

**Novel approach to treat root caries in the elderly**

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**1.0 Introduction**:

Dental caries is one of the most prevalent chronic diseases worldwide (Bagramian et al., 2009). It affects all ages and people remain susceptible to this disease throughout life. It is a complex disease that occurs as a result of acid produced by microorganisms found in dental plaque and modified by many factors such as saliva, diet and host factors. Root caries is a condition which more commonly affects the elderly due to increased gingival recession and poor oral hygiene and many are taking medications that may have a hyposalivatory effect (Saunders and Handelman, 1992). These people are usually overburdened with other medical problems and some have difficulty reaching dental professionals and receive little care. A simple effective method of treating such a cohort is urgently needed; the atraumatic restorative technique (ART) is one such method. Another way to improve the outcome for such individuals is to enhance the preventative properties of restorative materials. This can be done by utilising existing restorative materials as a vehicle for antimicrobial agents, whereby long-term sustained inhibition of dental caries is achieved. This literature review will explore these matters in more detail including some of the treatment concepts utilised in the treatment of root caries in the elderly.

**1.1 Dentalcaries**

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 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 secreted by cells. This provides protection from desiccation, host defences, and more importantly, provides resistance to antimicrobial agents (Selwitz et al., 2007).

In recent decades, the 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, the kinetics of demineralization, 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 in most individuals in the normal state (Marsh, 1994). Microbial fermentation of carbohydrate substrates causes the production of acid. Exposure to low pH will gradually lead to inhibition of acid-sensitive species and the selection of organisms with an aciduric physiology, such as *Streptococcus* *mutans* and *Lactobacilli*. These two microorganisms are not only able to survive in an acidic environment but are also able to produce more acid by means of metabolism and lower 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 that form the bulk 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 balance is tipped towards demineralisation, the loss of calcium and phosphate ions initiates the caries process (Banting, 2001).

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 to the mid-1990s from a mean DMFT (Decayed Missing Filled Teeth) of 17.3 in 1968 to 3.7 in 1995 ( Beck et al., 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 DMFS (decayed missing filled surfaces) with increasing age appeared to be linear (Broadbent et al., 2008). The findings of the Dunedin study showed that caries rate increases with age. This is particularly true with root caries, where MinQuan Du et al. (2009) in their study involving 2160 Chinese participants found that the prevalence of root surface caries in the elderly was 43.9% and in the middle aged was 13.1%.

**1.1.1 Risk factors of dental caries**

Dental caries is a complex disease caused by the interaction of multiple factors including biological, behavioural, and social factors (MacEntee et al., 1993).

Risk factors for dental caries are not stationary and change with time (Selwitz et al., 2007). Physical and biological risk factors for enamel or 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 the elderly (MacEntee et al., 1993). Physically dependent individuals usually depend 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 practiced, many residents had poor oral and denture hygiene. Since oral hygiene is one of the most influential factors in development of oral diseases in older adults, 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 caries risk factors in relation to lifestyle and behavioural factors are usually under the individuals control. For example, the frequency and nature of oral hygiene practices and dietary habits can affect one’s caries risk (Selwitz et al., 2007). High consumption of carbohydrates is one of the main drivers of caries (Palmer and Depaola, 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, physical form of 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 proximity of eating to bedtime (Palmer and Depaola, 1995). Increasing the frequency of sugar intake increases the odds of developing root caries. This has been shown when a group of individuals with restricted sugar intake due to diabetes were followed and compared to a matched group in terms of age, teeth and gingival recession; caries prevalence was found to be lower in the group that restricted sugar intake (Palmer and Depaola, 1995).

Root caries is found more regularly at the cementum-enamel junction, although it can 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 the dental plaque on the root surface as a by-product from carbohydrate fermentation, causes demineralisation of cementum exposing the collagen fibrils which are gradually broken-down by bacterially derived enzymes in 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).

**1.1.2 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 about 50 years ago, when he noticed the change in bacterial population from aerobic and facultative to predominately anaerobic as the plaque matured over 9 days (Ritz et al., 1967). Distinctive microflora lives 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, usually involves undisturbed accumulation of plaque as a result of inadequate oral hygiene habits. This is usually combined with an increase in the frequency of consumption of fermentable carbohydrates. If these processes continue, the environment increases the risk of the formation of 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 demineralized, subjected to proteolysis and bacterial invasion (Brailsford et al., 2001). The environmental factors driving the 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 external factors [oral hygiene and diet] may change over a very short period, with catastrophic effects on the microflora and on the underlying dentine (Brailsford et al., 2001).

Similarly, microorganisms of dental caries change as the condition or the state of the carious lesion changes (Bowden, 1990). A good example is the increase in the proportions of *Streptococcus mutans* and *Lactobacilli* in individuals with root caries (Preza et al., 2008). The presence of these two 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).

In a study conducted in Vancouver, British Columbia to identify the predictors of dental caries in the elderly, a high level of *Lactobacilli* was found to be a major predictor (MacEntee et al., 1993). 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. Nevertheless, 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 yet 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, in addition to *mutans* streptococci, have been reported to possess at least one of these two traits (Brailsford et al., 2001).

**1.2.0 Oral health in the elderly**

Increase in life expectancy has led to an increase in the number of elderly (Frencken, 2014). Elderly people experience an increase risk in chronic diseases such as cardiovascular disease, hypertension, cancer, diabetes and Parkinson’s disease (Petersen and Yamamoto, 2005). This problem of a growing aging population and the concurrent increase in chronic diseases, has led the WHO to highlight the need for strong health promotion strategies amongst older people (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 the elderly 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 increase 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 that oral health is a determinant factor for quality of life (Petersen, 2003), calling for urgent policy development to improve the oral health of the elderly (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.

Our aging population is growing. When this is coupled with an improvement in oral health, more and more people are retaining their natural teeth for longer (Du et al., 2009). However, the majority of elderly people have systemic chronic diseases that ultimately lead to decline in health. These diseases are often accompanied by social and environmental impairments, some of which may have negative effects on oral health such as dementia, Alzheimer’s disease, physical disability, xerostomia and solitude. These individuals usually require dental care but they are not necessarily able to source dental treatment (Frencken, 2014).

The elderly are at higher risk of 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), and a reduced salivary flow rate can impair plaque removal and reduce the buffering capacity of saliva (Imazato et al., 2006). In addition, some medications are known to cause a reduction in salivary flow rates (Saunders and Handelman, 1992). A study done to evaluate 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 such medication (Saunders and Handelman, 1992). Some medications are thought to 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., tipping the pH balance towards demineralisation leading to an increase in caries development (Thomson et al., 2002). The second way is through the development of symptoms of xerostomia as a result of changes in the quality of saliva, the perception of salivary flow, or 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). Indeed, in a study published in 2002, Thomson et al. reported that medication had not been found to be a risk factor for the occurrence of coronal or 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 the elderly (Narhi et al., 1998). This study also found that men were less affected by reduced salivary rates than women, but in contrast the incidence of root caries was higher in men. It has been concluded that caries incidence can be modified by adequate oral hygiene as well as preventive programmes, which makes the assessment of risk of root caries difficult, however 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).

**1.2.1 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 socioeconomic 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). In older populations, many barriers to access oral health services are present 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, elderly people living in rural areas were more likely to have poor oral health status and inadequate utilization of dental care (Vargas et al., 2003). All these factors make the elderly more vulnerable to oral diseases.

In New Zealand, ethnicity and socioeconomic status are likely to influence whether people visit 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 socioeconomic deprived areas had visited a dental health professional in the last 12 months, compared to 59% of adults in the least deprived areas (Ministry of Health, 2015). In addition, 70% of Maori 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 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. 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, the elderly 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 technique.

**1.3.0 Atraumatic Restorative Technique (ART)**

If preventative measures are not implemented properly, the risk of developing root caries in the elderly increases. Utilisation of preventive measures such as fluoride application, will likely reduce 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 atraumatic restorative technique (ART) are two examples of minimally invasive dentistry 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 has reflected not only on the preferences of children but also on 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).

The Atraumatic Restorative Technique (ART) is an 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). Unlike its counterpart 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 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 elderly who are home bound and also institutionalised patients. It can be done with no 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 using ART was found to be more effective in the management of dentally anxious patients, especially for the management of younger patients under 6 years old. In addition, ART was found to be more effective and easier to perform than using rotary instruments in older patients (Frencken et al., 2012). ART has been shown to be at least as effective as conventional restorative techniques when dealing with patients who are at an increased risk of caries from a reduced salivary flow following radiation therapy to the head and neck region (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).

When compared to conventional rotary instrumentation, the ART approach has been found to be less time consuming (Da Mata et al., 2014). The authors suggested two reasons for this; local anaesthesia was not used, and conventional restorations usually required polishing after the setting of material and this is not required in the ART technique. ART is quite a simple procedure when compared to more conventional methods. This 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 employing dental therapists (Da Mata et al., 2014), although currently this is 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, and therefore, an ideal restorative material would have been an adhesive material that required only hand mixing and no light curing since access to electricity and sophisticated equipment was difficult (Frencken et al., 1996). Glass ionomer cement had these properties and the extra advantages of chemical bonding to enamel and dentine and also providing fluoride release (Frencken and Holmgren, 1999).

The success of restorations placed using the ART technique 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 pressure (Mickenautsch et al., 2010).

**1.4.0 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, and this 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 soon after the mixing of 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). This fluoride, if available as ions, might contribute to caries prevention (Causton, 1981).

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:restorative interface (Lin et al., 1992). The basic component of the glass is a calcium aluminosilicate containing fluoride. 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 an acid and a base, with the product of the reaction forming a hydrogel salt acting as a binding matrix (Lin et al., 1992).

Fresh glass ionomer releases more fluoride than maturated glass ionomer material. This release is high in concentration particularly in the confined space between the restoration and the tooth which could lead to remineralisation of softer dentine but also has an effect on the remaining bacteria in the dentine (Forsten, 1991). 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 which can be easily removed with dehydration, and tightly bound water which 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 than protection from water 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, leading ultimately 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 and hearing. These harmful effects on health have prevented the use of nail varnish routinely in dentistry. On the other hand, petroleum jelly is fairly safe and has been found to be adequate in terms of protection of GICs (Brito et al., 2010).

Originally, calcium ( ) was the main constituent of the powder in GICs, however it has been replaced by strontium (Sr) (Ngo, 2010). The main reason for this was to make the GIC restorations radiopaque. Both and Sr 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. Further, Sr can replace in hydroxyapatite without causing any detrimental effect (Ngo, 2010). There is also some evidence that Sr can have anticariogenic properties, especially when combined with optimum fluoride levels (Curzon et al., 1978).

During the initial setting of GIC immediately after mixing, cross-linking of the polyacid chains by either the or Sr ions takes place. This cross linking is not stable and is susceptible to water intake 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-), , Sr and phosphate PO4−3 (Ngo, 2010). 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 like and PO4−3 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 from the GIC to carious dentine can be extensive (Ngo, 2010).

The F- and Sr contained in 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 intimate contact between the GIC and the partly demineralised dentine (Ngo et al., 2006).

Originally, medium viscosity GICs were the only available material 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 specially 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 ART (Turkun et al., 2008).

**1.4.0 Modifications of restorations in order to improve antibacterial properties**

There are a number of antimicrobial agents available but only a few were 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 the surface. This is because dental plaque is a complex ecosystem that is mostly responsible for the development of caries. 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 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.

**1.4.1 Chlorhexidine**

Increasing antimicrobial properties of restorative materials is a very important clinical property. 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 (Palmer and Depaola, 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 ionomers. Its chemical structure is shown in Fig 1.



**Fig 1**: Chemical structure of chlorhexidine

adapted from (Zeng et al., 2009)

Chlorhexidine is a cationic bisbiguanide with broad antibacterial activity and low mammalian toxicity with strong binding to skin and mucous membranes (Jones, 1997). Chlorhexidine has broad spectrum antimicrobial activity against gram-positive and gram-negative bacteria (Hennessey, 1973), *Candida* spp (Salim et al., 2013) and lipophilic viruses (Harbison and Hammer, 1989). Different concentrations of chlorhexidine have different effects. At low concentration it is bacteriostatic, whereas it is bactericidal at high concentrations (Jones, 1997). These effects are different on different microorganisms. For example, the mean of minimum inhibitory concentration (MIC) 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 the tested gram-positive and gram-negative bacteria, however this effect was reduced dramatically in the presence of biological fluids. For example, addition of serum required a four times increase in chlorhexidine concentration to produce the same effect. Similarly, in the presence of 5% sucrose, S*treptococcus* *mutans* required significantly higher concentrations, and this was attributed to the binding of chlorhexidine to polysaccharides thus reducing the available 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).

**1.4.2 Modification of GIC with chlorhexidine**

Ribeiro and Ericson (1991), and Hoszek and Erickson (2008) have investigated the effect of adding chlorhexidine to GIC restorative material and luting cement in two different forms: chlorhexidine digluconate and chlorhexidine diacetate. It was found that addition 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 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%) did increase the setting time and decrease 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; higher concentrations of chlorhexidine diacetate (more than 2%) did however, have 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 2004 in a mobile dental clinic as part of the Division of Public Oral Health of the University of Johannesburg, South Africa investigated whether chlorhexidine diacetate 1% modified glass ionomer is 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 most of the teeth restored were permanent molars with at least one large occlusal cavity. 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).

The effect on the 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 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). The mechanical tests were performed in a universal testing machine at a crosshead speed of 1mm/min. A compressive load was applied along the diameter of the GIC disks for the diametral tensile strength test while it was applied along the long axis in the case of compressive strength test (Farret et al., 2011).

Similar results have been obtained with resin-modified GICs by Sanders et al. (2002) who tested the diametral tensite 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 adding 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 this 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 St. *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 has less effect on the mechanical properties of high viscosity glass ionomer cement 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). This however was an old study which was conducted before the introduction of high viscosity glass ionomer in the mid 1990’s and therefore may not be completely relevant. Another study has shown that addition of low concentrations of chlorhexidine digluconate to GIC had minimal effect on mechanical properties but concentrations of chlorhexidine digluconate exceeding 2.5% had significantly lower hardness when compared to the control but diametral tensile strength, compressive strength and biaxial flexural strength had insignificant decrease (Turkun et al., 2008).

To date, only one ongoing *in vivo* study in the Leeds Dental Institute has investigated the use of chlorhexidine digluconate modified GIC as a restorative material using ART. Many laboratory tests have been performed in order to determine the best formulation, 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 (unpublished data).

**1.5.0 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 risk and modifying factors contribute to the caries process and therefore determine the individual’s risk. The elderly 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 elderly are generally most in need of dental care but are not necessarily able to always get access to it due to mobility issues or lack of proper support. Simplifying dental treatment and improving antimicrobial properties of restorations are just a few of the examples that can be used to help such people. ART is a technique that only uses hand instruments in the majority of cases so it may be used to provide treatment in a 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 aim of this study is to investigate the effectiveness of GIC modified with chlorhexidine digluconate, when applied to root caries in elderly patients using the non-invasive, simple approach of ART. The hypothesis is that modified GIC with chlorhexidine restorations will reduce the quantity of microorganisms in samples obtained from plaque around restored teeth and unstimulated saliva. Other objectives of this study are to investigate the ART effectiveness and acceptability to participants.

**2.0 Experimental Approach and Methods**

**2.1.0 Study design:**

This study is designed as a non-randomised trial study with a split mouth design where one root caries lesion in one side of the mouth will be restored with chlorhexidine modified GIC to act as test and in the contralateral side another lesion will be restored using conventional GIC to be the control. If participants have only one root caries lesion, then a tooth from the contralateral side will be chosen and sampled for microbiological analysis for comparison.

**2.1.1 Participants:**

The study will be conducted at the University of Otago Faculty of Dentistry. A total of 34 participants will be recruited from patients attending the Removable Prosthodontics clinics. Power calculations from previous studies (Percival et al., 1991; Marsh et al., 1992; Percival et al., 1994) have demonstrated that the inclusion of 30 participants is sufficient to detect trends or statistically significant changes with 80% power and a Type 1 error rate of 5%. In this study, 4 more participants will be recruited to allow for dropouts or loss to follow up during the trial period. As these are repeated measurements, this pilot study will generate estimates of Intra Class Correlation (ICC) which will then be used to determine the sample size for future clinical trials.

**2.1.2 Inclusion and exclusion criteria:**

Patients attending the Removable Prosthodontics clinics who are 60 years of age or older will be screened as part of the normal clinical procedures and those who are identified as having at least one root caries lesion will be invited to participate in this study. Once participants accept the invitation, an information sheet and consent forms will be provided to the patients and only those who provide informed consent and are willing to follow the research schedule for the period of the study will be included. The invited participants will have their medical history checked and those who have received antibiotic therapy in the past month will be excluded from the study. In addition, those who had or are currently undergoing radiotherapy will be excluded. Moreover, those who have active periodontal disease that may require root surface debridement during the study will be excluded. Any participant currently using or having used a chlorhexidine product in the last four weeks or who is allergic to chlorhexidine will also be excluded. Those with significant dental problems such as acute infections or significant discomfort will be referred as appropriate for management.

**2.2.0 Methodology:**

**2.2.1 Participants and clinical procedure:**

Once recruited, participants who satisfy the inclusion criteria will be fully informed of the procedures involved and will be given clear instruction to not use any antimicrobial mouthwashes for a period of at least 6 months. After informed consent is obtained, participants will be assessed in the Department of Oral Rehabilitation 4N clinic. They will receive a full oral health assessment at baseline and a medical history will be recorded.

At the appointment when the ART will be applied, oral hygiene instructions will be given to the participants. They will be reminded not to use any mouthwashes and to brush teeth with toothpaste and a soft tooth brush twice daily, which will be supplied. One root caries lesion will be restored with the modified GIC and another carious lesion in the contralateral side will be restored using conventional GIC (Fuji IX). Which tooth receives which material will be determined randomly using a flip of a coin. If, however, no carious lesion in the contralateral side is found, then a tooth will be chosen to act as control and a plaque sample will be obtained for microbiological analysis. Plaque samples will be obtained from the control and the test teeth as well as unstimulated saliva samples before application of the ART and 1, 3 and 6 months after application. At the final visit (6 months after applying the ART), another full examination will be performed by a different operator to record survival of the restorations using the modified Ryge’s criteria to evaluate factors such as marginal defects, wear, and the need to replace or repair restorations (Ryge, 1980).

Preparation of the root surface carious lesions and application of the ART will follow the WHO ART guidelines (Frencken et al., 1996). Modified GIC will be applied using the ART technique to clinically visible root surface caries lesions, identified using visual and surface texture criteria (Banting et al., 1980; Banting et al., 1985; Banting, 2001) as follows:

Root caries is identified if:

1- There is 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 is located either at the cementum-enamel junction or wholly on the root surface.

GIC will be modified with 5% chlorhexidine digluconate according to the following method.

GIC Fuji IX GP GC Japan comes in powder and liquid form that is usually mixed for 25-30 seconds before application. The liquid will be modified by the addition of equal amount of 10% chlorhexidine digluconate 1/1 (w/w). This will generate a liquid with 5% chlorhexidine digluconate which can be mixed with the powder according to the manufacturer’s recommendations so that 1/2 a scoop of powder and one drop of liquid will be mixed for 10 seconds, then another half scoop will be added to the mixture and mixed for 15-20 seconds.

**2.2.2 ART application and GIC modification**

The restoration will be placed according to the ART method (Frencken et al., 1998). First the tooth will be isolated, plaque will be removed from the tooth surface with a wet cotton wool pellet, then the outer carious dentine will be removed with excavators. After that, any unsupported thin enamel/cementum will be broken off with a hatchet making sure that the enamel/cementum does not contain any carious spots. The cavity will be cleaned with wet and dry cotton wool pellets to ensure no plaque or debris are present. After dentine conditioning (using GC Dentin Conditioner made up of 10% polyacrylic acid) for 20 seconds according to the manufacturer’s recommendation, the cavity will be washed and gently dried with cotton wool pellets. High viscosity glass ionomer cement Fuji IX will be hand mixed for 20 seconds after modifying the liquid with 5% chlorhexidine digluconate. A small amount of the mixture will be inserted into the cavity using a flat plastic instrument or ball burnisher and packed in place, ensuring all cavity areas are filled properly. Petroleum jelly will be applied on the surface of the restorative material. Excess restorative material will be removed with a carver.

At baseline, 1 month, 3 months and 6 months after ART application, the survival rate, marginal defects and wear of the ART restorations will be recorded by a different clinician than the one who placed the restorations. Clinical evaluation and assessment forms will be based on the modified criteria proposed by Ryge 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) (Ryge, 1980). All participants will have pre-treatment and post-treatment photographs taken at baseline and at 6 months recall.

**Table 1:** Modified Ryge criteria for clinical evaluation of restorations (Ryge, 1980)

|  |  |  |
| --- | --- | --- |
| Category | Inspection type | Rating scale |
| Anatomic form | Visual inspection with mirror and explorer | 0 = The restoration is continuous with the existing anatomic form  1 = Slightly under/over contoured |
| Secondary caries | Visual inspection with mirror and explorer | 0 = No visible evidence  1\* = Visible evidence |
| Marginal adaptation | Visual inspection with mirror and explorer | 0 = Continuous with existing anatomic form  1 = Explorer catches but no crevice visible  2\* = Obvious crevice at margin, dentine or lute exposed |
| Surface roughness | Tactile diagnostics with explorer | 0 = Smooth  1 = Slightly rough  2 = Rough |
| Colour-match | Visual inspection | 0 = Very good/good, almost invisible  1 = Slight mismatch  2\* = Obvious/gross mismatch outside of normal range |
| Gingival health | Visual inspection with explorer | 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 |

* Unacceptable

To assess the acceptability of the treatment, participants will be given a questionnaire after the administration of the ART, and at 1 month, 3 months and 6 months after. The questions will be about the smoothness of the restoration, pain experience during and after the treatment, satisfaction with aesthetics, changes in taste, and anxiety and discomfort experienced during the procedure.

Microbiological analyses will be undertaken on samples of whole unstimulated saliva and dental plaque from the supragingival margins of the carious tooth collected prior to ART application and after 1, 3 and 6 months. At baseline, a clean explorer will be used to remove the plaque from the supragingival margins of the test and control teeth. Then the plaque sample will be placed in an anaerobic container and sealed immediately. To obtain an unstimulated saliva sample, patients will be asked to rinse their mouths with 10 ml sterile water and expectorate into a sterile container. Samples will be well-sealed, de-identified and transported to the microbiology laboratory in the Faculty of Dentistry for microbiological analysis.

**2.2.3 Sampling for microbiological analysis**

In this study, changes in cariogenic bacteria in plaque and saliva samples will be compared before and after administration of the ART. Samples will be serially diluted under anaerobic conditions and inoculated onto selective and non-selective media. Mean viable counts will be expressed as proportions of the total viable counts of the sample. Representative colonies will be sub-cultured for confirmation of identification. A portion of each sample will be stored in buffer at -80°C for 16S ribosomal RNA (16S rRNA) gene analysis. This is the gold standard technique to determine, qualitatively and quantitatively, the bacterial species present. New Zealand Genomics Ltd (NZGL) will be contracted to amplify and sequence the 16S rRNA genes and perform the subsequent bioinformatics.

Microbial loads before and after intervention will be compared using a paired t-test if data are normally distributed; otherwise, the equivalent non-parametric Wilcoxon Signed Ranks test will be applied. A P value of ≤ 0.05 will be considered statistically significant. Further analysis exploiting the repeated measures of microbial load (at baseline, one, three and six months) will be carried out. Data will be normalised by suitable transformations if not normally distributed.

**2.3 Timeline:**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activities | Jan–Mar 2017 | Apr-Jun 2017 | Jul-Sep 2017 | Oct-Dec 2017 | Jan–Mar 2018 | Apr-Jun 2018 | Jul-Sep 2018 | Oct-Dec 2018 |
| Recruitment and assessment |  |  |  |  |  |  |  |  |
| Participant consent, baseline samples |  |  |  |  |  |  |  |  |
| GIC-modified ART application |  |  |  |  |  |  |  |  |
| Collection of 1-month samples |  |  |  |  |  |  |  |  |
| Collection of 3-month samples |  |  |  |  |  |  |  |  |
| Collection of 6-month samples |  |  |  |  |  |  |  |  |
| Microbiological analyses |  |  |  |  |  |  |  |  |
| Data analysis and thesis writing |  |  |  |  |  |  |  |  |

**2.4 Potential outcomes and outputs**

The generated data will be published in a peer-reviewed journal and, if ART with modified GIC proves effective, they will form the basis of a follow-on randomised clinical trial aimed at examining its effectiveness, acceptability and cost-effectiveness in the prevention of root caries in elderly in care homes and/or frail elderly people who are housebound. Future studies will be aimed to investigate ART use for treatment of other vulnerable groups, for example, dental-phobics, individuals with mental or physical handicap and other special-needs groups, particularly where they have limited access to dental care. ART is a simple approach that will permit the training of dental therapists or other oral health care personnel to place ART restorations, which will improve the cost-effectiveness of the treatment. The ease with which ART can be applied on a domiciliary basis will further improve cost-effectiveness by reducing the need to transport patients to dental surgeries, and treatment will be applied in a less stressful environment.

**2.5 Dissemination**

The participants in the study will be informed of the outcome in writing at the end of the pilot study. The findings will be reported in University of Otago Faculty of Dentistry research meetings to staff and students (postgraduate and undergraduate) and it will be published

**2.6 Cost estimate**

Direct Salaries ($23,171)

A 0.1 FTE Professional Practice Fellow and 0.1 FTE dental assistant are needed for ART administration and clinical research and support.

Materials and Consumables ($2,100)

Materials and consumables are needed for clinical intervention (modified-GIC, disposable hand instruments), printing forms and clinical documents, and laboratory investigations (sterile storage containers, microbiological media, and chemicals).

Operating expenses ($24,600)

Bench fees are charged by the Faculty of Dentistry Molecular Biology Laboratory ($3,000 per year x2). Costs are also associated with genomics DNA purification, analysis and bioinformatics support ($15,600). The costs of treatment of each patient (for root caries only) will also be covered by this grant ($75 per participant x 35 = 2,625).

Other costs ($2,500)

Financial support is also needed to cover costs of appropriate equipment for clinical photography.

**Details of expenditure**

|  |  |  |
| --- | --- | --- |
| **Year 1 - 2017** | | |
| **Activities** | **Cost per unit** | **Total cost** |
| Direct salaries |  | 11,497 |
| Materials and consumables |  | 1,050 |
| Operating expenses |  | 4,800 |
| Other costs – clinical photography equipment | 2,500 | 2,500 |
| **Year 2 - 2018** | | |
| **Activities** | **Cost per unit** | **Total cost** |
| Direct salaries |  | 11,675 |
| Materials and consumables |  | 1,050 |
| Operating expenses – molecular studies |  | 19,800 |
| **TOTAL** | | **$ 52,372** |

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