 1980; Banting et al., 1985; Banting, 2001) as follows:
1- There was a discrete, well-defined, and discoloured cavitation on the root surface;
2- The explorer entered easily and displayed some resistance to withdrawal;
3- The lesion was located either at the cementum-enamel junction or on the entire root surface.

Figure 3-1. Root caries lesion identified in one of the study participants.

3.3.2 Preparation of GIC-CHX

Glass ionomer cements (GIC), such as Chemfil® Superior (DENTSPLY), are normally prepared by mixing the GIC powder with deionised or distilled water to form a cement. In this study, the modification of GIC was done according to methods described previously by different studies (Kabil et al., 2017; De Castilho et al., 2013). The water was replaced with an aqueous solution of 5% chlorhexidine digluconate (Lab Express International, Fairfield, USA) (CHX) to prepare the modified GIC (GIC-CHX). The glass ionomer cement was mixed, as per the manufacturer’s instructions, in the ratio of 1 scoop of powder to 1 drop of liquid (Figure 3-2). This equates to a powder to liquid ratio (wt/wt) of 7.4:1 which equates to 0.6% (wt/wt) CHX in the cement placed in the lesion. Mixing was done by the dental assistant on a waxed pad at the chair side, and the cement was placed on the excavated root cavity lesion as per standard practice. Chemfil® GIC is available in seven shades, but only the light-yellow shade was used in this study to enable easy assessment of colour changes. Prior to GIC placement, the tooth was conditioned with an aqueous solution of polyacrylic acid. After placement, the restoration was varnished using the supplied varnish (Chemfil Varnish™, DENTSPLY).
3.3.3 ART application

The restoration was placed according to the ART method described by Frencken et al. (1996). First, the tooth was isolated, plaque was removed from the tooth surface with a wet cotton wool pellet and the outer carious dentine was removed with excavators. Any unsupported thin enamel/cementum was broken off with a hatchet to eliminate any carious spots. The cavity was then cleaned with water and dried using dry cotton wool pellets to ensure no plaque or debris were present. The dentine was conditioned using 10% polyacrylic acid (GC Dentin Conditioner™, GC CORPORATION, Tokyo, Japan) for 20 seconds. The cavity was then again washed and gently dried with cotton wool pellets. High-viscosity glass ionomer cement Chemfil® was hand mixed for 15 seconds by mixing 2 scoops with 1 drop of liquid for 5 seconds first, then adding another drop of liquid while mixing for a further 10 seconds. The liquid used was either sterile distilled water or 5% chlorhexidine depending on whether it was a test or control restoration. A small amount of the mixture was inserted into the cavity using a flat plastic instrument or ball burnisher and packed in place ensuring all cavity areas were filled properly. Proprietary varnish (Chemfil Varnish™, DENTSPLY) was applied on the surface of the restoration. Excess material was removed with a carver. Figure 3-3 shows the root caries lesion restored with GIC modified with 5% chlorhexidine.
3.3.4 Operator assessment of the restorations

At baseline, 1 month, 3 months and 6 months after ART application, the survival rate, marginal defects and wear of the restorations were recorded by the clinical operator and by another clinician. Clinical evaluation and assessment were based on the modified criteria proposed by Ryge (1980) to evaluate the integrity of the restorations, its anatomic form, the presence or absence of recurrent caries, marginal adaptation, surface roughness, colour-match and gingival health (Table 1). All participants had pre-treatment and post-treatment photographs taken at baseline and at 6 months.
Table 1. Modified Ryge criteria for clinical evaluation of restorations (Ryge, 1980).

<table>
<thead>
<tr>
<th>Category</th>
<th>Inspection type</th>
<th>Rating scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomic form</td>
<td>Visual inspection with mirror and explorer</td>
<td>0 = The restoration is continuous with the existing anatomic form 1 = Slightly under/over contoured</td>
</tr>
<tr>
<td>Secondary caries</td>
<td>Visual inspection with mirror and explorer</td>
<td>0 = No visible evidence 1* = Visible evidence</td>
</tr>
<tr>
<td>Marginal adaptation</td>
<td>Visual inspection with mirror and explorer</td>
<td>0 = Continuous with existing anatomic form 1 = Explorer catches but no crevice visible 2* = Obvious crevice at margin, dentine or lute exposed</td>
</tr>
<tr>
<td>Surface roughness</td>
<td>Tactile diagnostics with explorer</td>
<td>0 = Smooth 1 = Slightly rough 2 = Rough</td>
</tr>
<tr>
<td>Colour-matching</td>
<td>Visual inspection</td>
<td>0 = Very good/good, almost invisible 1 = Slight mismatch 2* = Obvious/gross mismatch outside of normal range</td>
</tr>
<tr>
<td>Gingival health</td>
<td>Visual inspection with explorer</td>
<td>1= Healthy gingivae 2= Mild inflammation – slight colour change, slight oedema, no bleeding on probing 3 = Moderate inflammation – redness, oedema and glazing, bleeding on probing. 4 = Severe inflammation – marked redness and oedema, tendency to spontaneous bleeding</td>
</tr>
</tbody>
</table>

* Unacceptable

To assess the acceptability of the treatment, participants were given questionnaires immediately after the administration of ART, and at 1, 3 and 6 months. The questions were directed to investigate how participants perceived the ART and how satisfied they were with the appearance of ART restorations. At baseline, the questionnaires were about
the comfortability of the treatment, pain during the procedure, anxiousness compared to conventional restorative methods and the time taken to complete the ART procedure. At the 1, 3, and 6 months review appointments, the questionnaires were about the smoothness of the restored teeth compared to other teeth, presence or absence of pain on the treated teeth, satisfaction with the appearance of the restored teeth and if there was any taste change since the time of placing the restorations.

### 3.4 Results

#### 3.4.1 Recruitment

Recruitment was finished after 14 participants due to a slower than expected rate of recruitment. Only nine participants were eligible to take part in the study. Figure 3-4 shows the number of participants recruited during 9 months, and the CONSORT-style flow of participants diagram is presented in Figure 3-5.

![Figure 3-4](figure.png)  
**Figure 3-4.** Number of recruited participants each month during the study.
3.4.2 Participants characteristics

In total, 18 restorations were placed using both conventional GIC (n=9) and modified GIC (n=9) in nine participants (four men and five women), with a mean age of 67.2 years. All participants were followed for 6 months. Just under half of the participants were over 70 years of age (44.4%, n=4), a third of participants were between the age of 60 and 65 years and the remaining participants were between 50 and 55 years of age (22.2%, n=2). The majority of participants were New Zealanders of European descent (66.7%, n=6), one participant was Māori, one was Middle Eastern and one was South African (11.1%, n=1). Smoking status was also recorded for participants, the number of ex-smokers and non-smokers were even (44.4%, n=4) and only one participant was a current smoker. The majority of the participants brushed their teeth twice a day (66.7%,

**Figure 3-5.** Flow diagram of participants following CONSORT style.
n=6). One participant brushed irregularly, and two participants brushed once a day (22.2%).

### 3.4.3 Participant’s acceptability of ART

The majority of participants (89%, n=8) did not feel anxious during the ART procedure and all participants were not anxious during all subsequent visits (Figure 3-6). The majority of the participants felt no pain during ART procedure (89%, n=8), although one participant reported feeling a little pain. Participants thought that time taken for restoration placement using ART was either less than expected (66.7%, n=6) or as expected (33.3%, n=3). In the first 3 months, all participants were satisfied with their treatment (100%, n=9). At 6 months, two participants recorded neutral for satisfaction with the ART treated teeth (22.2%) (Figure 3-7). The majority of participants (88.9%, n=8) felt no pain in the teeth and although ART was a comfortable procedure, only one participant indicated there was little pain in the ART-treated tooth. The majority of the participants reported that the restorations were smooth after 1 month (77.8%) and after 3 months (83.3%) months. While at 6 months (77.8%) of participants felt the restorations smooth (Figure 3-8). A summary of patients’ perceptions of ART treatment is shown in Table 2.

**Figure 3-6. Anxiousness of participants**
Figure 3-7. Participants’ satisfaction with ART treated teeth at different time points investigated.

Figure 3-8. Participants’ perception of the roughness of the ART treated teeth over 6 months period.
Table 2. Summary of patients’ baseline assessment of acceptability

<table>
<thead>
<tr>
<th>Responses</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>9 (100.0)</td>
</tr>
<tr>
<td><strong>Comfort of ART</strong></td>
<td></td>
</tr>
<tr>
<td>Very comfortable</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>Comfortable</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td><strong>Pain during application of ART</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>A little pain</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td><strong>Anxiety during treatment</strong></td>
<td></td>
</tr>
<tr>
<td>Not at all anxious</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>A little anxious</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td><strong>Anxiety throughout clinical visit</strong></td>
<td></td>
</tr>
<tr>
<td>Not at all anxious</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>A little anxious</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td><strong>Time taken to complete ART procedure</strong></td>
<td></td>
</tr>
<tr>
<td>Less than expected</td>
<td>6 (66.7)</td>
</tr>
<tr>
<td>As expected</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td><strong>Satisfaction with appearance</strong></td>
<td></td>
</tr>
<tr>
<td>Very satisfied</td>
<td>6 (66.7)</td>
</tr>
<tr>
<td>Satisfied</td>
<td>3 (33.3)</td>
</tr>
</tbody>
</table>

3.4.4 Clinical operator assessment

The clinical operator assessment of the appearance and condition of the restorations was done for control and test restorations. The appearance and condition of control GIC restorations were satisfactory at 1 month. At 3 months, the majority of the GIC restorations were considered satisfactory by the operator (89.9%, n=8) and only one was unsatisfactory (11.1%, n=1). However, at 6 months, a third of the GIC restorations were unsatisfactory (33.3%, n=3) Figure 3-9. Similar results for GIC-CHX at 1 and 3 months, however at 6 months the number of satisfactory restorations stayed the same as 3 months with only one GIC-CHX being unsatisfactory (11.1%, n=1) Figure 3-10.
**Figure 3-9.** Operator’s satisfaction with GIC restorations over the 6-month period

**Figure 3-10.** Operator’s satisfaction with GIC-CHX restorations over the 6-month period
3.4.5 Operator’s assessment of ART restorations

The anatomic form of both control and test restorations were assessed according to the modified Ryge criteria (Ryge, 1980). At the 6-month examination, 83.3% of the GIC-CHX restorations (n=8) were continuous with the anatomy whereas (44.4%, n=4) of the GIC restorations were either under or over-contoured Figure 3-11. No secondary caries was detected for either of the two types of restorations.

![Figure 3-11](image)

**Figure 3-11.** The anatomic form for the GIC-CHX and GIC restorations during time period investigated.

At baseline, all restorations were well adapted with no explorer catches around the margins, with exception of one control restoration. At the one-month review, most of GIC-CHX restorations (66.7%, n=6) were continuous, while the majority of the GIC restorations had a catchy margin with no obvious crevice. At three months, the majority of control restorations (66.7%, n=6) had a margin catch, and both test and control restorations had one restoration with an obvious crevice. At 6 months, most of the test restorations (55.6%, n=5) were still well-adapted with only one restoration (11.1%) with defective margins, while most test restorations had either margins catches (44.4%) or an obvious crevice (33.3%) Figure 3-12.
Figure 3-12. Marginal adaptation of the restorations at different time periods.

The clinical operator assessed the surface roughness of the restorations according to modified Ryge criteria. At baseline, the majority of GIC-CHX and GIC restorations were smooth (77.8%, n=7 and 88.9%, n=8 respectively), while at 1 month the majority of both test and control restorations were slightly rough (77.8%, n=7) and one control restoration was scored as very rough (11.1%). At 3 months, (44.4%, n=4) of the test GIC-CHX restorations were smooth and (55.6%, n=5) were slightly rough. As for control GIC restorations, the majority (88.9%, n=8) were slightly rough. At 6 months, the majority of test GIC-CHX restorations (66.7%, n=6) were considered smooth, while the majority of control restorations (88.9%) were either slightly rough or very rough Figure 3-13.
Figure 3.13. Assessment of surface roughness of the ART restorations.

The health of the gingiva near the restorations was assessed and the result is depicted in Figure 3-14. At baseline, the majority of GIC-CHX restorations had either healthy gingiva (44.4%, n=4) or mild inflammation (44.4%, n=4) and only one (11.1%) had moderate inflammation. As for GIC restorations, 55.6%, n=5 had healthy gingivae, two restorations were associated with mildly inflamed gingiva and one was rated either moderately inflamed or severely inflamed (11.1%). At 1 month, all GIC-CHX restorations the gingiva was rated either healthy (44.4%, n=4) or mildly inflamed (55.6%, n=5). As for the control GIC restorations, two were associated with moderately inflamed gingiva (22.2%), three mildly inflamed (33.3%) and 44.4% had healthy gingiva. At 3 months, more restorations had healthy gingiva (66.7%, n=6) while only two had mildly-inflamed gingiva (22.2%) and one had moderately inflamed gingiva (11.1%). Most of GIC restorations (66.7%, n=6) had mildly inflamed gingiva and the remaining restorations (44.4%, n=3) had healthy gingiva. At 6 months, overall gingival healthy deteriorated for both types of restorations. Third of GIC-CHX restorations had either moderately inflamed gingiva, mildly inflamed or healthy gingiva. The majority of GIC restorations had moderately inflamed (44.4, n=4) or mildly inflamed (44.4%, n=4) and only one had healthy gingiva (11.1%) Figure 3-14.
Figure 3-14. Assessment of gingival health around the treated teeth.

3.4.6 Survival of restorations

Two different operators assessed the restorations at 6 months and the survival of the restorations was based on the assessment of the blinded operator. The survival rates for control GIC and GIC-CHX restorations were 66.7% and 88.9% respectively, and the main reason for failure was gross marginal defects. However, no statistically significant differences in the survival rates were found between the two different types of restorations (P value = 0.302 Long-rank (Mantel-Cox) test Chi square 1.07) Figure 3-15.

Figure 3-15. Survival of control and test restorations over time
3.5 Discussion

This study investigated the clinical effectiveness and patient acceptability of a modified glass ionomer cement to treat root caries and placed using the atraumatic restorative treatment (ART) approach.

The slower than expected recruitment of participants has resulted in total recruitment of 14 participants with nine of those being eligible for the inclusion in the study. Different reasons resulted in the exclusion of the other five participants and these were detailed in Figure 3-5. The strict inclusion and exclusion criteria set out by the protocol limited the number of eligible participants severely. The protocol followed a previous study from a different centre with the intention of creating a multicentre study. This has caused the research team to amend the ethics and approval was obtained to lower the age of eligible participants to 50 from 60 years of age. This initially has helped in further recruitment, but this was slow as well. Although there is a great number of patients registered in the Faculty of Dentistry, they are usually seen by different departments for different reasons. The author was actively involved in recruiting participants in the Oral Health clinic by collaborating with oral therapists and their clinical tutors and by providing recruitment information detailing inclusion and exclusion criteria. Moreover, the Department of Oral Rehabilitation was another source for participants. There is usually a window in the beginning of the year when patients are screened before the construction of removable prostheses and those who require stabilisation and restorations are usually treated by the students in order to commence their treatment. Most of the clinical tutors were approached directly or by email before the screening and assessment of patients commenced to identify eligible participants and informing the author. This process, although time consuming, was the only effective method in obtaining most of the participants in this study. The Urgent Care clinics of the Faculty of Dentistry was another potential source of participants however, even with continuous active recruitment, no participants were included from the clinic. In future studies, possibly obtaining ethics to be able to approach potential participants in rest homes may solve this problem of slow recruitment.

The majority of the patients in this study found the ART procedure to be comfortable. This is in agreement with previous studies (Frencken and Holmgren, 1999;
Honkala and Honkala, 2002) which confirms that ART is a pain-free procedure. Because ART requires only hand-held instruments to remove caries and prepare the cavity for restorations, usually no local anaesthesia is required. In a previous study, a group of children were treated using the ART, only a few participants required local anaesthesia (Frencken et al., 1998), while in a more recent study local anaesthetic was not required and patients did not exhibit pain after undergoing the ART (Honkala and Honkala, 2002). The fact that ART is considered largely to be a painless simple procedure it could potentially be used by other oral health professionals such as oral therapists.

In this study, 89% (n= 8) of the participants were not anxious at all during the ART procedure; one participant reported being slightly anxious and during the subsequent appointments, all participants were not anxious. This could be due to the fact that patients were aware that the procedure required only hand instruments with no drilling or local anaesthesia required. This makes the ART a potentially suitable treatment modality for dentally-anxious patients (Frencken et al., 1998). A study conducted in South Africa involving outpatients attending public oral health clinics tested whether using ART would result in less dental anxiety compared to conventional restoration placement (Mickenautsch et al., 2007). The study implemented the Short Form of the Dental Subscale of the Children’s Fear Survey Schedule (CFSS-SF) and Corah’s Dental Anxiety Scale (DAS) to determine the level of anxiety of participants after application of ART over conventional methods. They found that the mean CFSS-SF for the ART group was significantly lower than the conventional group and the mean DAS score for ART was also lower than the conventional group. However, no association was found between level of dental anxiety and number of extracted vs restored teeth. Therefore, it was concluded that ART resulted in less dental anxiety for patients (Mickenautsch et al., 2007).

In this study, participant satisfaction with ART was also investigated and all participants were satisfied with the treatment at baseline and at all subsequent appointments. A previous study conducted in Zimbabwe in 1996, showed that 95% of secondary school students who had never received any dental restorations previously were satisfied with the ART procedure and the restorations placed (Frencken et al., 1996). Small and medium size cavities were found to be very easy to restore with ART;
however, large cavities required extensive excavation by the operator, which might take longer than the conventional method using rotary instruments.

The majority of the patients reported that the ART procedure took less time than expected. This seems to be in agreement with a previous randomised control trial which investigated the cost-effectiveness of ART restorations in older adults patients (da Mata et al., 2014). The study found that ART took less time compared to conventional methods. The fact that no local anaesthesia was required, and only simple hand-held instrumentation was required, could be likely reasons why ART was less time-consuming. With the required training, ART may be used also by other oral health professionals such as oral health therapists. The ART would have a cost reduction effect due to the difference in labour cost among other operational costs. Ultimately, this would benefit patients by providing cost-effective treatment.

The cost-effectiveness of ART versus conventional restorative methods was assessed in a randomised clinical trial involving 82 adult patients in Ireland (da Mata et al., 2014). The study found that the ART was more cost-effective compared to conventional restorative treatments where the average cost for the ART and conventional restorations were €16.86 and €28.71 respectively, resulting in a cost-effective ratio of 0.18 and 0.29. In addition, they predicted that if a properly-trained dental hygienist was employed to provide ART the cost-effective ratio will be reduced to 0.14 (da Mata et al., 2014). However, it was stated that this was not possible in Ireland because of their current dental hygiene curriculum which does not allow dental hygienists to perform restorative procedures. In New Zealand, the current scope of practice set out by the Dental Council states that dental therapists are only able to treat children and adolescents up to the age of 18 years (Dental Council of New Zealand, 2011). The oral health therapy scope of practice introduced recently by the Dental Council allows oral health therapists to treat patients of any age but provision of restorative care is limited to patients under the age of 18 years. This situation might change in the near future if these scopes of practice are amended to allow oral health therapists to treat patients 18 years and over, and for oral health therapists to provide restorative care for the same group.

There was no perceived change in taste for patients who received the GIC-CHX restorations. This indicates that small amounts of chlorhexidine present may not have
significant effect on taste, even though it is known to have such effects when used as a mouthwash (Lang et al., 1988). Moreover it is known that chlorhexidine will form insoluble salts when bound to GIC therefore reducing the effect on taste (Sanders et al., 2002). From the results, the setting time of the modified GIC restoration was similar or quicker than the conventional GIC restorations, which indicated that modification with chlorhexidine does not affect the setting time. A previous study found that altering the powder to liquid ratio affects the setting time of GIC restorations, and a high powder to liquid ratio of GIC was found to shorten the setting time of GIC (Crisp et al., 1976). However, this was not observed in this study as the powder to liquid ratio was always standardised.

In the present study, restorations using GIC-CHX were compared to control (unmodified) GIC restorations at baseline, 1, 3 and 6 months according to the modified Ryge Criteria (Ryge, 1980). At 6 months, two independent operators assessed the restorations. One was blinded and the other was the same operator who placed the restorations. At the 6-month examination, marginal adaptation was also assessed. Survival of the restorations was based on the assessment of the blinded operator to minimise operator bias. According to the modified Ryge Criteria, the presence of an obvious crevice at the margin of restorations is considered a unacceptable. Therefore, any restoration with such a characteristic was considered a failed restoration. In this study, the survival rates of GIC-CHX and GIC restorations were 88.9% and 66.7%, respectively. Among the reasons for this difference could be the size and location of root caries lesions, since they were not identical in all patients, with some of the GIC lesions located slightly subgingivally making access more difficult. Another possible reason for this difference could be that GIC-CHX restorations eliminated the remaining bacteria in the cavity, preventing the development of secondary caries. A study by Lo et al. (2006) showed that secondary caries was one of the main reasons for the failure of ART restorations when conventional GIC was used. Secondary caries usually develops from the residual caries left in the prepared cavity, since the ART treatment may result in carious tissue being left behind in the cavity (Lo et al., 2006). Although fluoride-containing restorations such as GIC are known to have a cariostatic effect, it is not known whether the level of fluoride release was sufficient to inhibit demineralisation (Pereira et al., 1998). However, the incorporation of antibacterial agent in GIC may eliminate the remaining bacteria in the cavity, preventing the development of secondary caries. De
Castilho et al. (2013) showed that the incorporation of chlorhexidine in resin-modified GIC eliminated all bacteria in the cavity when tested by re-entry into the cavity after 3 months (De Castilho et al., 2013). Another study, showed much reduced count of caries associated bacterial in dentine samples treated with GIC modified with chlorhexidine diacetate after 7 days (Takahashi et al., 2006). In this study, there was no secondary caries with either GIC-CHX or control GIC. This could be related to the anti-caries properties of GIC, as the fluoride released from GIC replaces the calcium ions in hydroxyapatite, generating fluorapatite which is more resistant to mineral dissolution in acid and also enhances remineralisation (Featherstone, 1999). As patients were only followed up for 6 months, the long-term survival of the restorations is unknown.

The perceived surface roughness for both types of restorations were also assessed. At 6 months, 83.3% of GIC-CHX restorations were noted as smooth compared to only 11.1% for GIC restorations. This could be due to faster wear of GIC-CHX in comparison to GIC which makes the rough finish to GIC-CHX wear faster and creates a smoother surface. Marti and co-workers showed that the hardness of GIC decreased when chlorhexidine was added, which resulted in accelerated wear of the material (Marti et al., 2014). Therefore, GIC-CHX restorations should not be placed on occlusal surfaces where the occlusal loading is expected to be high.

In summary, the 6-months GIC-CHX survival was slightly better than GIC restorations. This could possibly be attributed to the effect of CHX on the remaining bacteria in the cavity. It was also shown that ART has a high acceptance by participants and the operator found it to be an easy and fast way to restore carious root lesions. ART could potentially be a cost-effective treatment modality specially if other oral health professionals are trained to perform it. This would also be possible if the current dental therapists scope of practice in New Zealand is amended to allow provision of restorative treatment for people over the age of 18 years.
4 Microbiological analysis of plaque and saliva samples

4.1 Introduction and background

Older people experience a number of oral diseases, as well as high susceptibility to chronic and systemic illnesses. Poor oral health such as tooth caries, tooth loss and severe periodontal disease, has a significant negative effect on the quality of life of older adults and is, therefore, an important public health issue (Petersen and Yamamoto, 2005). Dental caries is the most prevalent chronic disease in the world and indeed in New Zealand, affecting all age groups including the older population (Foster Page and Thomson, 2012). It is a complex multifactorial disease caused by the interaction of biological, behavioural, and social factors (MacEntee et al., 1993).

Several conditions are considered major risk factors in the development of dental caries in older people. These include loss of periodontal attachment (Gilbert et al., 2001), reduced salivary flow rates in cases of dry mouth (Saunders and Handelman, 1992), use of removable partial dentures instead of simple fixed prosthodontics (Jepson et al., 2001), cognitive decline as a result of Alzheimer’s disease (Avlund et al., 2004), medical conditions such as stroke (Maupomé et al., 2002), use of medications such as used to treat asthma (Thomson et al., 2002), lack of manual dexterity (Curzon and Preston, 2004) and difficulty in comprehending oral care instructions (Curzon and Preston, 2004).

Retaining natural teeth into old age has wide benefits for both oral and general health, resulting in improved diet, nutrition, self-esteem and quality of life. However, these benefits can only be realised if oral health is preserved. One of the major dental conditions affecting older people is the high prevalence of root surface caries (Hugoson et al., 2000; Fure, 2004; Imazato et al., 2006; Vilstrup et al., 2007; Vieira and Gati, 2011) which, if untreated, can lead to tooth loss (Slade et al., 1996).

Atraumatic Restorative Treatment (ART) has been used in caries management for over two decades. ART has significant advantages compared to conventional restorative techniques with regard to surgical removal of caries: it requires minimal intervention and minimal cavity preparation, is pain-free since there is no use of electrical drills or anaesthetics, it has high restoration survival rates and is very cost-effective.
(Frencken and Holmgren, 1999; Mjör and Gordan, 1999; Yip and Smales, 2002). ART relies mostly on hand instruments for opening the cavity and removing soft carious tissues, and for filling the cavity with adhesive restorative materials.

Glass ionomer cements (GIC) have long been used as restorative materials for sealing pits and fissures in teeth and are regarded as a cost-effective approach to prevent caries development. To improve its antimicrobial activity, agents with bactericidal activity have been incorporated into GIC. Among these agents, the addition of chlorhexidine has showed a significant increase in antimicrobial activity with minimal effects on the mechanical properties of GIC (Jedrychowski et al., 1983; Takahashi et al., 2006). Previous studies at the University of Leeds have demonstrated enhanced activity against cariogenic bacteria grown in biofilm culture of fluoride-releasing GIC modified with 10% chlorhexidine digluconate (Millett et al., 2005). This modified GIC was more effective in delivering a greater and longer lasting anti-biofilm activity compared to conventional GIC, with no adverse effects on setting time or physical and bonding properties of the material (Millett et al., 2005).

The benefits of using ART with conventional GIC for the management of root caries among older adults was demonstrated in Finnish and Chinese patients (Honkala and Honkala, 2002; Lo et al., 2006), with high survival rates of root restorations and overall patients’ satisfaction with ART. However, limited data are available on the clinical effectiveness of ART using chlorhexidine digluconate-modified GIC in populations with high caries risk and with high Decayed, Missing, Filled Teeth (DMFT) scores.

4.1.1 Modification of GIC with added chlorhexidine

Much research has been conducted to improve the antimicrobial properties of restorative materials. Jedrychowski et al. (1983) tested the addition of two antibacterial compounds, chlorhexidine gluconate and chlorhexidine dihydrochloride to composite resin and glass ionomer restorative materials in different concentrations. The antibacterial effect was tested in microorganisms commonly found in the oral cavity such as S. mutans and Lactobacillus acidophilus (Jedrychowski et al., 1983). It was found that chlorhexidine gluconate demonstrated significantly greater inhibition than chlorhexidine.
dihydrochloride. The study also tested the effect of addition of chlorhexidine on the mechanical properties of GICs. It was found that addition of 5% chlorhexidine gluconate altered the adhesive shear strength values but there was a significant increase in compressive strength values (Jedrychowski et al., 1983). A more recent in vitro study found that the addition of 0.5% chlorhexidine digluconate to GIC resulted in increased antimicrobial properties with no significant effect on the mechanical properties or setting time. However, higher concentrations of chlorhexidine digluconate (e.g. 1%-2%) increased the setting time and decreased the mechanical properties of the GIC (Marti et al., 2014).

Chlorhexidine diacetate in a powder form had been used to modify GIC in order to improve the antimicrobial properties of the restorative material. Takahashi et al. (2006) tested different concentrations and found that the addition of 1% of chlorhexidine diacetate improved the antimicrobial properties of GIC without significantly affecting the mechanical properties, bonding abilities or setting time. However, higher concentrations of chlorhexidine diacetate (more than 2%) had a significant detrimental effect on the compressive strength of GIC (Takahashi et al., 2006).

Moreover, Ribeiro and Ericson (1991) and Hoszek and Ericson (2008) have investigated the effect of adding chlorhexidine to GIC restorative material and luting cements in two different forms: chlorhexidine digluconate and chlorhexidine diacetate. It was found that the addition of two variants of chlorhexidine had an inhibitory effect on Streptococcus mutans and this effect was dose-dependent (Ribeiro and Ericson, 1991; Hoszek and Ericson, 2008).

The increased antimicrobial activity of GIC modified with chlorhexidine has been tested not only in vitro but also in vivo. A study in a mobile dental clinic as part of the Division of Public Oral Health at the University of Johannesburg, South Africa, investigated whether chlorhexidine diacetate 1% modified glass ionomer was more effective than conventional glass ionomer in inhibiting the growth of microorganisms left in infected and affected dentine under a restoration (Frencken et al., 2007). The majority of participants in this study were between the ages of 6-11 years and majority of the restored teeth were permanent molars with at least one large occlusal cavity. A statistically significant difference in the reduction of microorganisms such as S. mutans
and lactobacilli in infected and affected dentine samples was reported with chlorhexidine-containing GICs compared to the conventional GIC over a 7-day period (Frencken et al., 2007). In another in vivo study, comparable results were found with regard to microleakage when GIC modified with 1% chlorhexidine diacetate was evaluated in the primary dentition. The authors concluded that GIC modified with chlorhexidine could be a useful alternative in clinical use, in particular when ART is used (Mathew et al., 2013). In another study, the antibacterial and micromechanical properties of GIC following the addition of 10% and 18% chlorhexidine digluconate were investigated (Farret et al., 2011). It was found that both concentrations increased antimicrobial properties against the tested strains of S. mutans and the zone of inhibition was larger with higher concentrations. On the other hand, both concentrations of chlorhexidine had little influence on the physical properties of GIC, such as diametral tensile strength, compressive strength and shear bond strength (Farret et al., 2011).

From the literature, it is clear that chlorhexidine digluconate had greater inhibition zones specially for S. mutans and lactobacilli, and this effect was concentration-dependent (Farret et al., 2011). As the concentration of the chlorhexidine digluconate increased, there was an increase in the inhibition zone. Several studies have clearly shown this phenomenon (Hoszek and Ericson, 2008; De Castilho et al., 2013). On the other hand, the inhibition zones of chlorhexidine diacetate (which is in powder form) is not concentration-dependent (Takahashi et al., 2006). It also appears that chlorhexidine digluconate had a lesser effect on the mechanical properties of high-viscosity glass ionomer cement when compared to other forms, which has been reported in several studies (Millett et al., 2005; Farret et al., 2011).

This study investigated the clinical effectiveness and antimicrobial properties of GIC modified with chlorhexidine digluconate (GIC-CHX) in root caries management of older patients attending the University of Otago dental clinics.
4.2 Materials and Methods:

Following ethical approval (approval number 16/CEN/174).) and once the participants were recruited and root caries lesions were identified, plaque samples were collected before the placement of restorations for both test and control teeth. Saliva samples were also collected at baseline. All samples were then transferred to the microbiology laboratory for analysis of bacterial viability in tested micro-organisms. This procedure was repeated at 1, 3 and 6 months to test for the effect of GIC-CHX compared to GIC on the microbial count.
Figure 4-1. Flow diagram of the microbiological study
4.2.1 Statistical analysis

Mean count of cariogenic bacteria from the test (GIC-CHX) and control (GIC) plaque samples were compared at baseline, 1, 3 and 6 months post-treatment. Statistical analysis was performed using Prism version 7.00 for Windows (GraphPad Software, La Jolla California, USA). Bar graphs were reported as the mean ± standard error of the mean (S.E.M). Statistically significant differences were determined using one-way analysis of variance (ANOVA). If differences were detected, multiple comparisons were made using Tukey’s multiple comparison tests at a confidence level of 95% (P<0.05).

4.2.2 Sampling for microbiological analysis

4.2.2.1 Procedure for collection and processing of supra-gingival plaque samples

Prior to sampling, a sterile 1 ml microfuge tube was pre-labelled with a unique patient identification code, the corresponding month of the study (0, 1, 3 or 6), the specimen type (GIC-CHX = A, GIC = B, Saliva = D), and date of collection. All sample details were recorded in electronic and paper formats. Each plaque sample was collected using sterile interdental brushes (Figure 4-2). The head of the interdental brush was then cut (Figure 4-53) and placed into the sterile Eppendorf tube (Figure 4-34) containing 1 ml sterile reduced transport fluid (RTF) (Appendix 1). The microfuge tube containing RTF was weighed using a digital balance (model number ENTRIS623-1S, Sartorius Lab Instruments GmbH & Co. KG, Goettingen, Germany) (Figure 4-5) before and after placement of the head of interdental brush containing plaque samples so as the weight of the plaque could be determined. The above procedure was conducted aseptically to reduce the possibility of contaminating the dental plaque sample by the operator. The microfuge tubes were kept on ice during sample collection and transport. After collection, the samples were transferred to the Molecular Biosciences Laboratory (University of Otago, Faculty of Dentistry).

To identify the weight of the TePe™ brush head First the Eppendorf tube was weighted and was found to be 1.484g. The TePe brush head was cut off at the end of the bristles area to obtain a standard cut as much as possible. See Figure 4-3. The brush head was cut directly inside the Eppendorf tube without touching the tip and the
Eppendorf tube was closed immediately. Eppendorf tube and TePe brush head weighted to obtain the total weight and was found to be 1.491g. The weight of the TePe brush was found by subtracting the weight of the Eppendorf tube from the total weight and was found to be 0.007g. This process was repeated few times and the weight of the brush head was consistently 0.007g.

4.2.2.2 Formula to determine the weight of sampled plaque

The following formula was used to calculate the weight of plaque ($W_p$) which was in the calculation of colony forming units per mg of plaque.

$$W_p = \{(W_t - W_e) - 7mg\}$$

$W_t$ = Total weight of microfuge tube containing RTF and brush head containing plaque sample

$W_e$ = Weight of microfuge tube and RTF only
7 mg = Weight of TePe® brush head determined previously

$W_p$ = Weight of plaque sample
4.2.2.3 Formula to calculate CFU/mg for plaque samples:

The colony-forming units (CFU) per milligram of plaque samples (CFU/mg) taken from both test and control teeth were calculated according to the following formula:

$$\frac{CFU}{mg} = \frac{(N_c \times DF \times V_t)}{(V_c \times W_p)}$$

$N_c =$ Number of colonies counted for each plate
$DF =$ Dilution factor
$V_c =$ Volume of culture plate (0.1 ml)
$V_t =$ Total volume (1 ml)
$W_p =$ Weight of plaque

4.2.2.4 Formula to calculate CFU/ml of saliva suspension:

$$\frac{CFU}{ml} = \frac{(N_c \times DF)}{V_c}$$

$N_c =$ Number of colonies
$DF =$ Dilution factor
$V_c =$ Volume of culture plate (0.1 ml)
4.2.2.5 Serial dilution procedure

A serial dilution is the stepwise dilution of a substance in solution. Twelve tenfold dilutions were performed; each dilution is 1/10th the concentration of the previous dilution. The first 10-fold dilution is termed the $10^{-1}$ dilution. Each subsequent dilution was made from the previous dilution. Prior to the experiment, 900 µl sterile RTF was aliquoted into each sterile microfuge tube. The selective media (Fort Richard Laboratories Ltd, Auckland, New Zealand) (Mutans selective agar, Rogosa agar and Sabouraud agar) were kept refrigerated until the day of the experiment and pre-equilibrated to ambient temperature (22°C) on the bench 30 minutes before the experiment. Serial dilutions were made by vortexing the neat sample in order to remove and disperse microorganisms evenly into the solution. Then transferring 100 µl of the neat sample to a microfuge tube labelled $10^{-1}$ containing 0.9ml sterile RTF as described previously. This tube is then inverted few times to mix the contents evenly before 0.1ml is transferred to the next tube labelled $10^{-2}$. This process was repeated until the twelve ten-fold dilution was reached.

After the dilutions were completed, 100 µl of the samples were plated onto the corresponding plates in duplicates. Then the samples were spread using glass spreaders flame-sterilised with 99% (vol/vol) ethanol. The plates were then incubated in aerobic or anaerobic chambers as shown in Table 3.

Table 3. Recommended incubation time

<table>
<thead>
<tr>
<th>Media</th>
<th>Anaerobic/Aerobic</th>
<th>Temperature</th>
<th>Incubation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columbia Blood Agar (CBA) (total bacterial count)</td>
<td>Anaerobic</td>
<td>37 °C</td>
<td>2-3 days</td>
</tr>
<tr>
<td>Rogosa agar (Lactobacilli)</td>
<td>Anaerobic</td>
<td>37 °C</td>
<td>2-3 days</td>
</tr>
<tr>
<td>Sabouraud agar (fungi, e.g. <em>Candida</em>)</td>
<td>Aerobic</td>
<td>30 °C</td>
<td>3-5 days</td>
</tr>
<tr>
<td>Mutans Selective Agar (MSA; mutans streptococci, e.g. <em>Streptococcus mutans</em>)</td>
<td>Anaerobic</td>
<td>37 °C</td>
<td>2-3 days</td>
</tr>
</tbody>
</table>
All plates were checked for growth after 2 to 3 days of incubation. However, some required re-incubation for a total of 3 days to obtain full microbial growth. Columbia blood agar (CBA: Columbia agar base + 5% sheep blood) plates were used as non-selective medium for the enumeration of total viable bacteria under anaerobic conditions. The selective media used were: Rogosa agar (RA) for lactobacilli, Sabouraud agar (SA) for *Candida* and Mutans Selective Agar (MSA) for mutans streptococci (Figure 4-6).

![Figure 4-6. Representative plates showing the different agar types; (A) Rogosa medium for lactobacilli, (B) Columbia Blood Agar for total anaerobic bacteria, (C) Sabouraud medium for yeast and (D) Mutans Selective Agar for mutans streptoccoci.](image)

### 4.2.2.6 Procedure for collection and processing of saliva samples

Due to low salivary flow in most participants, 10 ml of sterile distilled water (Baxter, Old Toongabbie, NSW, Australia) was given to participants to rinse with and then expectorate the contents back into the container. The collection of saliva was done prior to ART application at the baseline visit and, subsequently, at the 1-, 3-, and 6-month visits after the ART application. Collected samples were transferred to the Molecular Biosciences Laboratory for analysis. Samples were serially-diluted and inoculated onto non-selective and selective media as described for plaque samples in section 4.2.2.5.
4.3 Results

In total, 18 restorations (GIC= 9; GIC-CHX =9), were placed in nine participants. Plaque samples were collected from around all 18 restorations (cervical and interproximal regions) at baseline, and after 1, 3 and 6 months to test for local effect of GIC-CHX on microorganisms present in plaque samples of the two different restorative materials. Saliva samples were also collected to test the general effect on microorganisms present in saliva.

4.3.1 Microbiological analysis of plaque samples:

The following graphs (Figure 4-7 to Figure 4-10) show the mean counts of the tested cariogenic microorganisms for plaque samples taken from around the cervical margins and interproximal areas of GIC and GIC-CHX restorations at each point time investigated.

After 1-month the mean counts of total viable anaerobic bacteria in both the GIC and GIC-CHX samples tended to be lower although these differences were not statistically significant ($P>0.05$) (Figure 4-7). At 3 months, both levels of total viable bacteria returned to levels similar to baseline, while at 6 months there was a slight drop in the total bacteria, with a greater reduction for the GIC-CHX restorations.
Figure 4-7. The mean count of total anaerobic total bacteria number (Log10 (CFU/mg)) for plaque taken from both GIC-CHX and GIC restorations at each time period investigated.
Figure 4-8 represents the mean counts of *Candida* in plaque samples for both types of restorations. The mean counts of *Candida* at 1 and 3 months for plaque taken from the control GIC restorations tended to be lower, although these differences were not statistically significant. However, for the test GIC-CHX restorations, the mean number of fungal colonies remained the same. At 6 months, the levels of *Candida* increased in plaque samples for both types of restorations.

![Graph showing mean count of Candida](image)

**Figure 4-8.** Log$_{10}$ (CFU/mg) of Candida found in plaque samples taken from both GIC-CHX and GIC.
The mean counts of lactobacilli in plaque samples for both types of restorations is represented in Figure 4-9. At 1 month, the mean lactobacillus count was slightly lowered in plaque samples collected from around GIC-CHX restorations; however, at 3 months, the level of lactobacilli returned to the baseline level. At 6 months, levels of lactobacilli were slightly higher than baseline levels. However, these results were not statistically significant ($P>0.05$).

![Mean count of lactobacilli](image)

**Figure 4-9.** The mean count ($\log_{10}$ (CFU/mg)) of lactobacilli found in samples of plaque taken from both GIC-CHX and GIC restorations.
At 1 month the mean counts of *S. mutans* in both the GIC and the GIC-CHX samples tended to be lower although these differences were not statistically significant (*P > 0.05*) after analysing the data using the one-way ANOVA test.

**Figure 4-10.** Log$_{10}$ (CFU/mg) of *Streptococcus mutans* found in plaque taken from both GIC-CHX and GIC restorations.

### 4.3.2 Microbiological analysis of saliva samples:

Saliva samples were also collected for microbiological analysis, to measure the general effect of GIC-CHX in the oral cavity. The same microorganisms sampled in plaque samples were investigated. The results are demonstrated in the following graphs (Figure 4-11 to Figure 4-14).

The level of total anaerobic bacteria in saliva samples was similar at all time points investigated, with no significant differences at 1, 3 or 6 months when compared to baseline (Figure 4-11).
Figure 4-11. Log$_{10}$ (CFU/ml) of total viable anaerobic bacteria per ml of saliva suspension at each time point investigated.

After 1, 3 and 6 months the mean count of *Candida* colonies in saliva samples tended to be lower although these differences were not statistically significant ($P > 0.05$) (Figure 4-12).

Figure 4-12. Log$_{10}$ (CFU/ml) of *Candida* per ml of saliva suspension
The mean count of lactobacilli enumerated in saliva is shown in Figure 4-13. At 1 and 3 months post-treatment, the mean count was similar to baseline level. At 6 months the mean count of lactobacillus colonies tended to be lower, although this result was not statistically significant ($P > 0.05$).

![Figure 4-13. Log$_{10}$ (CFU/ml) of lactobacilli per ml of saliva suspension at each time interval.](image)

The number of *Streptococcus mutans* colonies remained similar at baseline, 1 and 3 months. At 6 months, the mean count tended to be lower although not statistically significant ($P > 0.05$) (Figure 4-14).

![Figure 4-14. Log$_{10}$ (CFU/ml) of *Streptococcus mutans* per ml of saliva suspension at each time interval.](image)
4.4 Discussion

Dental caries is the most common chronic disease in the world (Frencken et al., 2012). The population is growing older and more people are retaining their natural teeth late in life (Du et al., 2009). Older people are more likely to report xerostomia and have salivary gland hypofunction; reduced resting salivary flow rates, increases the risk of caries significantly (Percival et al., 1994). There are many other factors which increase the risk of caries as we age. For example, poor oral hygiene, attachment loss, cognitive decline, medications, presence of removable partial dentures and lack of manual dexterity have all been implicated in increasing the risk of caries (Saunders and Meyerowitz, 2005). Many restorative approaches can be undertaken in the treatment of carious lesions. Minimal invasive dentistry is based on minimising structural tooth loss during the restorative process (Walsh and Brostek, 2013). However, due to the minimal preparation nature of the procedure, carious tissues and (possibly) viable microorganisms are likely to remain in the carious lesion. These remaining microorganisms may initiate secondary caries. Conventional restorations often lack the ability to eliminate root caries-causing microorganisms (De Castilho et al., 2013). Previous studies have shown that oral microorganisms are susceptible to chlorhexidine (Emilson, 1977) therefore, the incorporation of antimicrobial agents such as chlorhexidine into restorative materials is a key step in order to improve their efficacy (Palmer et al., 2004). Different types of chlorhexidine, mainly chlorhexidine diacetate in powder form and chlorhexidine digluconate in liquid form, and in various concentrations (1% to 18%), have been added to GIC in order to increase their antimicrobial efficacy (Jedrychowski et al., 1983; Millett et al., 2005; Takahashi et al., 2006; Frencken, Imazato, et al., 2007; Turkun et al., 2008; Hoszek and Ericson, 2008; Farret et al., 2011). The main aim of this study was to investigate the efficacy of glass ionomer cement modified with 5% chlorhexidine which was found to increase antimicrobial properties of the GIC without a negative effect on the physical properties (Jedrychowski et al., 1983).

When comparing the mean count of the total anaerobic bacteria in plaque samples obtained from around the unmodified GIC (control) and GIC-CHX (test) restorations after 1 month from the time of placement, there was slight decrease in the mean count of the total anaerobic bacteria compared to baseline. Although there was a difference observed between control and test restorations, this difference was not statistically
significant \((P > 0.05)\). The slight reduction in potentially cariogenic bacteria (lactobacilli and mutans streptococci) after 1 month could be due the improvement of participants’ oral hygiene habits by brushing more effectively following the instructions given at the time of the initial consultation, thus, reducing the plaque accumulation and number of cariogenic bacteria. Another possible reason could be related to the elimination of carious dentine during the ART application and changing of the environment that was harbouring these bacteria before application of the ART. Furthermore, the potential antibacterial effect of fluoride release in GIC restorations should not be ignored (Palenik et al., 1992). However, some studies have suggested that the concentration of released fluoride is not high enough to promote significant antibacterial effects in GIC restorations (Takahashi et al., 2006).

As for plaque samples taken from around the GIC-CHX restorations, the total anaerobic bacterial count was expected to be lower when compared to plaque taken from control GIC restorations. However, this was not the case for all tested microorganisms. This study sampled nine participants, and further differences between the two types of restorations might have been demonstrated with a larger sample size. Moreover, it is possible that the amount of chlorhexidine used might not have been sufficient to create an effective antimicrobial effect. Another important factor is that participants were using tooth paste containing sodium lauryl sulphate (SLS), which has been suggested to counteract the effects of chlorhexidine (Barkvoll et al., 1989). Chlorhexidine digluconate is a cation which can form salts easily with anions such as phosphate, sulphate and SLS. Therefore, SLS may counteract the effect of chlorhexidine by forming insoluble salts leading to reduction in antimicrobial efficacy. This is the reason why a minimum period of 30 minutes or more is required between tooth brushing and chlorhexidine mouthwash rinsing (Barkvoll et al., 1989).

In this study, microbial count did not decrease significantly in plaque samples around the GIC-CHX restorations. Previous studies have shown that incorporation of chlorhexidine in restorative materials increased the antibacterial effect for up to 90 days (Palmer et al., 2004; Takahashi et al., 2006; Hoszek and Ericson, 2008; Turkun et al., 2008). However, these were \textit{in vitro} studies, which could explain why the same effect was not observed in this clinical study. Studies performed in the oral environment are different in many ways when compared to laboratory-based studies. For example, saliva
is produced and secreted continuously, and humans are estimated to swallow saliva about 585 times on average per day (Lear et al., 1965). Given the fact that only a small amount of CHX was added to the GIC, the high clearance rate of saliva could have reduced its effect significantly. In addition, fluid intake might have diluted the effect of chlorhexidine on the surface of the GIC and reduced its antimicrobial effect. This was confirmed in a previous in vivo study (Du et al., 2012). The authors investigated antibiofilm effects of conventional GIC and GIC containing 2% chlorhexidine diacetate. Conventional GIC and CHXGIC specimens were bonded to the buccal surface of the molars and left untouched for 4 and 24 hours. The bacterial viability was analysed by confocal laser scanning microscopy, which revealed no significant difference in the viability of bacteria between the two restorations (Du et al., 2012). Moreover, an in vitro study performed to test for the antibacterial and physical properties of resin-modified glass ionomer cement (RMGIC) modified with chlorhexidine, the antibacterial effect was noted in the first 3 weeks and decreased in time corresponding to the decrease in available CHX (Sanders et al., 2002). The decrease in chlorhexidine, levels were attributed to elution of CHX from GIC and/or by forming insoluble salts with the GIC (Ribeiro and Ericson, 1991).

On the other hand, previous studies have found that the antibacterial effect of chlorhexidine was greatest in the micro-environment between the restoration and the cavity (Turkun et al., 2008). This area may lead to a high concentration of chlorhexidine, which could potentially eliminate the remaining bacteria in the cavity. This was observed in a study by De Castilho et al. (2013). The researchers conducted an in vivo and in vitro study to investigate the biological and mechanical behaviour of resin-modified glass ionomer cement (RMGIC) containing 1.25% chlorhexidine digluconate (CHX). They observed that cavities restored with RMGIC-CHX had no detectable S. mutans after 3 months when compared to RMGIC without CHX (De Castilho et al., 2013). This could explain why in this study GIC-CHX had better marginal adaptation and less leakage when compared to control GIC, since the remaining bacteria were eliminated during cavity preparation. In this study, it was not possible to re-evaluate the number of bacteria in the cavities after placement of ART. Therefore, clinical assessments were performed to assess the clinical effectiveness of both restorations. This has been discussed in more detail in the previous chapter (see section 3.5, pages 47-48).
To test the general effect of adding 5% chlorhexidine digluconate to GIC in the oral cavity, the number of micro-organisms in saliva was counted before and after placement of the restorations. The majority of participants had reduced salivary flow; therefore, they were asked to rinse their mouth with sterile distilled water (10 ml). Samples were collected by encouraging participants to expectorate the contents back into the container. Microbiological analyses for all saliva samples showed that at baseline, and at 1, 3 and 6 months post-treatment, there was only a slight reduction in Candida and S. mutans. However, this reduction was not statistically significant ($P>0.05$). The levels of both total anaerobic bacterial count and lactobacilli were similar for the whole duration of the study. The reduced number of Candida colonies after 1 month could be due to several reasons, including improved oral and denture hygiene after the first appointment. For participants with maxillary dentures or removable partial dentures, it has been observed that the levels of Candida were much reduced when they did not sleep with the denture in the mouth the night before the appointment, compared to those who did sleep with their dentures in the mouth. Dentures are considered a reservoir for Candida, protecting these microorganisms from being washed out by saliva (Williamson, 1972). It is possible that as time passes, participants could have relaxed their oral hygiene habits, which resulted in Candida counts being similar to the baseline level.

Overall, it appears that the amount of chlorhexidine incorporated in GIC had a limited effect in reducing the levels of the tested microorganisms. In this study, the levels of S. mutans decreased slightly at the 1-month review compared to lactobacillus levels. This confirms that S. mutans are more sensitive to the presence of chlorhexidine in the oral cavity, as shown in a previous study (Sari and Birinci, 2007). Sari and Birinci (2007) showed that low concentration (0.2%) chlorhexidine gluconate mouthwash significantly reduced S. mutans counts in saliva taken from orthodontic patients for up to 4 weeks. However, it had no effect on lactobacillus counts (Sari and Birinci, 2007). In this study, GIC-CHX restorations had no significant effect on lactobacillus counts found in saliva, while it promoted a small reduction on S. mutans for up to 1 month after restoration placement. However, this result will need to be confirmed with a larger sample size. At 3- and 6-months post-treatment, the antimicrobial effect of GIC-CHX reduced significantly, and the mean count of S. mutans returned to baseline level. A possible explanation of this reduction of antimicrobial activity could be due to reduction in available chlorhexidine. This has been shown previously that the concentration of CHX
in modified GIC with CHX reduces overtime due to the continuous elution of CHX in addition to the formation of insoluble salts of CHX with glass ionomer, therefore reducing the available chlorhexidine (Ribeiro and Ericson, 1991; Sanders et al., 2002).

In conclusion, addition of chlorhexidine to GIC restorations appears to have minimal effect on the total viable anaerobic bacterial count and lactobacilli, however there appears to be a reducing effect on S. mutans found in saliva and plaque for up to 1 month post placement of the restoration with no measurable effect at 3 and 6 months. It would be interesting to see if larger sample size will generate the same set of results.
Chapter 5
5 Summary and future directions

5.1 Summary

The main objective of this study was to investigate the clinical effectiveness of ART when using glass ionomer cement (GIC) modified with 5% chlorhexidine as a restorative material in the treatment of root caries. To achieve this, it was necessary to have a control restoration (conventional GIC). The distilled water that was mixed with GIC was replaced with 5% chlorhexidine solution, so that two different restorative materials were tested in a split-mouth design study.

To test for the local and general effects of chlorhexidine in the GIC, plaque and saliva samples were collected before restoration placement at baseline and 1, 3 and 6 months after placement. Differences in microbial counts at these time points were used to investigate the antimicrobial effect of the two restorations over the 6-month period.

The atraumatic restorative treatment (ART) approach was also assessed in this study from the participant’s and from the operator’s points of view. The method of assessment included questionnaires distributed to the participants and the operator at baseline 1, 3 and 6 months. Moreover, both types of restorations (test and control) were assessed using modified Ryge Criteria at baseline and all subsequent visits and from this information survival of the restorations over 6 months period was determined.

The following points highlight the main findings of this study:

- There was a slight reduction in the number of cariogenic bacteria (*Streptococcus mutans*) and *Candida* in plaque and saliva samples after 1 month for both types of restorations, although this result was not statistically significant;
- GIC-CHX did not promote a greater reduction of microbial numbers in plaque samples when compared to conventional (non-chlorhexidine-modified) GIC. The reasons for this could be related to the inactivation of chlorhexidine by sodium lauryl sulphate (SLS) present in most toothpaste preparations, and the
lower concentration of chlorhexidine added to GIC which could have been diluted and subsequently cleared by saliva, food and fluids;

- The majority of participants found that ART took less time than expected and all participants were not stressed during ART and found it to be pain-free treatment modality. In addition, no participants exhibited dental anxiety during their subsequent clinical visits;
- Patients indicated that there was no change in taste perception after ART was administrated except one participant at the 6-month review appointment;
- The operator found ART to be an easy and fast treatment method; and
- Restoration of carious root surfaces using GIC-CHX resulted in higher survival rate of restorations (89%) compared to control GIC (67%). The main reasons for failure of restorations were gross marginal defects.

Although this result might suggest that GIC-CHX did not have a significant effect on reducing bacterial count in both plaque and saliva samples, the effect on the micro-environment between the tooth cavity and the restorations might have been more substantial. This is beneficial in minimally-invasive dentistry (MID) procedures such as ART, where infected dentine and its associated microorganisms are likely to be left behind after cavity excavation. The elimination of these microorganisms may improve the restoration adaptation and minimise secondary caries, and therefore improve the survival of the restorations as it was shown in this study.

It is important to mention that even though every effort was made to standardise the variables in the study, it was near impossible to find carious lesions of the same nature, size and location among the participants of the study.
5.2 Hypotheses and aim of the study

The hypotheses of this study proposed in section 1.2 are stated as follows:

“root caries lesions restored with glass ionomer cement (GIC) modified with chlorhexidine (CHX) will have a reduced load of microorganisms in plaque samples when compared to teeth restored with conventional GIC.”

“the microbial count of unstimulated saliva will be reduced after application of GIC modified with chlorhexidine.”

Both hypotheses are rejected by this study. Small sample size made statistical analysis not possible to detect true difference in the microbial count in plaque and saliva samples between the different types of restorations. Although a slightly lowered mean count of S. mutans was noted at the 1-month, it is not clear if this is clinically relevant.

The main aim of this study as proposed in section 1.3 is stated as follows:

The main aim of this study was to investigate the clinical effectiveness of ART when using glass ionomer cement (GIC) modified with 5% chlorhexidine as a restorative material in the treatment of root caries.

Within the limitations of this study, the main aim of this study was achieved. The majority of participants found ART to be a stress and pain free procedure. Moreover, the survival rate of the GIC-CHX restorations was higher compared to GIC restorations. However this should be treated with caution because of the small sample size

5.3 Future directions

Within the limitations of this study, the placement of a glass ionomer cement modified with 5% chlorhexidine using the ART approach appears to provide a simple and potentially cost-effective treatment which can also be considered for use in outreach dental services to restore root surface carious lesions, particularly those at higher risk such as institutionalised older adults and possibly special care groups. A larger number of patients, however, is required to confirm the validity of this finding.
The potential effect of a SLS-based toothpaste in inactivation of chlorhexidine was not investigated this study. A future study investigating the effects of SLS-based toothpastes on CHX-GIC restorations will be interesting to determine if there is a value in using SLS free toothpastes when restoring carious lesions with GIC modified with chlorhexidine.

Further clinical studies investigating different concentrations of chlorohexidine-modified GIC are necessary to test the efficacy in reducing microbial counts in plaque and saliva.
6 References:


Kanagaratnam S (1997). Dental caries patterns and the utilisation of dental services


Takahashi N, Nyvad B (2011). The role of bacteria in the caries process: ecological


7 Appendices:

7.1 Appendix A: Reagents and media composition and guidance on preparation of media

**CBA (Columbia Blood Agar)**

- CBA base: 39.0g
- Defibrinated sheep blood: 50ml (5%)
- Distilled water: 950ml

Suspend 39g CBA in 950ml of distilled water. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C in water bath and add 5% sterile defibrinated blood. Mix the blood gently and pour plates in sterile single vent plates.

**Rogosa Agar**

- Rogosa powder: 82g/L
- Distilled water: 1 litre
- Glacial Acetic Acid: 1.32ml

Suspend 82g Rogosa powder in 1 litre of distilled water and bring to the boil to dissolve completely. Add 1.32ml glacial acetic acid and mix thoroughly. Heat to 90-100°C for 2-3 minutes with frequent agitation. Then pour plates in sterile single vent plates.

Refer to:

**Sabouraud agar**

- Sabouraud dextrose agar: 65g
- Distilled water: 1 litre

Suspend 65g Sabouraud dextrose agar in 1L distilled water and autoclave at 121°C for 15 min. Cool down the in water and pour pour plates in sterile single vent plates.

**Reduced Trasport Fluid (RTF)**

- K2HPO4: 0.45g
- KH2PO4: 0.45g
- NaCL: 0.90g
- (NH4)2SO4: 0.1875g
- Na2CO3: 0.40g
- Dithiothreitol: 0.20g
0.1 M EDTA 10ml (0.8766g/30ml)
Distilled water 1000ml
Filter sterilize into sterile containers and keep in the fridge (Hoover and Newbrun, 1977).

7.2 Appendix B: Chlorhexidine digluconate (CHX) dilution protocol

Purpose:

To describe the protocol for the dilution of CHX (20%) to CHX (5%) with sterile distilled water. This procedure should be carried out in the laboratory located in the third floor of the Dental School.

Materials and reagents and equipment required:

- A 25mL aliquot of CHX (20%).
- Note: CHX (20%) is initially supplied in 1 gallon, and aliquoted under strict aseptic conditions into 25ml sterile tube.
- Clean dry liquid dispenser bottle
- Sterile distilled water (Baxter 100ml sealed bottle)
- Eppendorf pipettes and sterile pipette tips
- Empty 25ml sterile tubes
- Laminar Flow Cabinet

Measure 3x 25ml from the sterile distilled water (Baxter water for irrigation) and dispose of the remaining water from the bottle.

Return the total amount of 75ml sterile distilled water back into the bottle.

Pipette 25ml from chlorhexidine into the bottle to make a total of 100ml liquid.

Close the lid immediately and shake by inverting the bottle multiple times to homogenise the solution and this will result in 5% chlorhexidine solution.

Label the bottle clearly and date it and store in cool dark place.

Notes:

Dispose of the pipette tips into a clinical waste bin.
7.3 Appendix C: Specimen storage & freezing of bacteria using glycerol – SOP

7.4 Procedure for specimen storage

Saliva and plaque samples should be refrigerated or frozen down as soon as possible after collection. When samples remain at room temperature for periods of time longer than a few hours there is also opportunity for bacterial growth.

1. Remaining saliva and plaque samples should be stored at -80°C. Samples will be stored in glycerol (at a final concentration of 30%) with clear labelling.
2. The position and place in the -80°C freezer shall be recorded electronically and in the study specific laboratory book.

Freezing Bacteria Using Glycerol

Purpose

Bacteria can be preserved for many years when frozen at -80°C using a solution of 30% (v/v) glycerol. The process is simple and requires screw cap micro tube and sterile glycerol. Glycerol and bacterial culture in broth are mixed, dispensed into tubes and then frozen.

Preparation of 60% (v/v) glycerol solution (e.g. 100ml):
Transfer sterile 40ml of BHI (brain heart infusion) into a small screw-cap plastic bottle, add 60ml 100% sterile glycerol under laminar flow cabinet.

Procedure

1. Label 1ml-sterile screw-cap microtubes with the isolate’s ID and or number, and date
2. Transfer a bacterial suspension (0.5ml) to a sterile microtube (maximum 2ml) and add 0.5ml (same volume as remaining sample) of the sterile 60% (v/v) glycerol solution. Mix by vortexing.
3. Place the tube(s) in the freezer and record their location.
### 7.5 Appendix D: Template for recording of counts

<table>
<thead>
<tr>
<th>Sample</th>
<th>Baseline</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient ID: Time of sample (please circle)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start date of incubation:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plate one colonies count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plate two colonies count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean colonies count x dilution factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean colonies count x dilution factor / 0.1 ml (volume of plated sample) / weight of plaque = CFU/ng</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Columbia</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 °C anaerobic</td>
<td>D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rogosa</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 °C anaerobic</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sabouraud</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 °C anaerobic</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutans Selective</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 °C anaerobic</td>
<td>D</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A = plaque from lesion restored with CHX-GIC
B = plaque from region restored with GIC
C = plaque from healthy tooth
D = unstimulated saliva
7.6 Appendix E: Procedure for the disposal of biological waste

Purpose

An autoclave is a specialized piece of equipment designed to deliver heat under pressure to a chamber, with the goal of decontaminating or sterilizing the contents of the chamber.

Required training to operate the Autoclaves are necessary to prevent accidents and safe use of the equipment.

There are available SOP’s for all autoclaves with detailed instruction how to operate the autoclaves.
7.7 Appendix F: Participants questionnaires at baseline:

Thank you for your participation in this study. Please read and answer the following questions on both pages.

Section 1:

General characteristics:

1) What is your age:
   - 50-59
   - 60-65
   - 66-70
   - 70 or more

2) Gender:
   - Male
   - Female

3) Ethnicity: Please specify your ethnicity
   - New Zealand European
   - Maori
   - Islander
   - Asian
   - Other

4) Smoking status:
   - Past smoker
   - Never smoked
   - Current smoker

5) Frequency of tooth brushing:
   - Irregular
   - Regular once a day
   - Regular twice or more a day

6) Have you refrained from brushing this morning:
   - Yes
   - No

Section 2:

Participants acceptability of the treatment:

1) How comfortable was the ART treatment?
   - Very comfortable
   - Comfortable
   - Neutral
   - Uncomfortable
   - Very uncomfortable
2) How much pain did you feel during the ART treatment?
   None   A little pain   A lot of pain

3) How anxious did you feel during the ART treatment?
   Not at all anxious   A little anxious   Very anxious

4) How anxious did you feel throughout your clinical visit today?
   Not at all anxious   A little anxious   Very anxious

5) How much time did you feel that the ART procedure took?
   Less than expected   As expected   More than expected

6) How confident were you that the dentist was able to apply the ART treatment?
   Very confident   Confident   Neutral   Unconfident   Very unconfident

7) How satisfied are you with the appearance of the ART treatment on your tooth?
   Very satisfied   Satisfied   Neutral   Unsatisfied   Very unsatisfied
Patient’s questionnaires at 1, 3 and 6 months:

Have you refrained from brushing this morning?

Yes [ ] No [ ]

Participants acceptability of the treatment:

1) How smooth does your treated tooth/teeth feel compared to your other teeth?

Very smooth [ ] Quite smooth [ ] Neutral [ ] A little rough [ ] Very rough [ ]

2) How much pain have you had in your tooth/teeth following ART treatment?

None [ ] A little pain [ ] A lot of pain [ ]

3) How anxious did you feel throughout your clinical visit?

Not at all anxious [ ] A little anxious [ ] Very anxious [ ]

4) How satisfied are you with the appearance of the ART treatment on your tooth/teeth?

Very satisfied [ ] Satisfied [ ] Neutral [ ] Unsatisfied [ ] Very unsatisfied [ ]

5) Have you experienced any taste change since receiving treatment?

None [ ] A little change [ ] A lot of change [ ]
Operator's assessment of acceptability of the ART restorations:

Baseline operator questionnaire:

8) Polyacrylic used?
   Yes ☐   No ☐

9) Proprietary varnish used?
   Yes ☐   No ☐

Baseline Acceptability assessment (operator):

1) How easy was the ART procedure?
   Very easy ☐   Quite easy ☐   Neutral ☐   Quite difficult ☐   Very difficult ☐

2) How long did the procedure take?
   Less than expected ☐   As expected ☐   More than expected ☐

3) How satisfied are you with the appearance of the ART restoration?
   Very satisfied ☐   Satisfied ☐   Neutral ☐   Unsatisfied ☐   Very unsatisfied ☐

4) Time taken for GIC-CHX to set:
   Quicker than GIC ☐   same as GIC ☐   Longer than GIC ☐
Operator's follow up assessment of acceptability at 1, 3 and 6 months:

1) How satisfied are you with the appearance of the GIC on the patient's tooth?
   \[
   \begin{array}{cccccc}
   \text{Very satisfied} & \text{Satisfied} & \text{Neutral} & \text{Unsatisfied} & \text{Very unsatisfied} \\
   \hline
   \end{array}
   \]

2) How satisfied are you with the condition of the GIC on the patient's tooth?
   \[
   \begin{array}{cccccc}
   \text{Very satisfied} & \text{Satisfied} & \text{Neutral} & \text{Unsatisfied} & \text{Very unsatisfied} \\
   \hline
   \end{array}
   \]

3) How satisfied are you with the appearance of the GIC CHX on the patient's tooth?
   \[
   \begin{array}{cccccc}
   \text{Very satisfied} & \text{Satisfied} & \text{Neutral} & \text{Unsatisfied} & \text{Very unsatisfied} \\
   \hline
   \end{array}
   \]

4) How satisfied are you with the condition of the modified GIC on the patient's tooth?
   \[
   \begin{array}{cccccc}
   \text{Very satisfied} & \text{Satisfied} & \text{Neutral} & \text{Unsatisfied} & \text{Very unsatisfied} \\
   \hline
   \end{array}
   \]
### Appendix G: Appendix I tables of raw data of microorganisms count for different media

Table 4. Colony forming units (CFU) of total viable bacteria per mg of plaque taken from both test and control teeth over a period of 6 months.

<table>
<thead>
<tr>
<th>Participants</th>
<th>Baseline</th>
<th>1 M</th>
<th>3 M</th>
<th>6 M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHX-GIC</td>
<td>GIC</td>
<td>CHX-GIC</td>
<td>GIC</td>
</tr>
<tr>
<td>1</td>
<td>1.02E+09</td>
<td>2.30E+09</td>
<td>7.30E+08</td>
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<tr>
<td>2</td>
<td>2.40E+13</td>
<td>7.90E+13</td>
<td>1.10E+07</td>
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<tr>
<td>3</td>
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<td>6.70E+13</td>
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<td>5.08E+08</td>
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<tr>
<td>4</td>
<td>2.74E+09</td>
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<td>1.38E+11</td>
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<tr>
<td>5</td>
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<td>1.75E+07</td>
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<tr>
<td>6</td>
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<tr>
<td>7</td>
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<tr>
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<td>9</td>
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</tr>
<tr>
<td>StDv</td>
<td>3.5542E+13</td>
<td>5.47E+13</td>
<td>3.96E+13</td>
<td>3.64E+13</td>
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</table>
Table 5. Log_{10} (CFU/mg) of total viable bacteria data from Table 1

<table>
<thead>
<tr>
<th>Participants</th>
<th>Baseline</th>
<th>1 M</th>
<th>3M</th>
<th>6 M</th>
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<td>11.05</td>
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<td>13.60</td>
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Table 6. Colony forming units (CFU) of yeast per mg of plaque taken from both test and control teeth over a period of 6 months.

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Table 7. Log \(_{10}\) (CFU/mg) of yeast in plaque samples taken from both control and test teeth.

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Table 8. CFU/mg of lactobacilli found in plaque taken from both test and control teeth over a period of 6 months.

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Table 9. Log$_{10}$ (CFU/mg) of lactobacilli found in plaque samples taken from both test and control teeth.

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Table 10. CFU/mg of *Streptococcus mutans* found in plaque samples taken from both test and control teeth at each time interval.

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Table 11. Log_{10} (CFU/mg) of *Streptococcus mutans* found in plaque taken from both test and control teeth at each time interval.

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Table 12. Log_{10}(CFU/ml) of total viable bacteria found in saliva suspension at different time intervals

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Table 13. Log$_{10}$ (CFU/ml) of yeast found in saliva suspension at different time intervals

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Table 14. Log\(_10\) (CFU/ml) of lactobacilli found in saliva suspension at different time interval.

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<td>StDv</td>
<td>1.163781</td>
<td>0.939947</td>
<td>1.013226</td>
<td>1.520228</td>
</tr>
</tbody>
</table>
Table 15 Log$_{10}$ (CFU/ml) of *Streptococcus mutans* found in saliva suspension at different time interval

<table>
<thead>
<tr>
<th>Participants</th>
<th>Baseline</th>
<th>1 M</th>
<th>3M</th>
<th>6 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.91</td>
<td>6.77</td>
<td>6.43</td>
<td>6.81</td>
</tr>
<tr>
<td>2</td>
<td>5.83</td>
<td>6.46</td>
<td>6.48</td>
<td>5.93</td>
</tr>
<tr>
<td>3</td>
<td>6.95</td>
<td>6.37</td>
<td>5.54</td>
<td>6.11</td>
</tr>
<tr>
<td>4</td>
<td>7.83</td>
<td>7.22</td>
<td>6.3</td>
<td>6.67</td>
</tr>
<tr>
<td>5</td>
<td>7.33</td>
<td>6.64</td>
<td>7.68</td>
<td>6.49</td>
</tr>
<tr>
<td>6</td>
<td>6.34</td>
<td>4.74</td>
<td>7.06</td>
<td>5.6</td>
</tr>
<tr>
<td>7</td>
<td>8.25</td>
<td>8.53</td>
<td>8.04</td>
<td>6.4</td>
</tr>
<tr>
<td>8</td>
<td>7.04</td>
<td>7.29</td>
<td>6.85</td>
<td>6.78</td>
</tr>
<tr>
<td>9</td>
<td>7.79</td>
<td>6</td>
<td>6.83</td>
<td>4.28</td>
</tr>
<tr>
<td>Mean</td>
<td>7.14E+00</td>
<td>6.67E+00</td>
<td>6.80E+00</td>
<td>6.12E+00</td>
</tr>
<tr>
<td>StDv</td>
<td>0.76009</td>
<td>1.02867</td>
<td>0.74665</td>
<td>0.800132</td>
</tr>
</tbody>
</table>
7.9 Appendix H: Poster presentations:

Academy of Australian and New Zealand Prosthodontists,

The 2018 AANZP Biennial Meeting was held on the 26th-28th of July 2018 in Melbourne, Australia.

Clinical evaluation of modified GIC restorations applied using ART

Hasan Ahmed, Jithendrap Ratnayake, Carolina Loch, Nicholas Heng, Karl Lyons, Paul Brunton
1. Faculty of Dentistry, University of Otago, Dunedin, New Zealand.

Background

Root caries is common amongst the older population. The risk of caries increases with irregular attenders and special needs groups. A simple, reliable and cost-effective therapeutic method is required to address this issue.

Objectives

To investigate the clinical effectiveness and patient acceptability of a novel restorative material to treat root caries placed using atraumatic restorative treatment (ART).

Methods

Two clinically-visible root surface carious lesions per patient (n=9) were restored using ART. One root carious lesion was restored with a conventional glass ionomer cement (GIC) and the other with GIC modified with 5% chlorhexidine digluconate (GIC-CHX). Patient acceptability and survival rates of the restorations were evaluated using questionnaires and the modified Ryge criteria (Ryge 1980) at baseline, and after 1, 3 and 6 months. Plaque samples were collected from around both restorations and microbiological analysis were completed at baseline, 1, 3 and 6 months.

Results

Summary of patients’ acceptability of ART

<table>
<thead>
<tr>
<th>Response</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comfort of ART treatment</td>
<td>9 (100.0)</td>
</tr>
<tr>
<td>Very comfortable</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>Comfortable</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Pain during ART treatment</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>None</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>A little pain</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Anxiety during treatment</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>Not at all anxious</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>A little anxious</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Anxiety throughout clinical visit</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>Not at all anxious</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>A little anxious</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>Time taken to complete ART procedure</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>Less than expected</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>As expected</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Satisfaction with appearance</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>Very satisfied</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Satisfied</td>
<td>7 (77.8)</td>
</tr>
</tbody>
</table>

There was some reduction in the mean count of total viable bacteria and St. mutans at 1 month for both restorations.

Mean counts of microorganisms at different time intervals

Conclusions

- Most participants found ART to be a pain-free technique and none experienced taste change.
- GIC-CHX restorations had higher survival rates compared to GIC restorations.
- There was no statistically significant difference in the mean bacterial count between GIC and GIC-CHX for the tested plaque samples.
- There was a reduction in the mean number of Candida and S. mutans in saliva suspensions; however, these results were not statistically significant.
- GIC-CHX restorations placed using ART appear to be a promissing treatment modality.

References

Clinical evaluation of modified-GIC restorations: Six month results
Hassan Ahmed, Jithendra Ratnayake, Karl Lyons, Carolina Loch, Paul Brunton
The University of Otago, Faculty of Dentistry, Dunedin, New Zealand

Background
Dental caries is a global disease affecting all different ages and sectors of the population. Despite the advancement in early detection and treatment, dental caries remains one of the most chronic diseases in the world. The aim of this study was to investigate the clinical effectiveness and patient acceptability of a modified glass ionomer cement to treat root caries lesions using the atraumatic restorative technique (ART). Interim findings are presented.

Methods
As part of an ongoing study recruiting 34 subjects, two clinically-visible root surface carious lesions per patient (n=9) were restored using ART. One root carious lesion was restored with a conventional glass ionomer cement (GIC) and the other with a GIC modified with 5% chlorhexidine digluconate (GIC-CHX) cement. Patient acceptability and survival rates of the restorations were evaluated at baseline, and after 1, 3 and 6 months using questionnaires and the modified Ryge criteria.

Results 1: Patients Acceptability

| Conduct of ART treatment | | |
|-------------------------|---------|
| Very comfortable        | 8 (88.9%) |
| Comfortable             | 1 (11.1%) |
| Pain during ART treatment | | |
| None                    | 8 (88.9%) |
| A little pain           | 1 (11.1%) |
| Anxiety during treatment | | |
| Not at all anxious      | 8 (88.9%) |
| A little anxious        | 1 (11.1%) |
| Anxiety throughout clinical visit | | |
| Not at all anxious      | 8 (88.9%) |
| A little anxious        | 1 (11.1%) |
| Time taken to complete ART procedure | | |
| Less than expected      | 6 (66.7%) |
| As expected             | 3 (33.3%) |
| Satisfaction with appearance | | |
| Very satisfied          | 6 (66.7%) |
| Satisfied               | 3 (33.3%) |

Discussion and Conclusions
- All participants indicated that ART technique used was not painful.
- Restoration of carious root surfaces with the modified GIC-CHX resulted in higher survival rates compared to GIC restorations.
- No secondary caries were found and the main reason for failure were gross marginal defects.
- ART may be a viable approach for use in outreach services to restore root surface carious lesions in the elderly. However, a larger number of patients (n=34) is currently being recruited to confirm the validity of this finding.

References

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