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A new system using artificial calculus to analyze the roughness of dental materials

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A NEW SYSTEM USING ARTIFICIAL CALCULUS TO ANALYZE THE ROUGHNESS OF DENTAL MATERIALS

by

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DEDICATION

This work is dedicated to my wife and my parents, who guide, inspire, and encourage me to learn more each day and support me in every step throughout my journey in dentistry by transfer their knowledge in dental field to me.

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I would like to express my deepest appreciation to my mentors Dr. Tang, Xiaoren and Dr. Serge Dibart, for their constant support, encourage and willingness to guide me and teach me. Without their persistence and determination to accomplish truly magnificent research, this would not have been possible.

A NEW SYSTEM USING ARTIFICIAL CALCULUS TO ANALYZE THE ROUGHNESS OF DENTAL MATERIALS

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ABSTRACT

Periodontitis is a primary oral disease that can cause teeth loss. Home care is needed to prevent periodontitis, such as daily brushing using toothpaste, mouthwash, and other teeth-cleaning tools. However, we still need clarification on 1) whether periodontitis is caused by dental calculus and 2) whether commercial mouthwash or toothpaste can effectively inhibit the growth of oral bacteria in dental plaque that causes periodontitis. Therefore, we are interested in the formation and role of calculus around teeth. Since it is difficult to use human calculus, and the commercial artificial calculus (Dental Calculus Set, Nissin Dental Products INC) cannot be inoculated with bacteria due to its specific function, we tried to find an artificial calculus that can mimic and act as a human calculus. Recently, we discovered that whole milk can be stably solidified on teeth under certain conditions. We hypothesize that the solid whole milk can be used as an artificial calculus to understand and study the formation of dental plaque; the degree of roughness of dental materials may affect the formation of calculus and accumulation of cells on the surfaces adjacent to oral soft tissues and its effect on the progression of periodontitis and corresponding prevention and treatment.

As aim one, we used 5 µl whole milk with 1 µl of Aluminum chloride hydrate to examine the Milk Artificial Calculus (MAC) time length to form at 37°C. We mixed it with E. coli and tested the cell survival over 1,5,10 days at room temperature, then checked the effectiveness under different treatments at 37°C overnight like mouthwash, three different concentrations of alcohol (35%, 70%, and 100%), nine types of toothpaste, and different pH percentage (3.5%-9%). As aim two, we tested the effect of dental provisional, permanent, and removable prosthetic polymer materials with varying degrees of roughness on calculus and cell adhesion.

Our results show that the Milk Artificial Calculus (MAC) dried and formed around 10 minutes, and cells can survive up to 10 days at room temperature. There was no significant difference in the number of cell growth between different alcohol concentrations compared to the control; only one of the nine toothpastes significantly suppressed the cell growth compared to the power, and only 3.5 pH percentage has a significant difference compared to the control. MAC can be used as a good and reliable substitute for calculus. Although the roughness of materials tested here is similar (0.32-0.37µm), their resistance to MAC builds up or cell adhesion differs. A dental material with a lower level of roughness is more effective in resisting the formation of MAC and the adhesion of cells.

In conclusion, Milk Artificial Calculus (MAC) can mimic human calculus because it can attach to teeth and other dental materials. It is reliable, cheap, and easy to make and use—cell survival for up to 10 days with different treatments. In general,

mouthwash, 70% ethanol, or various toothpastes can't suppress cell survival in the milk-formed artificial calculus.

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3D	Three Dimensional
BU	Boston University
GAGs	Glycosaminoglycans
GCF	Gingival Crevicular Fluid
IgE	*Immunoglobulin E*
ISO	International Standards Organization
MAC	Milk Artificial Calculus
PD	Periodontal Disease
PMNs	*Polymorphonuclears*
RCMP	Royal Canadian Mounted Police
RES	Resistance
RET	Retention

INTRODUCTION

1. Periodontal disease.

Periodontal disease (PD) is an inclusive term for inflammation of the gingiva (gingivitis) and periodontium (periodontitis). The disease progresses from gingivitis to periodontitis [76]. It may be affected by systemic conditions such as diabetes mellitus, collagen diseases, leukemia, or other disorders of leukocyte function, anemia, or vitamin deficiency. An increased serum C-reactive protein, a marker for inflammation and a risk factor for coronary artery disease [77] links it to atherosclerosis.

Prevalence and epidemiology: Prevalence increases directly with age: 15% at age 10, 38% at age 20, 46% at age 35, and 54% at age 50. Men have a higher prevalence and severity than women. Prevalence is inversely related to increasing levels of education and income. Rural inhabitants have higher severity and prevalence than urban inhabitants [78]. Factors involved in host resistance include the following:

Gingival sulcus: A V-shaped crevice surrounding each tooth, bounded by the tooth on one side and the epithelium lining the free margin of the gingiva on the other. The sulcus is ideal for bacteria survival and is resistant to saliva's cleaning action. Gingival fluid (sulcular fluid) is a rich nutrient source for microorganisms. The depth of the gingival sulcus is a diagnostic parameter. A dentist should monitor patients with PD biannually [79].

Bacterial factors: bacterial plaque is the primary causative factor in PD. Bacteria secrete compounds detrimental to host defenses including endotoxins and exotoxins, free

radicals and collagen-destroying enzymes, leukotoxins, bacterial antigens, waste products, and toxic compounds [80].

Polymorphonuclear leukocytes (PMNs) or neutrophils are the first line of defense against microbial infection. PMN functional defects are catastrophic to the periodontium. PMNs are depressed in the elderly and those with diabetes, Crohn's disease, Chédiak-Higashi syndrome, Down's syndrome, and juvenile periodontitis thus establishing an extremely high risk for rapidly progressing PD [81]. Transient neutropenia and PMN function defects may cause alternating quiescence and exacerbation in PD. PMNs release numerous free radicals, collagenases, hyaluronidases, inflammatory mediators, and an osteoclast differentiation stimulator [81].

Macrophages and monocytes are present in increased numbers in PD. They phagocytize bacteria and debris. They are the primary source of prostaglandins in diseased gingiva and release abundant enzymes involved in collagen destruction [82].

Lymphocytes: produce lymphokines. Although other immune components overshadow their role, they promote PMN and monocyte chemotaxis, fibroblast destruction, and osteoclast activation [83].

The complement system: cascade may be activated by antigen- antibody complex (classic pathway) or by bacterial polysaccharide (alternative pathway). Activation initiates cascade (classic or alternative pathway) triggers immunologic and nonspecific resistance to infection and pathogenesis of tissue injury. Complement activation component regulate release of mediators from mast cells; promote smooth muscle contraction mediator chemotaxis of PMNs, monocytes, and eosinophils; and increase phagocytosis. The net

effect is increased gingival permeability, increased number of bacteria, and byproduct penetration, as well as a positive feedback cycle initiation. Other effects include solubilization of immune complexes, cell membrane lysis, neutralization of viruses, and killing of bacteria. In PD, complement activation by the alternative pathway in the periodontal pocket is a significant factor in tissue destruction [84].

Mast cells and immunoglobulin E (IgE): immunoglobulin bind to mast cells and causses degranulation. These cells when exposed to an allergen inflammatory mediator including histamine, prostaglandins, leukotrienes, kinins, serotonin, heparin and serine proteases). IgE complexes, complement components, mechanical trauma, endotoxins, and free radicals can initiate it. Increased IgE in the gingiva of PD patients suggests an allergic factor in the progression of PD in some patients [85].

Amalgam restorations: Faulty dental restorations and prostheses are common causes of gingival inflammation and periodontal destruction. Overhanging margins are ideal for plaque and bacteria. Silver amalgam decreases the activities of antioxidant enzymes. Mercury accumulation depletes free radicals–scavenging enzymes, glutathione peroxidase, superoxide, dismutase, and catalase. Proteoglycans and glycosaminoglycans (GAGs) of collagen are sensitive to free radicals [86].

Miscellaneous local factors include food impaction, unreplaced missing teeth, malocclusion, tongue thrusting, bruxism, toothbrush trauma, mouth breathing, and tobacco.

2. Dental Calculus

Dental calculus is formed by the accumulation of dental plaque and interaction with bacterial biofilm. The formation of dental calculus requires many days of calcification, so calculus cannot easily be removed by toothbrush and toothpaste or mouthwash. Dental calculus is one of the critical factors in the development of gingivitis and periodontal diseases that lead to tooth loss [1]. Understanding periodontal pathogenesis is important to improving and managing this common and complex disease. According to Merriam-Webster's Collegiate Dictionary, pathogenesis could be defined as "the origination and development of a disease." For example, bacterial plaque may lead to periodontitis, and plaque accumulation may also determine gingivitis.

Dental calculus can be classified as supragingival calculus (Figure 1) that is located coronally to the gingiva and can be easily seen. Subgingival calculus is apical to the gingival margin, which is not easily seen on routine clinical examination [2]. The two most common areas for supragingival dental calculus are the buccal surfaces of the maxillary molars and the lingual surfaces of the mandibular anterior teeth. Saliva flows from the upper molars parotid duct of the facial surface. In some cases [3], a bridge-like structure of dental calculus may form between adjacent teeth.

Subgingival dental calculus may be extended to the base of periodontal pockets in patients with chronic periodontitis [4], but the calculus cannot reach the junctional epithelium. Thus, once dental calculus is formed, a recession of gingival tissues may occur [5]. Subgingival calculus is exposed after a few days and reclassified as supragingival

calculus. Supragingival calculus consists of newly deposited and previous formed subgingival calculus. Both supra-and subgingival calculus are present on radiographs [6].

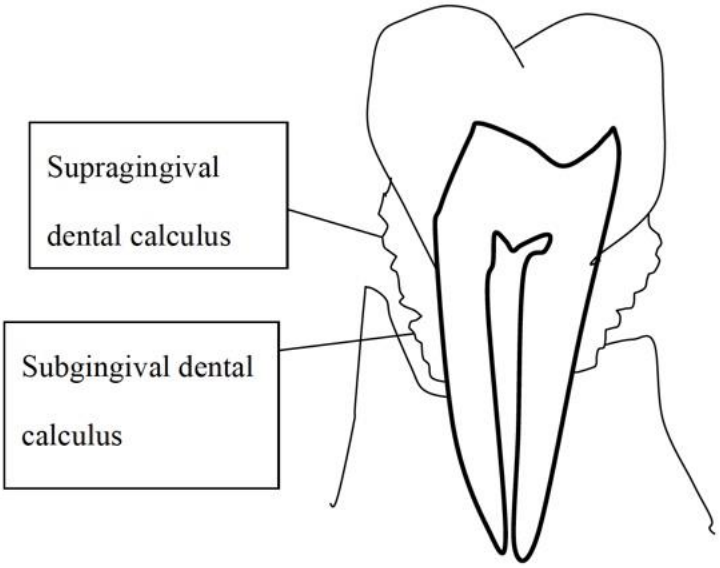


Figure 1 Site of attachment of dental calculus

Regular interdental cleaning (like Dental floss, dental tape, and interdental brush) is associated with a lower level of dental plaque and less chance of calculus formation [7]. Once dental calculus is formed, supra- and sub-gingival scaling using either hand or an ultrasonic device, can be used to remove it [8].

2.1 Formation process of dental calculus

Dental calculus can be classified as mineralized plaque, which requires plaque-induced calcification. The three steps in dental calculus formation are (1) formation of acquired pellicle, (2) plaque maturation, and (3) mineralization [9]. The first two steps are dealing with plaque formation. A plaque consisting of bacteria mixed into an intercellular matrix serves as an organic matrix for subsequent mineralization. In supragingival calculus formation, mineralization ingredients come from salivary gland secretions; in subgingival calculus, the ingredients come from gingival crevicular fluid (GCF). Formation starts with tiny crystals deposited on the plaque matrix, which is then wholly calcified for bacteria. The formation of dental calculus depends on the amount of bacterial plaque and secretion of fluids from salivary glands [10] or GCF. Saliva buffering may help regulate pH in the oral cavity, which could have substantial effects on oral health, which might lead to caries and calculus formation [11].

Hydroxyapatite, the primary form of calcium in human enamel, is a significant component of supragingival calculus [12]. Supragingival calculus usually form within two weeks once the oral environment is ideal for plaque accumulation and mineralization. Supragingival calculus formation are surgical and physio-chemical processes. When the pH of saliva increases, the hydroxyl groups are released. The

release of these molecules is positively correlated with phosphate and total protein content. Calculus formation is inversely proportional to the viscosity of saliva. Age, gender, and diet may affect calculus formation [13]. To a certain degree, mouthwash could decrease dental plaque [14].

The speed of dental calculus formation is different from person to person. Deposition speed for different teeth from the same person is also different, and it is related to various factors like metabolism, salivary components, GCF components, the amount of plaque, and the presence and/or type of food [16]. Soft and sticky foods are more likely to allow calculus deposition [3]. The speed of formation is also related to dental features such as a mal-aligned tooth or restoration surface. Children have less dental calculus than adults, which might be related to different strains of bacteria [17].

Although various attempts have been made to explain the calculus mineralization mechanism, a complete understanding is unknown. Plaque mineralization is influenced by the following two factors [18]:

(1) Core mineralization: Mineral deposition must be presented as core mineralization between plaque bacteria and epithelial cells and may be the primary substance of core material [19]. Plaque stromal cells are mainly protein-polysaccharide complexes, which may become a calcification center. Plaque bacteria, such as ciliated and actinomyces species, form a matrix that may the adsorbed salivary mineral on the tooth surface [20].

(2) Mineral deposition: Calcium-, phosphorus- and other minerals-containing salts or molecules supersaturated in saliva and a significant source of inorganic salts in supragingival calculus [21]. Subgingival calculus results from the exudates of mineral salts in GCF. Two pathways have been proposed to initiate the mineralization process [22]: (i) Carbon dioxide theory: when the saliva in the mouth flows out of the catheter, CO2 has such high tension, including calcium, phosphorus, and other mineral ions, which are all supersaturations. The tension of CO2 decreased by about half after entering the oral cavity. It increases saliva pH [23]. This theory explains why the supragingival calculus occurred in the significant salivary duct but cannot explain the subgingival calculus. The medical word "tension" is very confusing, such that the proper scientific term is "partial pressure." (ii) Ammonia formation theory: Ammonia proteolytic bacterial metabolism increases saliva or GCF's pH, which is related to protein solubility.

Some scholars have found that proteolytic activity was positively correlated with the formation of dental calculus.

2.2 Chemical properties of dental calculus

Different types of calcium phosphates have been found in calculus, brushite (CaH(PO4) × 2H2O), octa calcium phosphate (OCP, Ca4H(PO4)3× 2H2O), hydroxyapatite (HA, Ca5(PO4)3× OH)and whitlockite (β -Ca3(PO4)2)[24].

OCP was reported in the exterior layer of supragingival calculus and hydroxyapatite was present that was in the inner layer [25]. Brushite could only be found in freshly formed calculus less than two weeks old. The inorganic component of dental calculus is supposed to be close to bone, dentine, and cementum. The organic is less extensive in GCF-induced subgingival calculus, which contains proteins, lipids, carbohydrates, protein-polysaccharide complexes, desquamated epithelial cells, leukocytes, and various microorganisms [26].

2.3 Biology aspects of dental calculus

Dental calculus is the result of the deposition of dental plaque, which starts with biofilm formation [29], a thin layer of film formed on the tooth surface from salivery glycoproteins.

After cleaning the tooth surface, some of the components of saliva will quickly be attached, and a thin film of non-cellular material will be formed [30]. Once the film forms on the tooth surface, floating bacteria in the mouth will attach to it bacterial colonization [31]. streptococcus bacteria from blood are the first species to colonize on the tooth surface [32].

The nutrition sources for the supragingival and subgingival calculus are entirely different, the bacterial composition of supra- and subgingival plaque is also different [33]. Supragingival plaque has the non-calcified bacterial mass attached to the tooth surface or the surface of the prosthesis. Subgingival plaque is located under the gingival margin, and

is influenced by fluid from the gingival crevicular or periodontal pocket, which has no self-cleaning. Bacteria may survive under the gingiva, where fluid is nutritionally rich from the blood nutrients. Gingival crevicular or periodontal pockets of less oxidation-reduction potential tend to faster growth of anaerobic bacteria [34]. The pocket is a relatively stable environment that allows bacterial retention.

2.4 Detection of Dental Calculus

Supragingival calculus is located coronally to the gingival and is visible, but subgingival calculus usually cannot be seen by the naked eye. To detect dental calculus, three technologies are currently used:

(1) Perioscopy: can be used in curing periodontal diseases without surgery. Miniaturized digital video technology enables clinicians to diagnose and treat areas below the gum line, which should be without surgical discomfort and inconvenience. The periscope is not widely used because it is costly and complex to operate and individuals need to be trained before use [35].

(2) Optical spectrometry: Dect-Tar calculus detection utilizes light-emitting diodes and fiber-optic technologies. It can detect calculus by the absorption, reflection, and deflection of the red line. Dental calculus detection by optical spectrometry is signaled by an audible and luminous signal [36].

(3) Autofluroscene-based technology: Subgingival calculus is complex and can be identified by measuring to the probing or dental radiographs. Ultraviolet right can excite

some fluorescent chemicals that can distinguish calculus from a healthy and unaffected surface [35].

2.5 Pathogenic role of dental calculus

Dental calculus is the leading cause of periodontal disease. Epidemiological surveys confirmed that plaque and periodontal inflammation are positively correlated [37]. Although dental calculus may mechanically stimulate gums, its rough surface does not cause gingivitis [38]. Experiments in monkeys found that junctional epithelium in the sterile calculus can form hemidesmosomes and epithelial basement adhesion on the membrane and that calculus in vivo cannot induce inflammation or an abscess [39]. This study ruled out the possibility that calculus per se might be the original cause of periodontal disease, in which the effects could be secondary to plaque on the dental calculus [30]. Due to the presence of dental plaque, calculus must have direct contact with the tissue surface. For motion to occur. The porous structure of calculus can easily absorb large amounts of bacterial toxins. Some oral hygiene procedures may be hampered by such things as bleeding gums, deepening periodontal pockets, and alveolar bone resorption [40]. Calculus is a significant risk factor for periodontal disease.

3. Removal of dental calculus

Dental calculus may lead gingivitis and periodontitis. Periodontal disease is a significant risk factor for tooth loss. Dental calculus removal reduces oral microflora and the risk of transient bacteriemia. Poor oral hygiene is a predisposing factor in the development of infective endocarditis. Regular dental scaling improves oral hygiene by reducing the bacterial load from the periodontal pocket and reducing the risk of infective endocarditis [41].

3.1 Hand instruments

Scalers and curettes (Figure 2 & Figure 3) are the leading hand instruments for dental scaling. Curettes aim at root planning and debridement of subgingival calculus [36]. Since the naked eye cannot see subgingival calculus. Using only hand instruments for dental scaling may not totally remove the calculus [42].

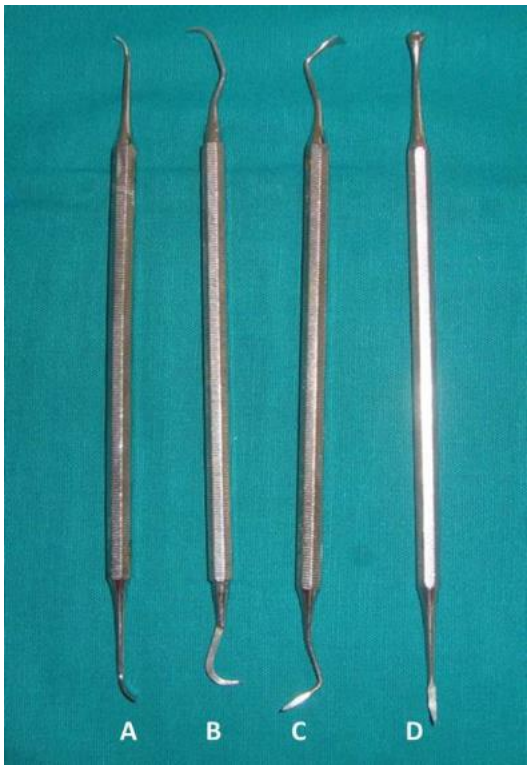


Figure 2 Supragingival scalers. (A) Surface scaler, (B) anterior sickle scaler, (C) Posterior sickle scaler, (D) universal scaler.

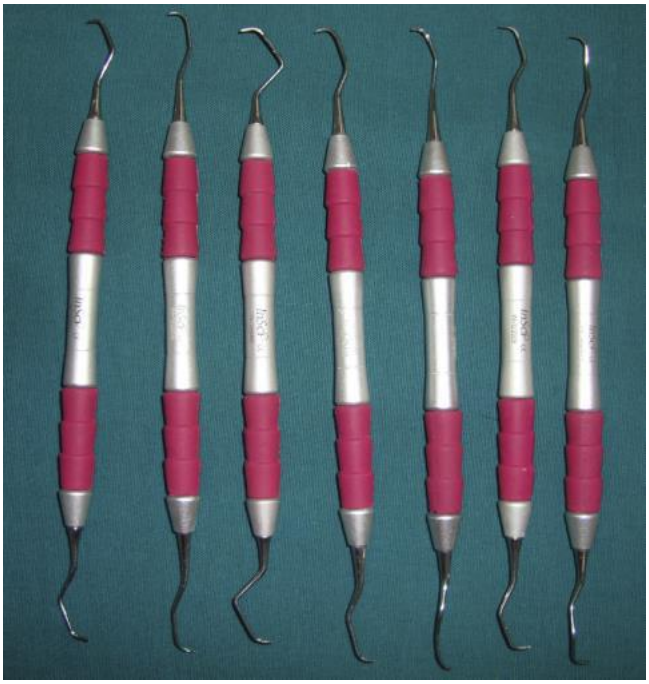


Figure 3 Gracey curettes for subgingival scaling (area specific curettes).

3.2 Ultrasonic instruments

Ultrasonic instruments are timesaving compared to hand instruments. These instruments oscillate at high speeds and generate thermal effects, cavitation, acoustic micro-streaming, and radiation force [43]. They are regarded as efficient and economically favorable [44]. Controversial opinions due to various operative techniques and instrumentation that might yield different results. After scaling by hand instruments, root surfaces will be flattened. Ultrasonic instruments will produce a root surfaces that will be undulated with a texture [45].

3.2.1 Magnetostrictive ultrasonic instruments:

The vibrations of magnetostrictive ultrasonic instruments are generated by resonating stack of metal strips [46]. It could be used to remove light to moderate subgingival dental calculus [47].

2.2.2 Piezoelectric ultrasonic instruments:

The vibrations of piezoelectric ultrasonic instruments are generated by oscillations using a piezoelectric crystal. The system has a significant increased calculus removal than magnetostrictive or hand instrumentation [36].

3.3 Other oral hygiene tools

Behavior changes affect periodontal diseases [48]. It is necessary to conduct independent research into other oral hygiene tools, such as toothbrushes, interdental aids, toothpaste, and mouthwash and gel-containing antibacterial agents [49], to evaluate their effects on calculus formation. Toothpaste could reduce plaque formation or inhibit plaque calcification by interfering with calcium phosphate crystal formation. In early anti-calculus drug research, pyrophosphate interfered with converting amorphous calcium phosphate to hydroxyapatite, which could prevent calcification [50]. Parotid saliva has pyrophosphate concentrations lower than those that inhibit calculus. Plaque and saliva content of pyrophosphate impacts calculus formation. Both laboratory and animal experiments

demonstrated that pyrophosphate adversely affects experimental calculus formation. Using pyrophosphate in toothpaste might be a simple method to inhibit the formation of dental calculus.

4. Periodontal tissue

4.1 Saliva

Human saliva consists of 99.5% water, and the remnant 0.5% are electrolytes, mucus, glycoproteins, enzymes and anti-bacterial compounds such as secretory IgA and lysozyme [51]. Saliva contains sodium, potassium, calcium, magnesium, chloride, bicarbonate, and phosphate ions [52]. These ions act as a buffer and maintain the pH of the oral cavity between 6.2 and 7.4. Precipitation of some salivary proteins and maintenance of oral pH may prevent dissolution of minerals [53]. Radiotherapy may reduce saliva secretion and protein production [54] with fluctuating values of sodium, potassium, and chloride ions during and after radiotherapy (Figure 4) [55].

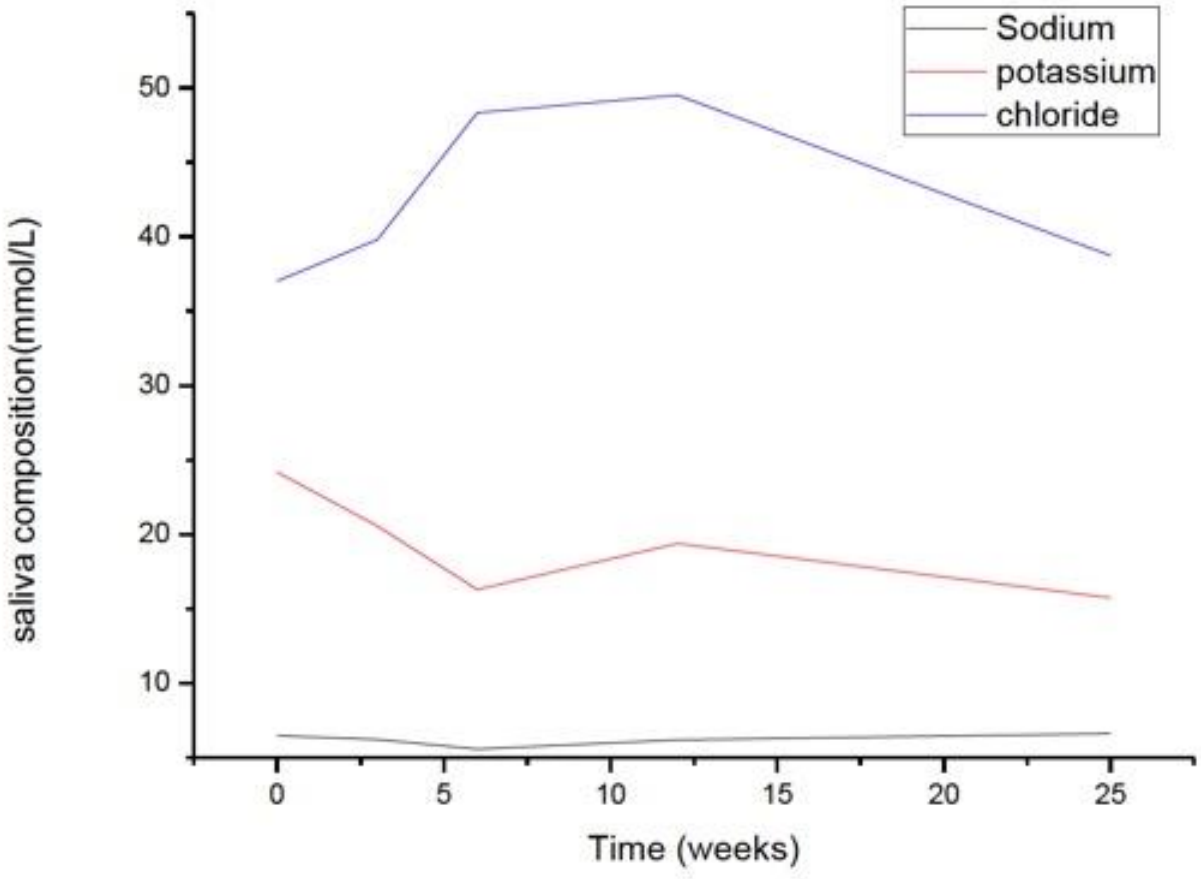


Figure 4 Mean values of sodium, potassium and chloride during and after radiotherapy

Saliva is one of the most crucial body fluids to maintain oral health [56]. Every day, an average of 600 ml saliva is secreted by three pairs of primary and many minor salivary glands. Saliva has lubricating, buffering, cleaning, anti-microbial, agglutination, film forming, digestion, and other functions [57]. Saliva is also essential to the oral immune defense system. The protective effects of saliva are closely related to its active

components, flow volume, and flow rate. Saliva dysfunction can cause severe oral hard and soft tissue disorders.

The physical properties of saliva provides physiological protection [58]. Effective saliva flow rate provides necessary lubrication to assist food delivery, bacterial removal, and epithelial shedding. Constant secretion of saliva disturbs antibacterial components, such as lysozyme, lactoferrin, catalase, and immunoglobulin-rich histone protein. The buffering effect of saliva helps to maintain homeostasis of enamel. Changes in the physical properties of saliva, such as reducing the flow rate or reducing buffering action, may lead to a decline in saliva defensive functions [59] and may increase the occurrence and development of dental caries and periodontal disease.

Salivary proteins are involved in the initial formation of plaque, in which a saliva film is first formed on a clean tooth surface, followed by bacterial attachment and colonization [60]. Saliva is also involved in the formation and mineralization of dental calculus.

Saliva is also rich in anti-microbial ingredients [61], including lysozyme, peroxidase, lactoferrin, secretory immunoglobulin A, IgG, and IgM. The presence of specific antibodies and pathogens in saliva have been confirmed [63].

Other salivary proteins, such as proline-rich proteins, cysteine-rich proteins, and mucin-rich histones, may affect the ecological environment of oral bacteria [64].

5. Artificial dental calculus

Subgingival scaling and root planning are the primary treatments for periodontal disease. The main goal for subgingival scaling is to remove dental calculus, while root planning removes residual calculus and diseased cementum. Clinically, this is a continuous process, so the two methods cannot be separated. Currently spatulating curettage is the mostly effective used tool. In order to remove plaque, calculus, and diseased tissue quickly and thoroughly, dental students and oral hygienists must have sufficient scaling training. Artificial dental calculus (Figure 5) is one of the training tools, combined with a tooth model, for scaling training.

The artificial calculus set contains a bottle of liquid and two bottles of powder. The instructions are: (1) after preparing the tooth model, mix the liquid and powder until it becomes a single small lump. (2) apply the lump quickly to the tooth model and shape it to the form and size as you like, and let it cure for 60 minutes. (3) place the tooth model into the jaw model and start scaling. The powders A and B have the same ingredients. The only difference between them is color, with one darker which is easier to distinguish.

The colors of artificial calculus formed from these two powders are quite different from real dental calculus. This artificial calculus may be too easy to remove and may not correctly represent real dental calculus. Users cannot use it to check dental materials' properties such as surface roughness or calculus adhesion to different surfaces or tissue damage from acidity or cytotoxins released from human calculus that leads to periodontal or dental diseases. This study aimed to find an artificial calculus into which bacterial cells can grow inside and adhere to different dental materials' surfaces.

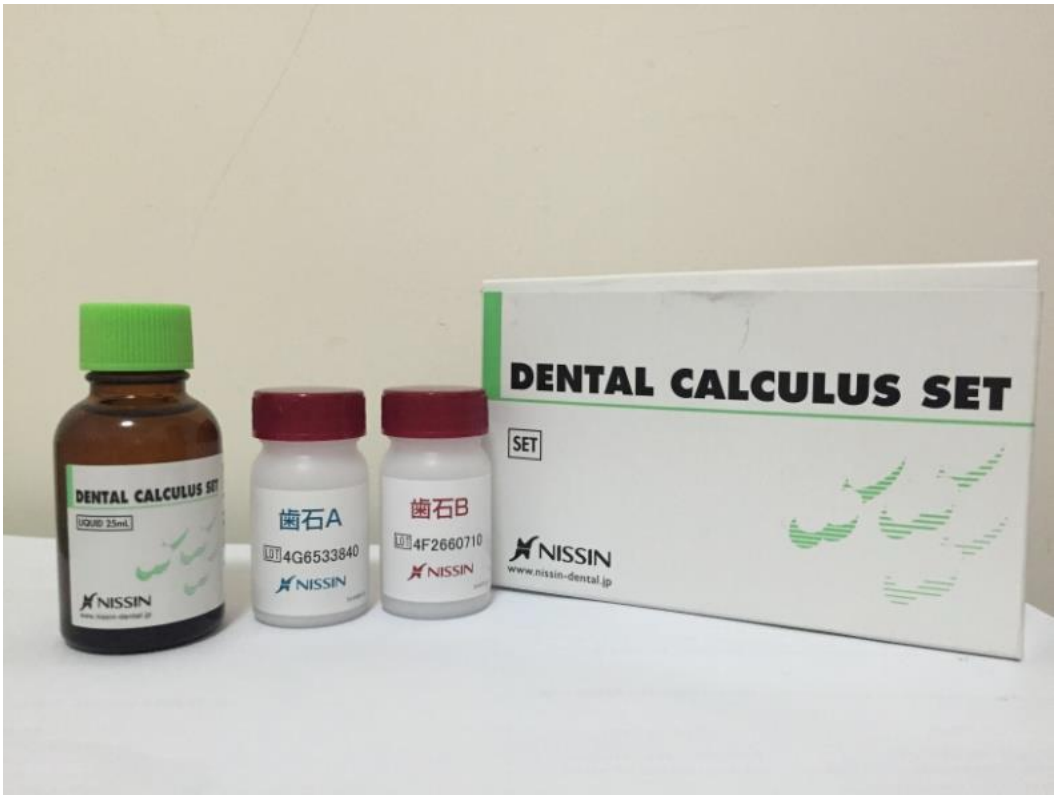


Figure 5 Artificial dental calculus (Nissin Dental, Japan)set for training purpose.

Hypothesis

We hypothesize that 1) The solid Milk Artificial Calculus (MAC) can be used to study the efficacy of home oral hygiene tools; 2) This MAC as a new system can be used to analyze the roughness of dental materials.

Aim

The aim of this study is:

- To evaluate the milk artificial calculus (MAC) under different treatment protocols.
- To determine the effect of surface roughness of different dental materials on milk artificial calculus (MAC) adhesion.

AIM ONE

MATERIAL AND METHODS

1.1 Cell lines

A. E.coli bacterial cells were stored in -80°C then took 2µl in 2% Lysogeny broth (LB) medium over night at 37 °C shaker incubator with 85 rpm speed. then spread on 2% LB medium agar over night at 37 °C shaker incubator with 85 rpm speed. Later one colony picked and grown in 2% LB medium over night at 37 °C shaker incubator with 85 rpm speed.

B. RAW cells (Cat#, TIB-202™, ATCC®) were grown in RPMI medium 1640 (Cat#: 11875-093, Life Technologies, NY) with 10% FBS (Fetal Bovine Serum, Sigma) at 37 °C in a 5% CO2 atmosphere.

1.2 Milk Artificial Calculus (MAC) formation

Whole milk (5 µl) was mixed with 1µl of pre- cultured E. coli ($5x10^2$) cells aluminum chloride hydrate (1 µl) was placed as one drop on Petri dish. Each plate was incubated at 37ºC for 0, 2, 4, 6, 8, 10 minutes. PBS (30 µl)was added to each spot for 5 minutes then transferred to 2% LB solid medium and incubated at 37ºC overnight. Colonies were measured and counted for analyzsis.

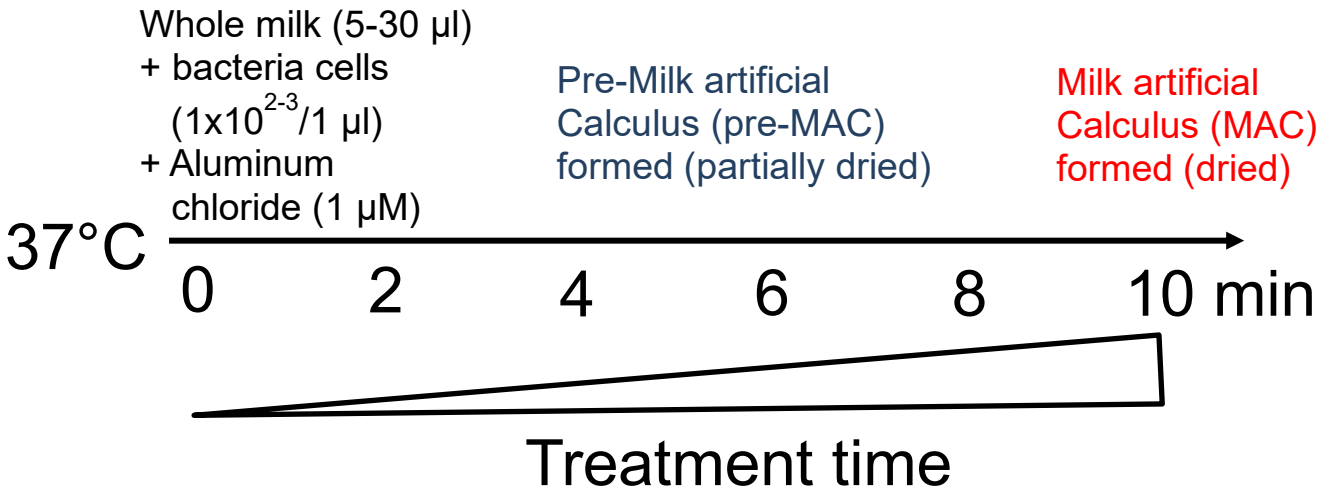


Figure 6 Time frame for formation of Milk Artificial Calculus.

1.3 Milk Artificial Calculus (MAC) storage time.

Whole milk (5 µl) was mixed with 1µl of pre- cultured E. coli ($5x10^2$) cells aluminum chloride hydrate 1 µl was added and incubated for 3 time periods (1, 5, 10) days at room temperature then transferred to 4°C. Each sample of Milk Artificial Calculus (MAC) was suspended in 30 µl pBS and placed into a plate with 2% LB solid medium and incubated at 37ºC overnight, Colonies were then measured and counted.

1.4 Milk Artificial Calculus (MAC) with different treatments.

In four small tubes, mix 5 µl of whole milk with 1 µl of pre-cultured *E. coli* (5 x 10^2 CFU) and 1 µl of aluminum chloride hydrate. Allow the mixture to dry for 10 minutes to form the milk artificial calculus (MAC). Then, to each tube, add 30 µl of the following treatments: diluted Sensodyne toothpaste (Pro-Enamel) to the first tube, Listerine mouthwash to the second tube, 70% ethanol to the third tube, and phosphate-buffered saline (PBS) as a control in the fourth tube. Let the samples sit at room temperature for 5 minutes. Afterward, collect all samples and plate them on 2% LB solid medium, incubating at 37°C overnight. Finally, measure and count the colonies that develop.

1.5 Milk Artificial Calculus (MAC) with different Alcohol concentration.

In four small tubes, mix 5 µl of whole milk with 1 µl of pre-cultured *E. coli* (5 x 10^2 CFU) and 1 µl of aluminum chloride hydrate. Allow the mixture to dry for 10 minutes to form the milk artificial calculus (MAC). Next, add 30 µl of different ethanol concentrations to each tube: 35% ethanol to the first, 70% ethanol to the second, 100%

ethanol to the third, and phosphate-buffered saline (PBS) as a control in the fourth tube. Let the samples sit at room temperature for 5 minutes, then wash each tube with 500 µl of PBS. Collect the samples and transfer them to plates containing 2% LB solid medium, incubating at 37°C overnight. Finally, measure and count the colonies that develop.

1.6 Milk Artificial Calculus (MAC) with different toothpastes (non-dried).

In ten small tubes, mix 5 µl of whole milk with 1 µl of pre-cultured *E. coli* (5 x 10^2 CFU) and 1 µl of aluminum chloride hydrate. Then, add 30 µl of each of the nine types of diluted toothpaste, labeled as follows: 1) Clinpro 5000 (3M), 2) Clinpro Tooth Crème (3M), 3) Enamelon (Premier), 4) Pronamel Active Shield (Sensodyne), 5) MI Paste Plus (GG), 6) ProMineralizer (Oral Health), 7) Remin Pro (Voco), 8) Gel Kam (Colgate), and 9) Plaque ProRelease (Colgate). The tenth tube will contain phosphate-buffered saline (PBS) as a control. Incubate all samples at room temperature for 5 minutes, then collect them and transfer to plates containing 2% LB solid medium. Incubate the plates at 37°C overnight, and then measure and count the colonies that develop.

1.6 Milk Artificial Calculus (MAC) with different toothpastes (dried).

In five wells of a 96-well plate, mix 5 µl of whole milk with 1 µl of pre-cultured *E. coli* (5 x 10^2 CFU) and 1 µl of aluminum chloride hydrate. Then, add 30 µl of each of the five types of diluted toothpaste, numbered 1 to 5, and use phosphate-buffered saline (PBS) as a control in the last well. Incubate the samples at room temperature for 10 minutes, then wash each well with 500 µl of PBS. After washing, add 30 µl of PBS to collect the samples

and transfer them to plates containing 2% LB solid medium. Incubate the plates at 37°C overnight, and measure and count the resulting colonies.

Additionally, a pH detector was used to examine the pH levels of all nine toothpastes, leading us to select different pH conditions for the experiment.

1.7 Milk Artificial Calculus (MAC) with different potential of hydrogen (pH).

In seven small tubes, mix 5 µl of whole milk with 1 µl of pre-cultured *E. coli* (5 x 10^2 CFU) and 1 µl of aluminum chloride hydrate. Add toothpaste (sample no. 4) to the first five tubes, each adjusted to different pH levels: 3.5%, 4.5%, 6.5%, 7.5%, and 9%. The seventh tube will contain only phosphate-buffered saline (PBS) as a control. Incubate the samples at room temperature for 5 minutes, then collect all samples and transfer them to plates containing 2% LB solid medium. Incubate the plates at 37°C overnight, and then measure and count the resulting colonies.

1.8 Statistical analysis

All experiments were performed in triplicate. Statistical analysis was conduct with the SAS software package.

RESULTS AIM ONE

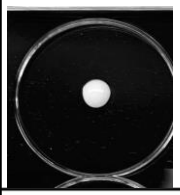
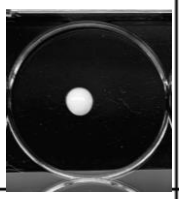
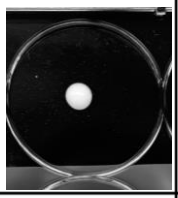
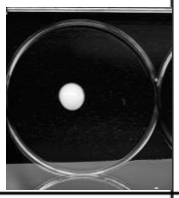
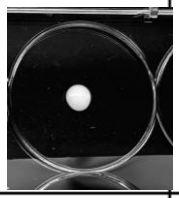
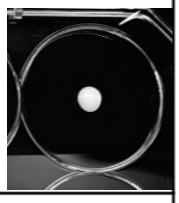
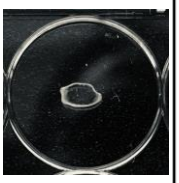
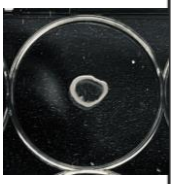
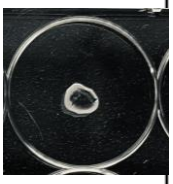
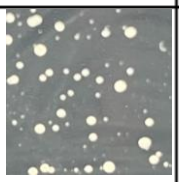
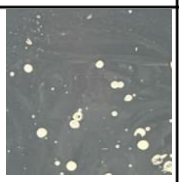
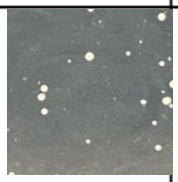
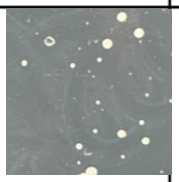
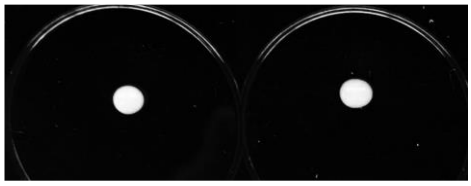
Time for treatment	0 min	2 min	4 min	6 min	8 min	10 min
5 µl milk with 1 µl *E.coli* cells ($5x10^2$) as a spot dripped on the plastic plate. Each spot was incubated at 37°C for different time range from 0-10 min.						
30 µl pBS was lightly added into each spot for 5 min, then transferred into LB solid medium and incubated at 37°C o/n.						
Cell colonies were grown on the LB medium and counted. Its numbers of colonies were shown below.						
Cell No. / comparison ratio (%)	80 (100 %)	79 (99 %)	55 (68.7%)	27 (33.7%)	27 (33.7%)	9 (11 %)

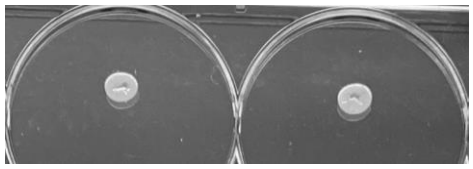
Figure 7 Time frame to form Milk Artificial Calculus (MAC)

The table in Figure 7 shows the time required for artificial milk calculus to harden. The first row shows the mixture of artificial milk calculus (MAC) with E.coli bacterial cells placed on petri plates and incubated for about 10 min to transform from liquid to solid. The second row shows the amount of MAC mixture left on the plastic plates after each period (0, 2, 4, 6, 8, 10 min). The third row shows of cell colonies grown that MAC on LB medium agar plates incubated at 37°C overnight. The fourth row shows that cells are still growing on the surface layer of MAC after 10 min compared to the percentage of cells grown at other times frames.

Whole milk (5-30 µl)+ E.coli bacteria ($1x10^{2-3}$/1 µl) + Alumnum chloride (1 µM)



Milk artificial Calculus (MAC) formed (dried)



MAC was washed with 30 µl pBS, then wash buffer was collected and plated into LB solid medium in plate o/n.

MAC was added with 30 µl pBS. All solution and MAC mixture were collected and plated into LB solid medium in plate o/n.

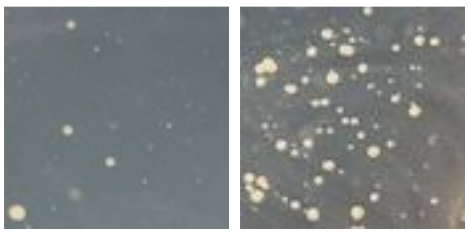
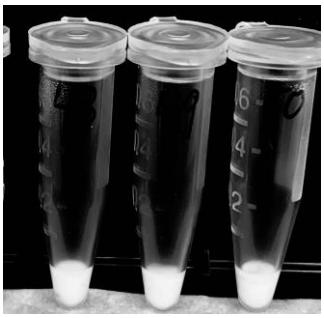


Figure 8 Cell survive on and inside MAC

After mixing whole milk (5-30 µl) with E.coli bacteria(1x102-3/1 µl) and Aluminum chloride hydrate (1 µM) required 10 min to become solid at room temperature, Washing with 30 µl pBS either entirely removed of MAC or only removed the superficial layer of MAC then culturing the solution on 2% LB agar plates overnight at 37' C, shows that the E.coli bacteria still survive inside MAC.



MAC was stored at room temperature for different time periods: 1, 5 or 10 days, then, transferred to 4°C.

1 day 5 day 10 day

Each MAC was suspended in 30 µl pBS, collected and plated into LB solid medium in plate o/n.

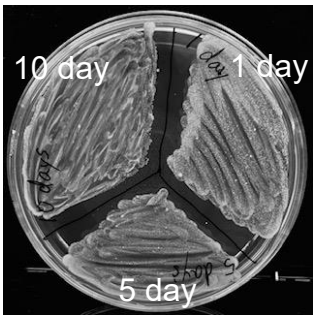
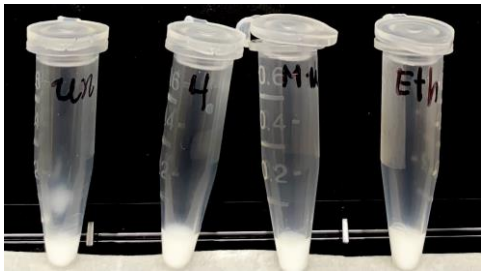


Figure 9 The time periods to storge MAC

MAC mixed with E. coli bacteria was incubated at room temperature for 1, 5, and 10 days. MAC was collected by washing with 30 µl PBS. The mixture was then placed on 2% LB agar solid medium at 37’ C overnight. The bacteria colonies were counted manually, the E. coli survival rate was not significant between 1, 5, and 10 days.

MAC with *E.coli*



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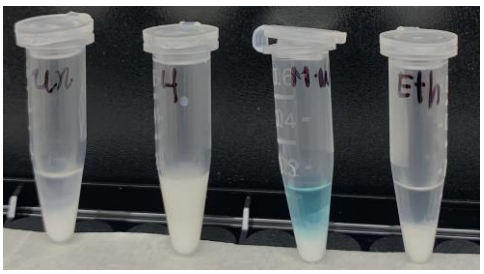


Plated into LB solid medium at 37°C o/n

untreat	Treated with paste	Treated with mouth wash	Treated with 70% Ethanol

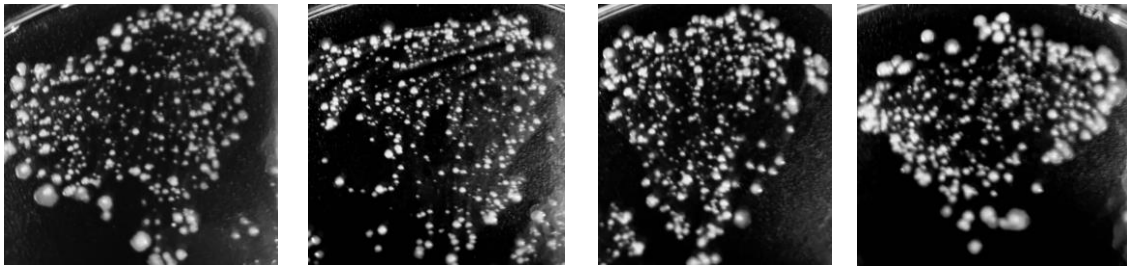
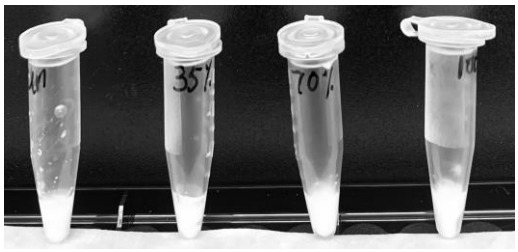


Figure 10 MAC treated with mouthwash, toothpaste and 70% ethanol.

MAC mixed with E.coli bacteria in were incubated three separate tubes at room temperature for ten minutes to become solid, Listerine mouthwash, Sensodyne toothpaste, and 70% ethanol, were added and samples were collected and placed on 2% LB agar solid medium at 37' C overnight. Bacterial colonies were counted manually, and the E.coli bacterial cell survival rate insignificant.

MAC with *E.coli*



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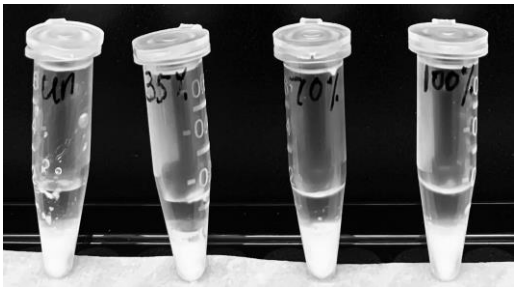
35%, 70% or 100% Ethanol



washed with 500µl pBS

Plated into LB solid medium at 37°C o/n

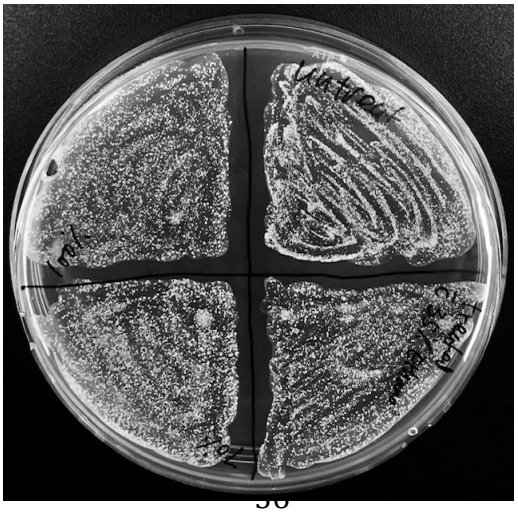
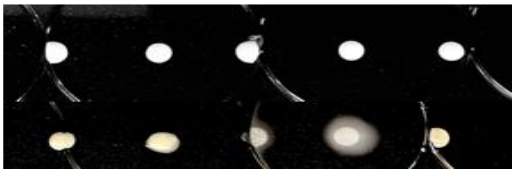


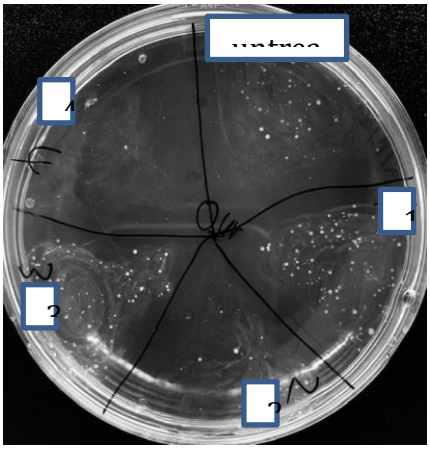
Figure 11 MAC treated with 35%, 70% , and 100% ethanol.

MAC mixed with E.coli bacteria in were placed in three separate tubes at room temperature for ten minutes to become solid, Ethanol concentration of 35%, 70%, and 100% were added, the samples were collected and placed on 2% LB agar solid medium at 37' C and incubated overnight. Bacterial colonies were counted manually, and the E.coli bacterial cell survival rate were calculated there were no significant differences between 35%, 70%, and 100% ethanol.

Pre-MAC+*E.coli* was respectively added with different brands of tooth paste and mixed well and incubated at room temperature for 5 min.



The mixture was collected and plated into LB solid medium (plates) and incubated at 37°C o/n. The results (cell colonies) shown and attached below.



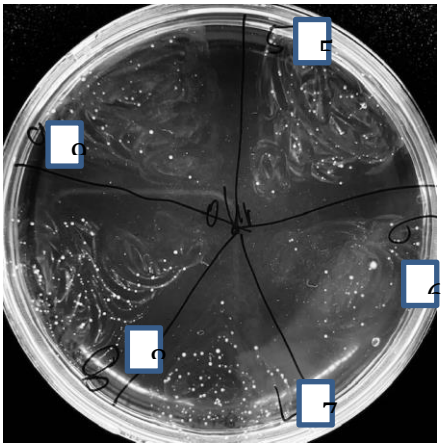


Figure 12 Pre-MAC treated with different toothpastes.

MAC mixed with E.coli bacteria were placed on a petri dish coverslip at room temperature for 5 minutes. Toothpastes ESPE Clinpro 5000, ESPS Clinpro Tooth Crème, Premier Enamelon, Sensodyne ProEnamel, MI Paste Plus, Oral Health Pro-Mineralizer, VOCO Remin Pro, Colgate Gel-Kam, Colgate Plaque Pro-Release were added. The samples were collected and placed on 2% LB agar solid medium at 37' C and incubated overnight, and then bacterial cell colonies were counted manually; the E.coli bacteria survival rate was not significant among all toothpastes except Sensodyne ProEnamel showed a significant reduction in survival rate of bacterial cells.

MAC with *E.coli* was formed in each well of 96 well plate

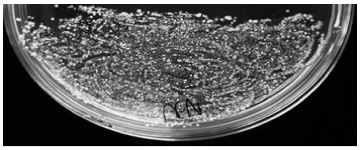
untreated as control and respectively treated with 5 different tooth pastes

Untreat 1 2 3 4 5 (paste)

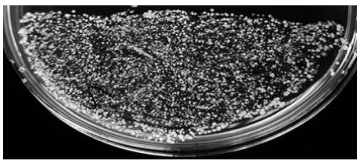
washed with 500µl pBS

All materials in a well were respectively collected and plated into LB solid medium (plates) and incubated at 37°C o/n. The results (cell colonies) shown and attached right side.

Untreat



1



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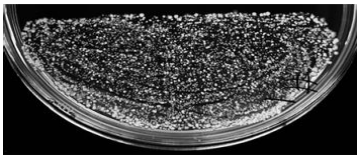

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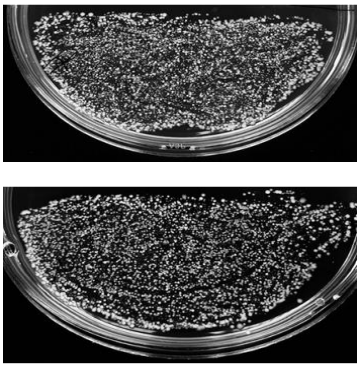


4

5 (paste)

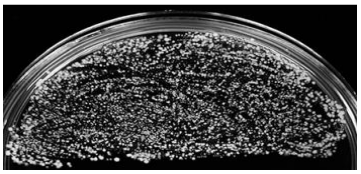


Figure 13 Solid MAC treated with four different toothpastes.

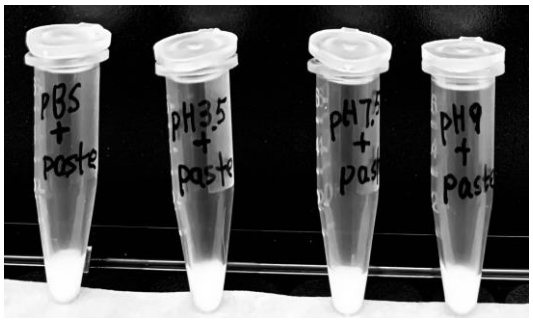
MAC mixed with E.coli bacteria were placed on a petri dish coverslip at room temperature for 10 minutes to become solid. Toothpastes including ESPE Clinpro 5000, ESPS Clinpro Tooth Crème, Premier Enamelon, Sensodyne ProEnamel were added. Each sample was washed with 500 µl PBS. The samples were collected and placed on 2% LB agar solid medium at 37' C and incubated overnight. Bacterial colonies were counted manually, and the E.coli bacterial cell survival rate was calculated. There were no significant differences between the treatments with the four toothpastes.

paste pH 6	paste pH 4	paste pH 7	paste pH 9

paste pH 6	paste pH 6	paste pH 5	paste pH 6	paste pH 6

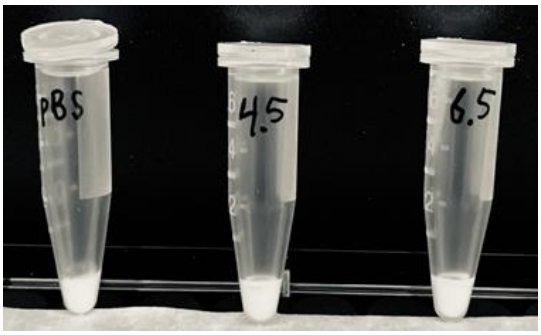
Figure 14 Toothpaste with different pH level.

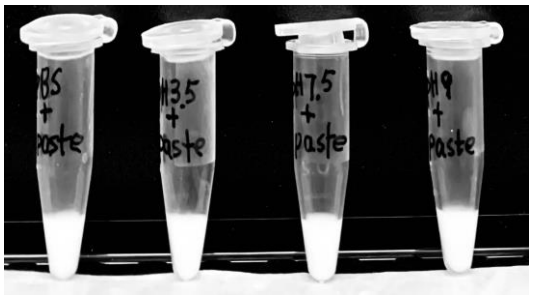
Figure 14 shows different brands of toothpaste tested with pH-indictor strips and indicates different pH levels for each toothpaste: ESPE Clinpro 5000 pH 6. Also, ESPS Clinpro Tooth Crème, Sensodyne ProEnamel, MI Paste Plus, and Colgate Plaque Pro-Release have a pH of 6. Premier Enamelon showed a pH of 5. Oral Health Pro-Mineralizer shows a pH 9, VOCO Remin Pro, at pH of 7, while Colgate Gel-Kam had a Ph 4.

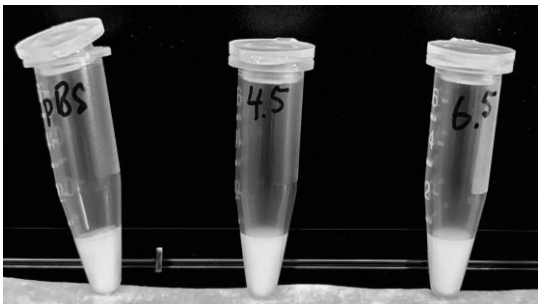


MAC With *E.coli*

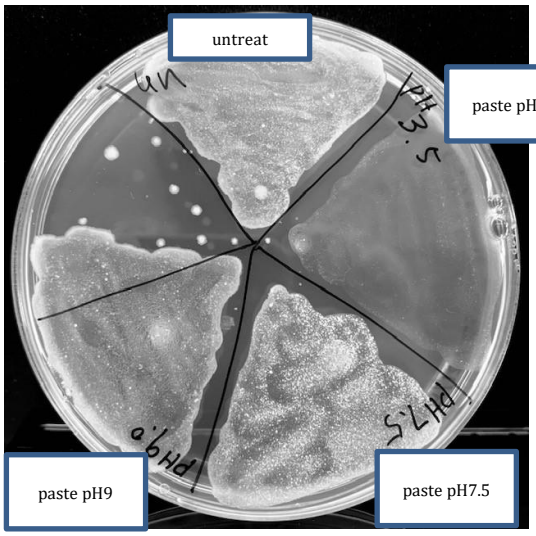
+







ph value of paste was adjusted to 3.5, 4.5, 6.5, 7.5, or 9



Plated into LB solid medium at 37°C o/n

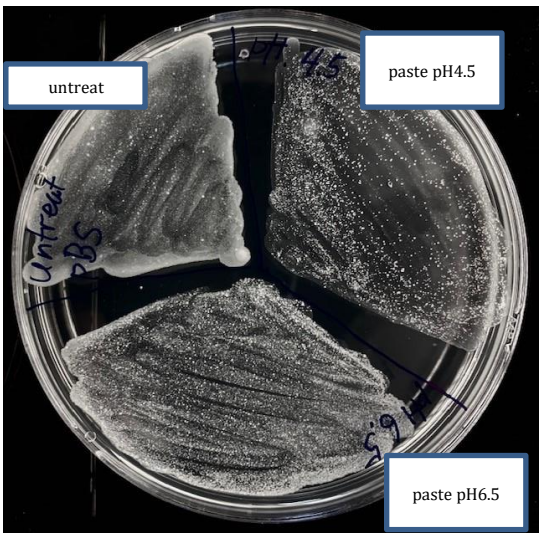


Figure 15 pH value of one toothpaste adjusted to different level.

MAC was mixed with E. coli bacteria in five separate tubes at room temperature for ten minutes to become solid. Sensodyne toothpaste with the pH adjusted to 3.5, 4.5, 6.5, 7.5, and 9 was added. The samples were placed on 2% LB agar solid medium at 37' C and incubated overnight. Bacterial colonies were counted manually and the E. coli survival rate calculated. The rate was not significant of different between pH levels 3.5, 4.5, 6.5, 7.5, and 9.

AIM TWO

MATERIAL AND METHODS

2.1 Polymer Dental Materials

Three brands of CAD/CAM denture base materials and two types of crown and bridge provisional materials, one 3D printed permanent tooth material and human adult tooth (< 1 μm as control) Table 1, were used to test cell adhesion and calculus formation. The CAD/CAM materials were sectioned into tiles using a diamond saw. The Rodin 3DP resin was printed into 2 mm thick bars using an Asiga Max printer and post processed according to manufactures instructions. The tested surfaces were polished with diamond grinding disk (Buehler Apex) to produce different roughnesses. The surface roughness values were tested by a stylus profilometer (Mitutoyo SJ-201).

Material Brand Name	Category	Lot Number	Composition	Roughness
Telio CAD	CAD/CAM block for provisional prosthesis.	ZOONG	Polymethyl methacrylate	0.34 µm
VITA CAD Temp	CAD/CAM block for provisional prosthesis	27900	Microfiller Reinforced	0.33 µm
KeyMill	CAD/CAM block for denture base.	20719ORG	Polymethyl methacrylate	0.37 µm
AvaDent	CAD/CAM block for denture base	112412	Polymethyl methacrylate	0.35 µm
Lucitone 199	CAD/CAM block for denture base	180828	Polymethyl methacrylate	0.32 µm
Rodin 3DP	3DP printed nanohybrid tooth material	A3-024FT0259D	Ceramic particle filled cross-linked polymethacrylates	0.09 µm, 0.17 µm or 0.77 µm

Table.1 Dental materials with their surface roughness.

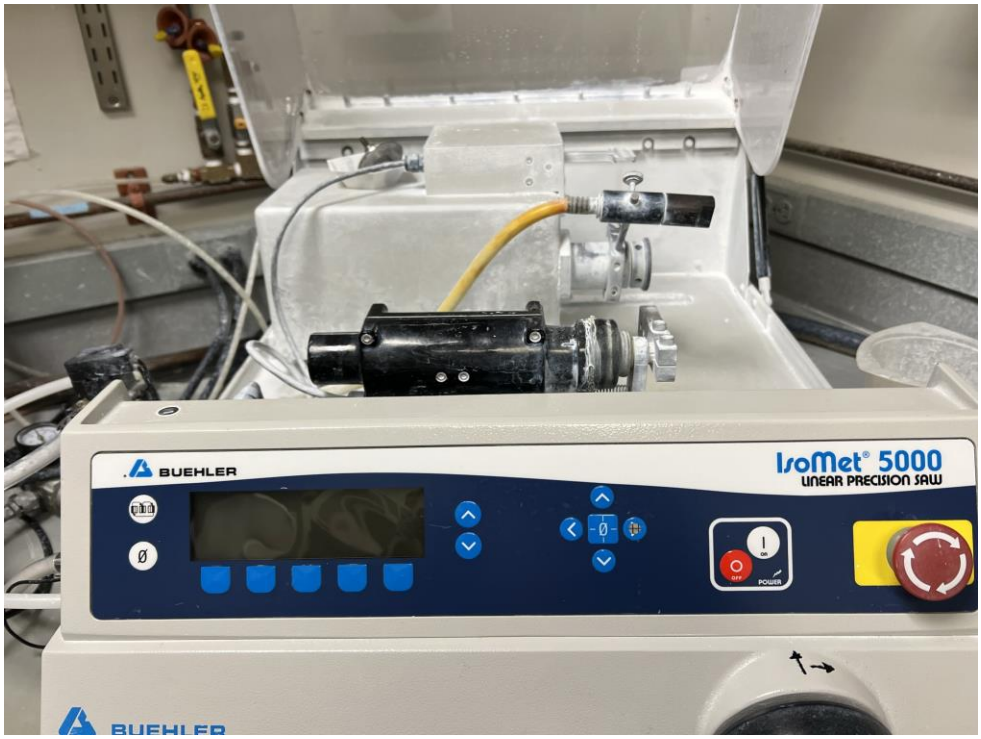


Figure 16 IsoMet 5000 saw (Diamond saw)


BUEHLER
For Polishing
SOP Scan this :
BUEHLER
EcoMet 250
BUEHLER

Figure 17 Buehler Apex (70,45,15,6 diamond grinding disk)

Figure 18 Mitutoyo SJ-201 (stylus profilometer)

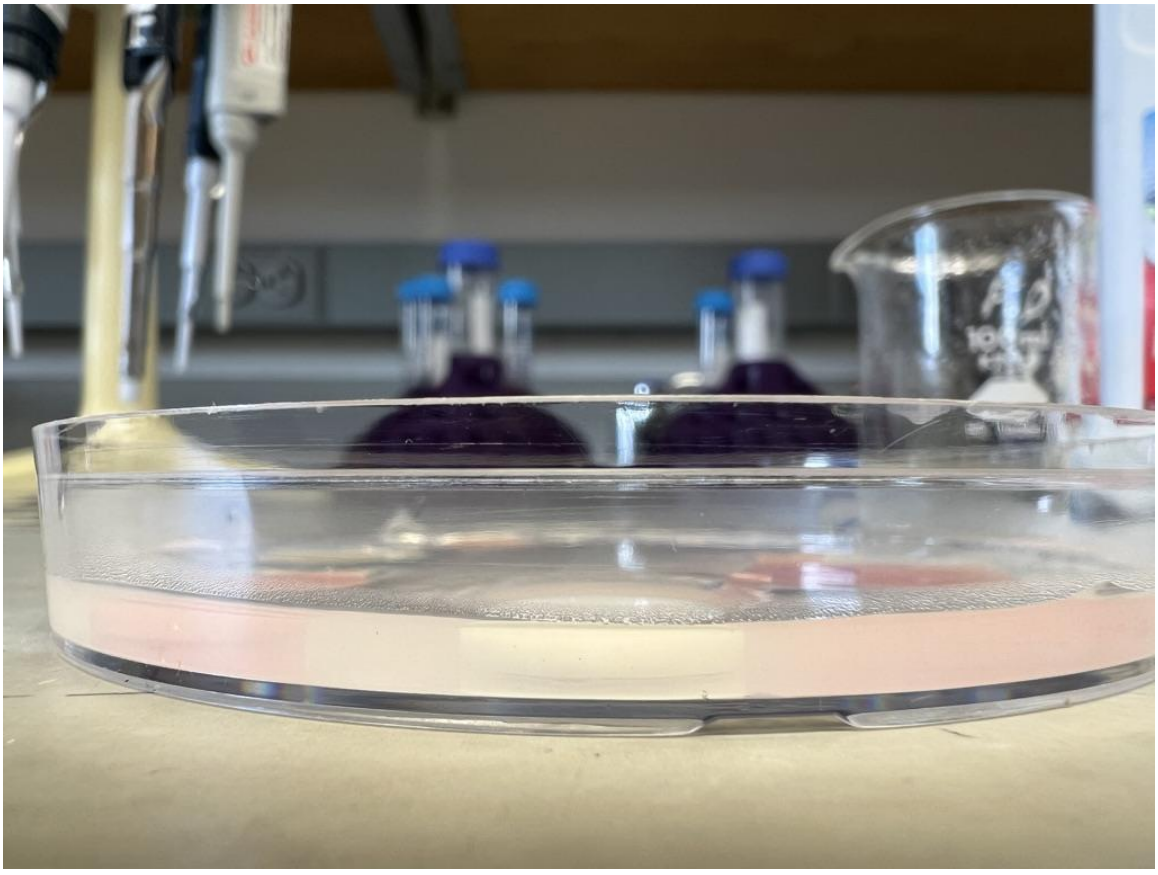


Figure 19 Dental materials imbeded in solid 2% LB agar medium

2.2 Measurement of whole milk adhesion on the dental material

All dental materials were placed in a petri dish with the flat surface with different surface roughness values facing up. A pre-warmed 2% agar solution was poured into the petri dish to fix the samples without submerging them during the experiment, and then allowed to cool for10 min at room temperature.

A mixture of 10 µl whole milk with 5 µl of 50% Ponceau Stain was coated on the surface of the dental material and incubated at room temperature for one hour until the milk was denatured and fixed. Water was added to the petri dish to about 5 mm above the surface of the dental material.

The dish was then placed on a shaker and rinsed at 180 rpm for 1 hour. The petri dish was taken out at 0 min and 1 hour and the retention rate of the denatured milk that remained on the dental material was photographed.

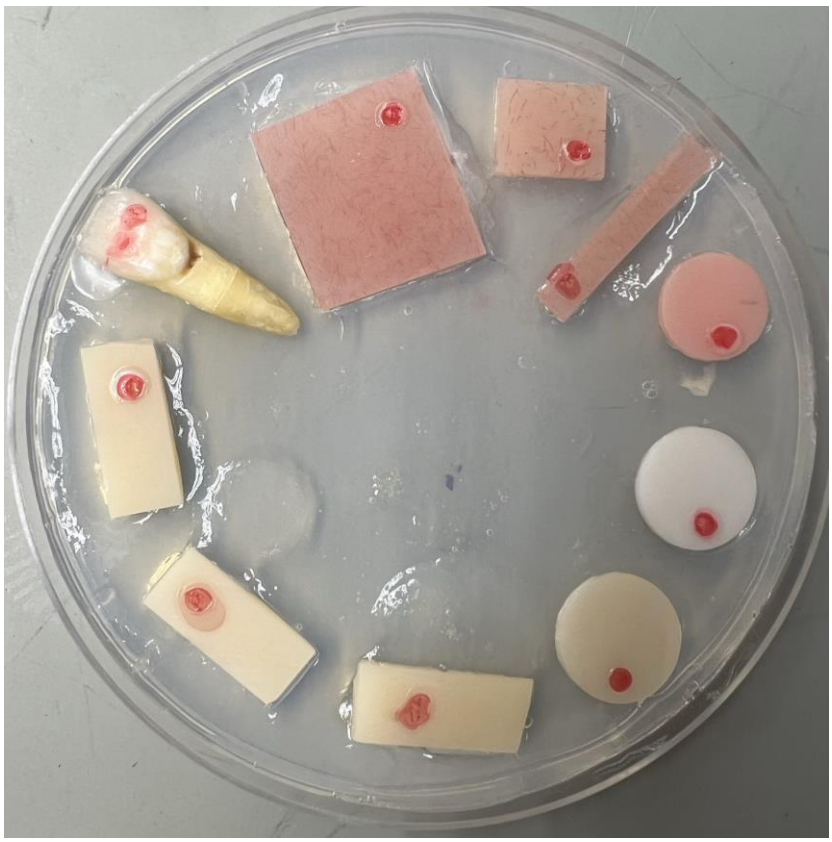


Figure 20 MAC on dental materials

2.3 Measurement of RAW cells adhesion on the dental material

Mouse RAW cells were grown in each well of 6 well plate with 2 ml RPMI medium 1640 (Cat#: 11875-093, Life Technologies, NY) plus 10% FBS (Fetal Bovine Serum, Sigma) at 37 °C, 5% CO2 until the cells attained 95% confluence. The cultured cells were suspended with Trypsin-EDTA solution (Cat# SLCB7154, Sigma) and $1x10^5$ cells with media were inoculated on the surface of the dental materials fixed in the 6 well-plate and incubated at 37 °C, 5% CO_2 for one hour.

The media were removed and the PBS solution added to the plate until the level was 5 mm above the cells, then the plate was placed on a shaker (Incu-Shaker, Benchmark Science) and rinsed at 180 rpm for 0 hour as control, 1 hour, or 2 hours. Cells were added with 10% formaldehyde solution for 30 min at room temperature, washed with pBS and stained with 4% Trypan blue (Cat# TB154, Sigma). The residual retention rate (cells were

rinsed for 1 or 2 hours) and its retention rate was counted and compared to the original retention rate (cells were rinsed for 0 hour as control).

The media were removed and the PBS solution was added to the plate with the volume about 5 mm above the cells adhered on the surface of the dental material. The plate was placed on a shaker and rinsed at 180 rpm for 0 hour as control, 1 hour or 2 hours as test group. The shaker was stopped at each time point. The cells in the plate were treated with a 10% formaldehyde solution for 30 min, washed with PBS, stained with 4% Trypan blue (Cat# TB154, Sigma) and observed. The residual retention rate (cells were rinsed for 1 or 2 hours) and its retention rate was counted and compared to the original retention rate (Cells were rinsed for 0 hour as control) and photographed.

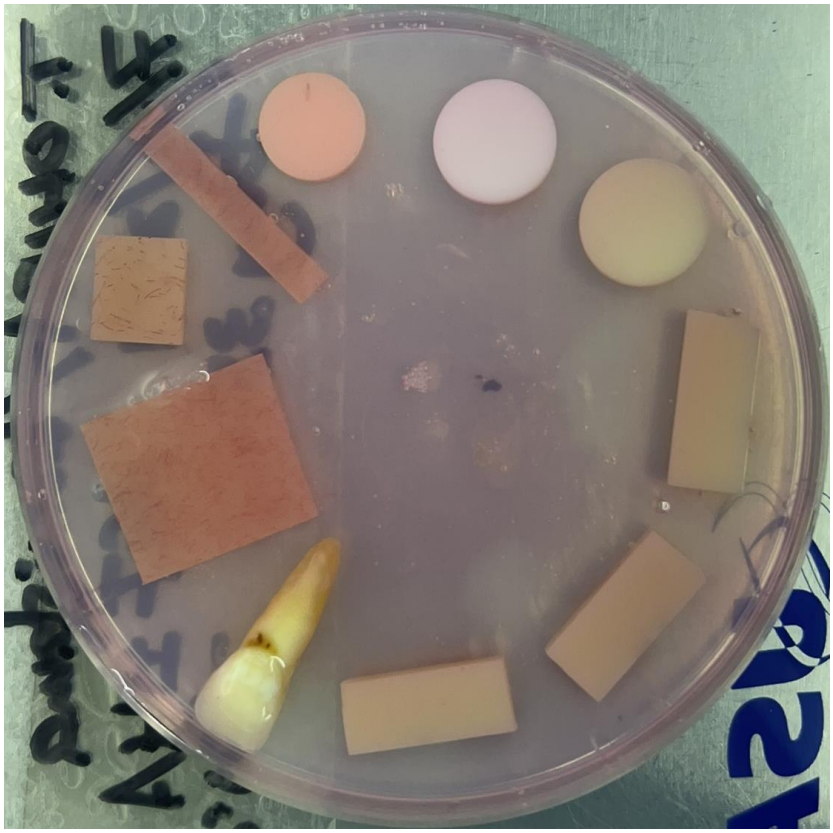


Figure 21 Cells impeded with media.

2.4 Statistical analysis

All experiments were performed in triplicate. Statistical differences were analyzed with the SAS software package.

RESULTS AIM TWO

Five dental materials plus human adult tooth as positive control were selected and resistance to milk adhesion determined. As shown in figure 22, Avadent (88%) or KeyMill by (85%) wwere most resistant to milk adhesion compared to Lucitone (62%), VITACAD Temp (65%), Telio CAD (70%) even though they had the almost same surface roughness (0.32-0.37 μm). The denatured milk was completely removed from an adult tooth after two-hours washing, lower roughness corresponded to being resistant to denatured milk adhesion.

Rodin 3DP polished to 0.09 μm roughness was most able to resist adhesion of denature milk compared to other degree of roughness (0.17 μm, 0.77 μm) as shown in figure 23. This supports the result figure 22, that the material with a low degree of roughness can better resist denatured milk adhesion.

Five dental materials plus an adult human tooth as control were tested for resistance to cellular adhesion. As shown in figure 24, Avadent was best able to resist cell adhesion

(78.3%) followed by KeyMill (70.2%), Lucitone (51.2%), VITACAD (42.2%) and Telio CAD (13.5%). The cells were completely removed from the surface of the adult tooth after two hours washing. When we tested cell adhesion to Rodin 3DP with different degree of roughnesses, Rodin 3DP polished with 0.09 μm roughness was better able to resist cells adhesion compared (0.17 μm or 0.77 μm) roughness as shown in figure 25.

DISCUSSION

Artificial calculus is used to test materials for the ability to prevent periodontitis [74]. Although artificial calculus is commercially available (Dental Calculus Set, Nissin Dental Products INC), commercial artificial calculus coated on the dental materials cannot be inoculated with cells or bacteria. This material cannot be used to test the effect or degree of roughness of dental material on resistance to cells and protein (plaque or calculus) adhesion. Our milk artificial calculus (MAC) with its high concentration of protein can be coated on the surface of the dental material [68], coagulates rapidly and hardens even at room temperature. After repeated tests, it was clear from aim one that our milk artificial calculus (MAC) is closer to the natural dental calculus than the commercial artificial calculus. Moreover, this artificial calculus formation is cost effective and is easily accessble.

Periodontitis is caused by oral bacteria attaching to teeth through calculus. If the tooth is severely damaged, it will be replaced with dentures formed from dental material.

The dental material used for replacement must be able to resist adhesion of oral bacteria. Since most oral pathogens cannot adhere to dental materials in the absence of calculus, we used RAW cells, since they can quickly adhere to dental materials.

In aim two, attachment of cells to natural tooth surfaces was significantly lower compared to prosthetic dental materials. The adhesion of cells and proteins is determined by two major factors: surface chemistry and surface topography. These factors can be evaluated by measuring surface hydrophilicity and surface roughness. The surface topography or roughness is a dominate factor, followed by surface texture [73]. A rougher surface is more conducive to cell or bacterial attachment. Surface roughness and chemistry of the material will either enhance cell or bacterial adhesion or resist it. The natural tooth surface, composed of hydroxyapatite, has a low roughness and higher hydrophilicity along with a pellicle coating, which leads to anti-fouling properties and less cellular and protein adhesion. The native tooth has self-cleaning properties through regular chewing and tongue movement. In contrast, 3D printed denture materials, primarily composed of poly (methylmethacrylate, PMMA), have relatively high hydrophilic characteristics with aliphatic chains and polarized ester bonds that may easily retain proteins and cells. This study provides evidence that a biofilm can form on PMMA-based prostheses.

Overall, our results in aim two indicate that: 1) Although the roughness of various materials is around the same, their resistance to the milk artificial calculus (MAC) or cell adhesion is different. For example, AvaDent material is more resistant than other materials as shown in figure 22 and 24. The dental material containing lower degree of roughness is

more resistant to artificial calculus formation or cell adhesion as shown in figure 23 and 25.

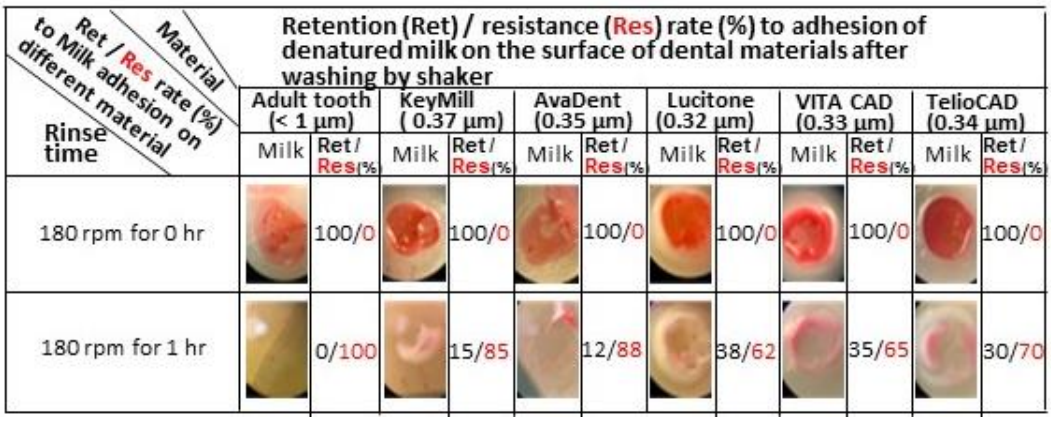


Figure 22 Retention or resistance rate of dental material to adhesion of denatured milk.

The denatured milk was fixed on surfaces of dental material (Adult tooth, KeyMill, Avadent, Lucitone, VITACAD, Telio CAD), then washed with water at 180 rpm for 0-1 hour by shaker . The rate as control was set to 100% (black highlighted) for retention (Ret) or 0% (red highlighted) for resistance (Res) of denatured milk adhesion to dental material by 0 hour washing, and their test rate by washing for 1 hour was measured and shown compared with the control value.

Ret / Res rate (%) to adhesion of milk on Rodin 3DP — Roughness / Rinse time	Retention (Ret)/resistance (Ret) rate(%) to adhesion of denatured milk on Rodin 3DP with its different roughness by washing					
	0.09 μm		0.17 μm		0.77 μm	
	Milk	Ret / Res (%)	Milk	Ret / Res (%)	Milk	Ret / Res (%)
180 rpm for 0 hr		100/0		100/0		100/0
180 rpm for 1 hr		5/95		15/85		20/80

Figure 23 Detection of Rodin 3DP to adhesion of denatured milk.

The denatured milk was fixed on three different degrees of roughness of Rodin 3DP, then washed with water at 180 rpm for 0-1 hour by shaker. The rate as control was set to 100% (black highlighted) for retention (Ret) or 0% (red highlighted) for resistance (Res) of denatured milk adhesion to Rodin 3DP by 0 hour washing, and their test rate by washing for 1 hour was measured and shown compared with the control value.

Dental Material / Ret) / Res rate (%) to cells adhesion on different material / Rinse time (180 rpm)	Retention(Ret)/resistance(Res) rate (%) of cells adhered to dental materials after washing by shaker											
	Adult Tooth		KeyMill		VITACAD		AvaDent		Lucitone		TelioCAD	
	cells Image	Ret/Res Rate (%)	cells Image	Ret/Res Rate (%)	cells Image	Ret/Res Rate (%)	cells Image	Ret/Res Rate (%)	cells Image	Ret/Res Rate (%)	cells Image	Ret/Res Rate (%)
0 hr												
1 hr		100 / 0		100 / 0		100 / 0		100 / 0		100 / 0		100 / 0
2 hrs		0 / 100		29.8/70.2		57.8/42.2		21.7/78.3		48.8/51.2		86.5/13.5

Figure 24 Retention or resistance rate of dental material to adhesion of RAW cells.

Cells were fixed on each surface of dental material (Adult tooth, KeyMill, VITACAD, Avadent, Lucitone, TelioCAD), then washed with water at 180 rpm for 0-2 hour by shaker . The rate as control was set to 100% (black highlighted) for retention (Ret) or 0% (red highlighted) for resistance (Res) to cells adhesion to dental material by 1 hour washing, and their test rate by washing for 2 hours was measured and shown compared with the control value.

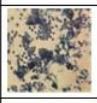
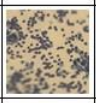
		Retention (Ret)/resistance (Res) rate (%) to cells adhesion on dental material after washing by shaker					
Material		Rodin 3DP tooth					
Roughness		0.77 μm		0.17 μm		0.09 μm	
Ret/Res rate (%) to cells adhesion on material		Cells Image	Ret / Res Rate %)	Cells Image	Ret / Res Rate (%)	Cells Image	Ret / Res Rate (%)
Rinse time (180 rpm)	0 hr						
	1 hr		100/0		100 / 0		100 / 0
	2 hrs		95.2/4.8		56.9/43.1		10.9/89.1

Figure 25 Detection of Rodin 3DP to adhesion of RAW cells.

Cells were fixed on three different degrees of roughness of Rodin 3DP, then washed with water at 180 rpm for 0-2 hour by shaker . The rate as control was set to 100% (black highlighted) for retention (Ret) or 0% (red highlighted) for resistance (Res) of cells adhesion to Rodin 3DP by 1 hour washing, and their test rate by washing for 2 hours was measured and shown compared with the control value.

CONCLUSION

We concluded that a Milk Artificial Calculus (MAC) can mimic human calculus for cell ability to attach to teeth and other dental materials—cells could survive for up to 10 days inside MAC with different treatments. Most toothpaste and different alcohol concentrations do not suppress bacterial growth inside MAC. Once artificial milk calculus (MAC) forms, it becomes harder to kill the cells inside which mostly resembles human calculus. MAC is reliable, cheap, and easy to make and use. Various materials have the same surface roughness, and their resistance to milk artificial calculus (MAC) differs. For example, AvaDent material is much stronger than other materials and resistant to MAC adhesion. The same dental material with lower surface roughness is more resistant to milk artificial calculus (MAC) adhesion.

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