

Dental Paste

BACKGROUND of INVENTION

Delivering a bioactive component intended to maintain and/or improve health in a human or an animal, such as a companion animal, presents varying challenges to formulators, particularly
5 depending on the target site and type of agent for delivery.

Many bioactive agents are susceptible to degradation and exposure to oxygen, heat, light and/or moisture may practically negate the health benefit of the bioactive. The formulation used to deliver the bioactive component may comprise a solid dose form, such as a capsule or tablet because inherent protection of the active is required.

10 Unfortunately, traditional encapsulation or other processing methods that provide a physical barrier to help reach/access the target site successfully are not always useful. For example, in the mouth, which defines the oral cavity of mammals, humans or animals such as pets, comprising the oral mucosa (gums, lips, cheeks, palate and floor of the mouth), the tongue and the teeth. Where the target site is in a structure in the mouth, the formulation is required to remain proximal and remain local for a
15 minimum period such that the bioactive component is delivered at the required efficacy.

However, many oral health products formulated for use in the mouth, such as oral washes, lozenges, and pastes, suffer the problem of unreliable and/or inconsistent delivery and a sufficient amount of the bioactive (on the mucosal membrane or tongue, for example) is never achieved which to yield a measurable effect.

20 This is especially true when the challenge concerns administering an oral health product to a dependent e.g. a child, elderly person, or an animal, such as a pet. Further, due to risk of involuntary ingestion of the active, undesirable effects associated with that specific challenge remain difficult to overcome; these include reliably controlling efficacy and toxicity and preventing the associated side effects, such as gastro-sensitivity and sickness, which may result.

25 Common diseases within the oral cavity often occur due to a change in the balance of the natural flora in the biofilm (plaque) covering the teeth and gums. The bacteria contained within the plaque is usually benign because the predominant components of the biofilm are typically commensal bacteria, such as *Lactobacillus*. However, if pathogenic bacteria become the dominating bacteria oral health can decline. As a result, tooth decay and/or gum disease may start to occur in the mouth of the
30 mammal, especially if teeth e.g. of pet animals, are not regularly cleaned to remove buildup of plaque.

Colonization of the pathogenic species is known to weaken and then dissolve dental enamel leading to caries, or lesions in the teeth and calcified deposits begin to form providing localized focus for the colony to continue to thrive on the teeth, thereby aggravating the problem. These processes contribute to the frequently recorded symptoms of poor oral health: such as malodor of breath,
35 inflammation and bleeding of the gingival tissue and gum.

To prevent oral disease and/or maintain oral health, oral care products including those used in veterinary field, often include a bactericidal agent to reduce the pathogen levels. However, since these agents are usually non-selective, they act to destroy all the natural biofilm including the commensal

species. This typically does not provide a useful remedy in mammals and especially pet animals, since a requirement to re-balance the flora is necessary to restore and/or maintain long-term oral health.

5 EP3424516 suggests a composition comprising binder lactic acid bacterium microorganism that is sensorically [sic] neutral and reduces *Streptococci* colonies. The composition may be added to pet food and does not suffer the taste/smell that the authors suggest typically prevent such compliance by a companion animal with use of effective bactericides. However, such a solution aims to reduce only *Streptococci* rather than effectively restoring the natural balance of the entire mucosal flora. The solution described is formulated and administered as part of a feed that must be masticated for application.

10 The invention herein comes about from a desire to overcome the challenges noted above and herein provides a dental composition, which effectively restores and/or maintains oral health in mammals and particularly in companion animals.

SUMMARY of INVENTION

15 The invention disclosed herein concerns a dental paste or gel comprising a composition having at least 2 to 4 % w/w of a thickening agent, preferably agar; 62 to 95 % w/w glycerol; and 0.03 to 3 % w/w of an epithelial-adhering protein complex (EAPC).

The epithelial-adhering protein complex may comprise one or more: 1) microbiome regulating proteins or a "protein fraction" and 2) a modified whey protein isolate (WPI) from milk.

20 The microbiome regulating protein (s) may be selected from lactoperoxidase, lactoferrin, lysosomal alpha-mannosidase, immunoglobulin G, angiogenin, ribonuclease 4 or quiescin sulfhydryl oxidase and/or combinations thereof. The modified WPI is formed from soluble native protein, protein aggregates and protein fibrils produced from whey protein isolates (WPIs).

25 The dental paste or gel may be applied effectively by brushing or coating structures in the oral mucosa directly. The novel form of the dental paste ensures the complex is maintained at the application location i.e. near the relevant structure, such as the gum and teeth. The composition is not washed away or swallowed immediately and remains stable upon delivery; when the active component is exposed to the target site, the selective antibacterial property is effective in-situ. Notably, bioactive components such as microbiome regulating proteins are routinely unstable and may lose their active capability prematurely, as compared to the lifetime of the paste product. The dental paste or gel
30 reduces and/or prevents the absorption of oxygen, moisture and/or light thereby protecting the microbiome regulating protein, such that its potency is unaffected during storage or delivery. The formulation acts as a temporary barrier and protects the protein complex and inherently its microbiome supporting selective anti-bacterial property. The dental paste or gel specifically stabilises the complex for longer, to ensure that the bioactive remains potent and effective when delivered
35 upon site-specific structures within the oral mucosa.

The gel or paste formulated according to the invention is nonetheless able to protect and maintain the selective anti-bacterial activity and delivers the bioactive protein complex locally within the oral mucosa for an effective period to enable a tangible effect to be observed from that bioactivity, concerning the restoration and maintenance of oral health in a mammal, or animal such as a dog.

Moreover, the dental paste or gel produced has a soft, malleable, and moist structure that can be applied easily to the teeth and gums of companion animals, such as cats and/or dogs, for example with a brush, or instant fluid gel coating applied in or to the surface of a toy. Advantageously, as compared to traditional delivery vehicles such as tablets or capsules, the formulation is palatable. The invention extends to a chew or animal toy with a coating of the dental paste or strip of gel of the invention.

In embodiments, the thickening component, preferably agar, is 2 to 3% w/w and in some preferred embodiments, the thickener or agar is approximately 2.6% w/w. The formulation of the paste having the combination of glycerol and at least one thickener, such as agar specifically maintains low water activity within the final dental paste. This is important to ensure the all the functions of the other essential components within the epithelial-adhering protein complex of the paste, namely stability and bioactivity, are maintained. If either is lost, the final product paste is unlikely to be suitable for use as a dental paste.

The thickening agent, preferably agar, serves to modify the viscosity of the glycerol in the final paste. In embodiments, other thickening agents can also be used in the formulation, including, for example, carrageenan and/or xanthan gum. However, agar is preferred. In addition, the texture of the paste can be further modified by addition of one or more other textural agents, which also may have a thickening affect, for example, rice flour, chitosan and/or silica.

In embodiments, the glycerol component is 80 to 95% w/w. In further embodiments, the glycerol component comprises at least 90 to 95% w/w.

In embodiments, the composition comprises at least 0.03% w/w epithelial-adhering protein complex, optionally this component is present in the range of at least 0.1 to 3%, 0.5 to 3%, 1 to 3%, 2 to 3% or 2.5 to 3% w/w.

The composition of the invention may comprise up to 32% w/w of other/further excipients designed to improve the texture of the composition including further thickening, or stabilising agents, such as chitosan or rice flour.

In embodiments, other gums and excipients may be utilised to improve the consistency of the composition, including but not limited to arable gum, xanthan gum, alginates, chitin, aloe extract, guar gum, hydroxypropyl cellulose, sodium casein, corn starch, carboxymethylcellulose, gelatin, agar, dextrin, methylcellulose, polyvinyl alcohol, microfiber cellulose, microcrystalline cellulose, seaweed cellulose, sodium polyacrylate, sodium polyphosphate, carrageenan or yeast cell wall.

The invention extends to a dental paste or gel according to any previous claim, for use as a medicament, optionally a veterinary medicament.

In embodiments, the paste or gel is for use in the treatment or prevention of oral disease, specifically canine oral disease mediated by an excess of pathogenic bacteria. The oral disease may be selected from but not limited to any of tooth decay, gum inflammation and/or calculus.

The paste or gel composition may also be for use in veterinary treatment to restore animal oral health, particularly canine or feline oral health.

The dental paste is useful to combat stubborn, adverse oral health conditions, such as a bacterial infection in an animal, particularly where disease may be due to an increase in pathogenic bacteria.

As described previously, an increase in the pathogenic bacteria typically causes adverse oral health including one or more of bad breath (halitosis), plaque and/or gum disease in animals. In
5 embodiments, an increase of pathogenic bacteria causing these adverse conditions may include bacteria from the genus *Psychrobacter*, *Porphyromonas*, *Pasteurella* and/or *Actinomyces*.

The adverse health of the animal may be due to a reduction in commensal bacteria, thereby creating an imbalance in the microbiota in the oral mucosa or specifically within the plaque. Such an imbalance may result in one or more conditions selected from bad breath (halitosis), plaque and/or gum disease
10 and so use of the paste can help restore that balance, especially in dogs, which has been duly evidenced herein. It is plausible it would be relevant to other animals particularly, such as cats, which regularly suffer periodontal disease and tooth loss, because of that decay.

The paste if partially swallowed would nonetheless be gentle on the digestive system and thus may be employed for frequent use and/or part of a long-term viable oral solution to maintain oral health
15 of the animal over a reasonable period of months or years.

In embodiments, when the dental paste is used for the prevention, treatment and/or improvement of oral health and more specifically, dental health, a suitable dosage regimen can be followed. To reduce the pathogenic numbers and improve oral health significantly such a composition may be most effective when the final product is used repeatedly daily. When applied as a single daily dose, the
20 product delivers a minimum of a 5, 15 or 25 to 50mg dose of the epithelial-adhering protein complex.

In some embodiments, particularly where lower volumes of paste product are required to deliver the effective daily dose e.g. in a single small ball of paste (suitable for brushing or direct application) on the teeth, the epithelial-adhering protein complex is provided at the higher range e.g. 1.5 to 3% w/w. In other embodiments where larger volumes of gel product are necessary e.g. to cover a much larger
25 surface area for coating a toy, a lower % composition may achieve the required daily dose for effect, for example, this may be as low as 0.03%.

Depending on the final formulation and product form, the paste or gel required to provide an effective amount of the active component, for a given single application, may weigh around 1g to 50g and have a relevant % of EAPC, in accordance with the scope of the invention.

30 Preferably, the product is applied daily for at least 5 of 7 consecutive days per week, and more preferably for at least 4 weeks.

A reduced frequency of the same dose, for example every other day or twice a week, may be utilised to maintain health via a suitably re-balanced microbiome of the oral mucosa, once the re-balanced microbiome has been established. However, regular administration will more effectively prevent re-
35 occurrence of pathogen build-up and therefore maintain a suitably re-balanced oral mucosa.

In embodiments, the invention therefore further relates to a method of improving or maintaining oral health comprising administering to a human or animal in need thereof the dental gel or paste composition as described above. In embodiments, the invention further comprises a method of

prevention or treatment of oral disease comprising administering to a mammal and/or animal in need thereof in the composition as described herein.

The invention further extends to a method for making a dental paste or gel, comprising the steps of at least:

- 5 providing glycerol at least from 50%, optionally 62% to 95% w/w, and optionally heating up to around 95°C;

adding and dissolving 2 to 4 % w/w thickening agent, preferably agar, in to the glycerol;

optionally adding one or more excipients 0 to 32% w/w to the glycerol and thickening agent combination and cooling, optionally to 40°C or below;

- 10 adding and dissolving 0.03 to 3 % w/w epithelial-adhering protein complex; and

cooling, optionally to approximately 18°C or below, to form the dental paste or gel.

- The dental paste resulting is formulated to deliver and maintain a local and tangible effect from the activity of the protein complex. As will be detailed further below, the novel formulated dental paste composition has been shown in the applicant's studies to retain significant bioactivity and at
- 15 surprisingly low concentration. This suggests the formulated form, in accordance with the embodiments of the invention herein, further enhances the effect of the EAPC beyond that which might be expected. In subsequent animal studies, evidence for the translation of bioactivity to a mammal has also been shown, inferring restoration and/or maintenance of oral and dental health is achieved when the dental paste of the invention is applied in the oral mucosa of a mammal.
- 20 Further, the texture of the paste or gel produced by this means is also more acceptable to allow for easier oral administration in certain mammal groups, such as infants, the elderly and companion animals, such as dogs. Once received into the mouth the recipient is more likely to accept the composition for example on a brush or other surface applicator, rather than automatically reject a hard or unpleasant tasting capsule or tablet. The dental paste or gel composition may further comprise
- 25 one or more excipients to optimise and improve palatability for the recipient.

BRIEF DESCRIPTION

Further aspects of the invention will become apparent from the following description, given by way of example and with reference to the accompanying figures, in which:

- Figure 1 is a graph showing the nil effect of the EAPC used in the paste of the invention on the growth of the commensal bacteria *L. acidophilus*;
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Figure 2 is a graph showing the bactericidal effect of the EAPC used in the paste on the pathogenic bacteria *E. coli*;

Figure 3 is a graph showing that the EAPC used in the paste adheres well to epithelial surfaces and retains its bactericidal activity towards the pathogenic bacteria *S. aureus*;

Figure 4 is a graphical representation of data demonstrating that the activity of a key microbiome regulating protein lactoperoxidase is sufficiently retained in the dental paste of the invention following manufacture (in accordance with the disclosure herein and storage thereafter);

5 Figure 5 is a graphic showing the effect of each paste sample on the growth rate of *B. subtilis* over a 12-hour 40 min period (760 min) in lab conditions;

Figure 6 displays graphically the concentration of microbiome regulating protein active in each type of paste sample required to inhibit growth of *B. subtilis* (relative level 0% for full inhibition);

10 Figure 7 graphically compares the abundance of six species of key pathogenic bacteria associated with canine dental disease after treatment with the trial dental paste of the invention versus the placebo in a canine microbiome trial; and

Figure 8 graphically illustrates the statistical relevance of the effect using the trial dental paste of the invention versus the placebo paste in the canine microbiome trial.

DETAILED DESCRIPTION

15 For the purposes of this specification, the term 'about' or 'approximately' and grammatical variations thereof mean a quantity, level, degree, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 0.1% to a reference quantity, level, degree, value, number, frequency, percentage, dimension, size, amount, weight or length. The term 'substantially' or grammatical variations thereof refer to at least about 50%, for example 75%, 85%, 95% or 98%.

20 The term 'comprise(s)' and grammatical variations thereof shall have an inclusive meaning - i.e. that it will be taken to mean an inclusion of not only the listed components it directly references, but also other non-specified components or elements.

The applicant's work comes from additional research, investigation and improvement of their recently developed formulations, specifically as it relates to the restoration, improvement and maintenance of oral health in companion animals, especially canines.

25 Microbiome regulating proteins

The enzyme lactoperoxidase is a protein that has been implicated in the microbiome regulating proteins isolated from bovine milk (NZ547859). This publication describes a combination of protein fractions from bovine dairy milk can be effective at preventing the growth of pathogenic bacteria such as *E. coli* and *S. aureus* while not effecting the growth of commensal bacteria from, for example, the *Lactobacillus* family. Formulations are disclosed in the art based on milk bioactive proteins that is extracted from milk. The components are produced naturally by the cow as an immune defense response against infection and inflammation. Such anti-inflammatory, antioxidant and antimicrobial action supports 'good' bacteria flora and kills 'bad' bacteria and has been shown to be safe in some clinical applications. However, their practical use in mammals or pet animals was not enabled for dental or oral application at that time. This is because, such proteins do not alone enable their useful properties to be delivered and formulations encompassing these agents were not found to be effective.

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Until the author's own research and development of these components there was no means by which microbiome regulating activity of such proteins and/or fractions thereof (or very similar types of functioning agent) could be exploited for effect in the oral mucosa successfully.

5 Nonetheless, the present applicant determined a method to successfully complex the microbiome regulating proteins that both stabilises the proteins and introduces a new physical function to enable the complexed proteins to adhere to an epithelial surface, upon topical application. Such an invention would enable a product to retain excellent protein functionality (selective bactericidal quality) but additionally have properties required to overcome unmet challenge (for example, local adherence, as would be relevant in the oral mucosa). This successful outcome of such a protein complex is due in
10 part to the important chemical and physical conditions under which the two components are reacted together in the applicant's process to form the complex, diligently researched, and controlled, to optimise the functional activity of both components.

This protein complex or "epithelial adhering protein complex" EAPC is one of the essential components of the present dental paste or gel of the invention disclosed herein. However, at the time
15 of making the present invention disclosure, the protein complex component remained undisclosed publicly.

A complete description of that component and how it is obtained is therefore provided herein, as well explaining for the first time how the EAPC is effectively used in the formulation and the manufacture of the novel dental paste of the invention and the embodiments thereof.

20 Epithelial-adhering protein complex "EAPC"

Disclosed herein is the applicant's protein complex comprising microbiome regulating proteins complexed with a modified whey protein isolate (WPI) from milk, wherein the complexed microbiome regulating proteins configured for topical application and, on application, to adhere to epithelial surfaces. The microbiome regulating proteins are selectively toxic to pathogenic bacteria, while
25 minimising any reduction in commensal bacteria population.

The microbiome regulating protein in the complex may comprise at least one or more of lactoperoxidase, lactoferrin, lysosomal alpha-mannosidase, immunoglobulin G, angiogenin, ribonuclease 4, quiescin sulfhydryl oxidase, and combinations thereof.

Microbiome regulating protein

30 For brevity of description, the word 'protein' or grammatical variations thereof as used herein is intended to encompass one protein, multiple proteins, a protein fraction, or protein fractions and reference to a singular protein should not be seen as limiting.

The microbiome regulating protein may be a blend of protein fractions from milk that are selectively toxic to pathogenic bacteria, but which minimise any reduction in commensal bacteria population.

35 The microbiome regulating protein may comprise lactoperoxidase, lactoferrin, lysosomal alpha-mannosidase, immunoglobulin G, angiogenin, ribonuclease 4, quiescin sulfhydryl oxidase, and combinations thereof. The protein may comprise a selection of some, or all the above listed proteins.

The microbiome regulating protein selected for the above method may initially be in dried powder form.

Modified Whey protein isolate (WPI)

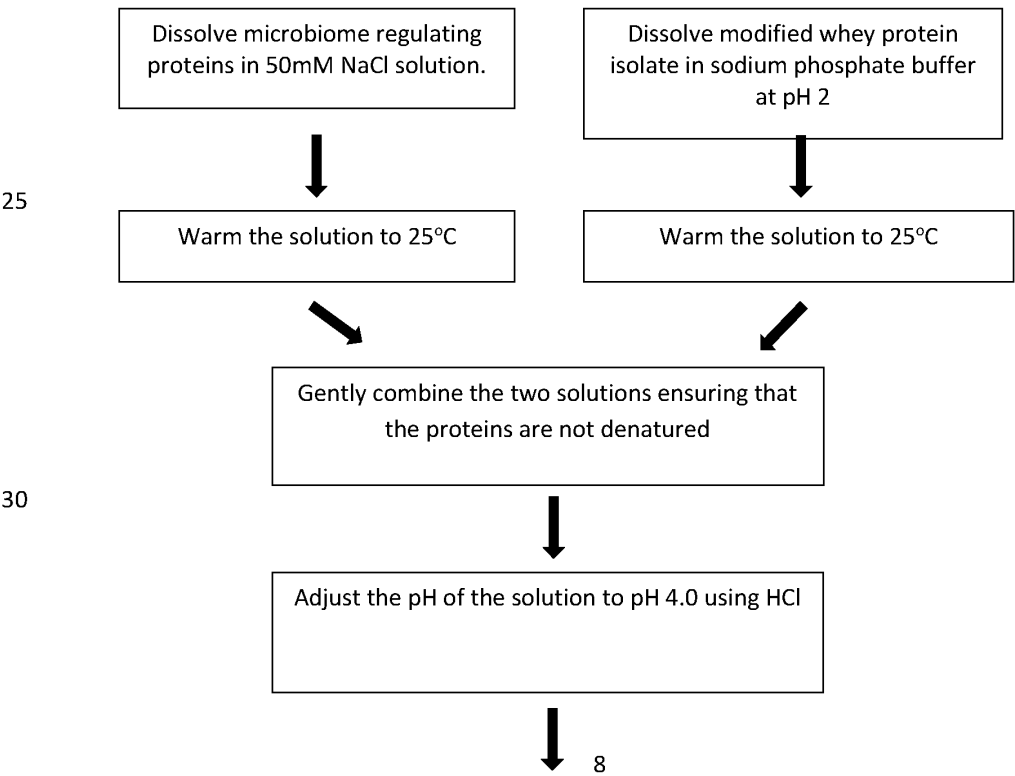
5 The term ‘modified whey protein isolate’ or grammatical variations thereof as used herein refers to soluble native protein, protein aggregates and protein fibrils produced from whey protein isolates (WPIs). The modified WPI selected for the above method may initially be in the form of an aqueous gel. The modified WPI selected may be derived from bovine species milk but may also be sourced from modified WPI obtained from other animals such as goats and sheep.

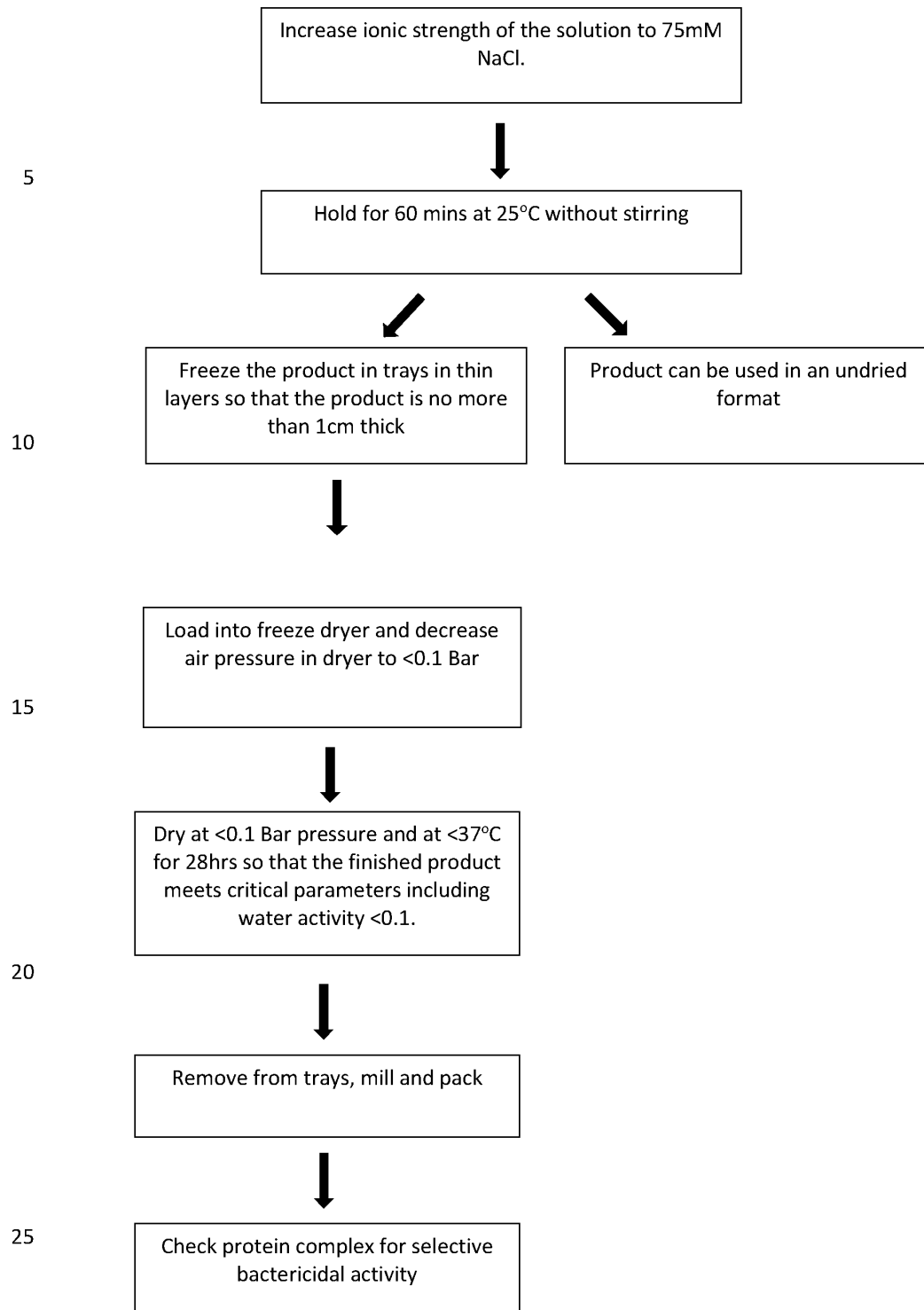
10 The gel enables binding of active ingredients together, as well as release of the agents in a controlled manner. It has shear thinning rheology (thixotropic) giving it good stability and positive skin feel. It has a semi-solid material composed of soluble native protein, protein aggregates and protein fibrils produced from whey protein isolates (WPIs) to form a modified WPI. The modified WPI gels of use herein typically have a viscosity from 0.25 to 4.5 Poise.

Epithelial-adhering protein complex (EAPC) method of manufacture

15 The method of producing the EPAC includes selecting a microbiome regulating protein in a powder form; selecting a modified whey protein isolate (WPI) from milk in a gel form; solubilising the microbiome regulating protein in an aqueous salt solution, the aqueous salt solution having an ionic strength of 25-200mM NaCl; blending together the solubilised microbiome regulating protein and modified WPI; adjusting the pH to 2.0-6.0; optionally adjusting the temperature to 18-37°C; holding
20 the blend at a pH of 2.0-6.0 and a temperature of 18-37°C for at least 30 minutes.

The following flow chart explains the process for producing the EAPC:





The resulting EAPC may be a semi-solid aqueous gel with a viscosity of approximately 0.25 to 4.5 Poise. The viscosity of the modified whey protein isolate is important to facilitate the complexing, and the optimum temperature for the most suitable viscosity is in the range of 18-37°C. Ionic strength of the mixture is important for the correct interaction of the protein groups on both components and the optimum ionic environment is maintained by 25-200mM NaCl at pH 2.0-6.0.

The ratio of microbiome regulating protein to modified WPI used in the method and/or present in the disclosed complexed protein may be : 1:1, or 1:2, or 1:3, or 1:4, or 1:5, or 1:6, or 1:7, or 1:8, or 1:9, or 1:10. In examples, the ratio may be from 1:1 to 1:10; i.e. 1 part microbiome regulating protein to 1-10 parts modified whey protein isolate. The ratio may be a 1:1 ratio.

- 10 It may be necessary to have at least as much modified WPI to protein to ensure the necessary extent of complexing is reached. Lower ratios could be used but with waste or non-complexed protein resulting from the method due to insufficient modified WPI being present at lower ratios.

Salt

- 15 The salt solution may comprise a mix of water and a chemical salt. The chemical salt may be NaCl although other salts such as MgCl may also be used. Salt may be added to the extent needed to provide the indicated ionic strength. The ionic strength of the salt solution may be approximately 25, or 50, or 75, or 100, or 125, or 150, or 175, or 200mM NaCl. As noted above, the ionic strength may vary from 25-200mM NaCl. The ionic strength be approximately 75mM NaCl. This ionic strength is maintained in the complexed blend of protein and modified WPI during and after holding.

- 20 This ionic strength range appears to be desirable for complexing or dissolution of the microbiome regulating protein into the modified WPI although, there may be some variation depending on the pH, temperature and holding time used. The salt solution is important to create the correct ionic nature of the solution and enable the powdered protein to dissolve and the protein to be correctly charged to create the complex.

25 Blending

Blending occurs gently so that no or minimal foaming of the mixture occurs during blending. Blending may be completed, for example, using a planetary mixer. Blending may continue during pH adjustment, temperature adjustment and/or holding. Gentle or slow blending of the protein may be important to minimise or avoid denaturing and loss of functionality.

30 pH

- The pH of the blend prior to pH adjustment may be approximately 6.5 to 7.5 or, around neutral pH 7.0. This start point in pH may be dependent on the pH of the water used to form the aqueous salt solution. The pH of the blend after pH adjustment may be approximately 2.0, or 2.5, or 3.0, or 3.5, or 4.0, or 4.5, or 5.0, or 5.5, or 6.0. In one embodiment as noted above, the pH may be from 2.0 to 6.0.
- 35 In one embodiment, the pH may be approximately 4.0. This pH range and value appears to be an optimum for complexing or dissolution of the protein into the modified WPI although there may be some variation depending on the ionic strength, temperature and holding time used. The acid conditions post adjustment appear to maintain the appropriate charges on the protein to ensure bonding of the complex. The pH may be adjusted by use of an acid. The acid may be hydrochloric acid

although other acids may also be used. In the inventor's experience, no buffers are required to maintain the reduced pH once adjusted.

Temperature

The temperature of the blend after any adjustment (if needed) may be approximately 18, or 19, or 20, or 21, or 22, or 23, or 24, or 25, or 26, or 27, or 28, or 29, or 30, or 31, or 32, or 33, or 34, or 35, or 36, or 37°C. As noted above, the temperature, post any adjustment may be from 18-37°C. In one embodiment, the temperature post adjustment may be approximately 25°C. The temperature may be adjusted to maintain the modified WPI at a desired viscosity to enable optimum complexing. This desired viscosity may be in the range of approximately 0.25 to 4.5 Poise. The temperature range and values described appear to be an optimum for complexing or dissolution of the proteins into the modified WPI although there may be some variation depending on the ionic strength, pH and holding time used. The temperature may be adjusted from ambient conditions (or retained at ambient conditions if the ambient temperature is within the desired range).

Temperature adjustment may be up or down depending on the difference between the desired temperature and the ambient temperature.

Holding time

The holding time may be at least 30 minutes. The holding time may be from 30 minutes to 24 hours. In one embodiment, the holding time may be approximately 60 minutes. In the inventor's experience, most of the complexing occurs within 30-60 minutes based on the chosen parameters. Extended durations could be used and based on the applicant's experience; there is no harm or risk of loss of functionality in holding the mixture for longer periods. This timing appears to be an optimum for complexing or dissolution of the protein into the modified WPI although there may be some variation depending on the ionic strength, temperature and pH used.

Drying

Optionally, drying may be completed in advance of subsequent formulation. In this embodiment, the dried complex may be a solid. Drying may be to a water activity of less than 0.6. In selected embodiments, the water activity may be as low as 0.2-0.3. Drying of the complex may be done under specific conditions of low temperature and low pressure over a time course that ensures the epithelial adhering nature and the selective bactericidal activity of the components of the complex are retained. Pressure during drying may be important in that, at low pressure the moisture boils off the gel at a lower temperature so that the drying can be undertaken at low temperatures. In addition, a very gradual lowering of pressure may be important so that the complexed protein solution in the dryer does not foam. Drying may be completed by freeze-drying or any other gentle drying process using a non-denaturing temperature/pressure e.g. less than 30°C, in a vacuum. The complexed protein remains functional when rehydrated post drying.

Only through careful experimentation and balance of these parameters, were the useful effects obtained in the resulting protein complex. Indeed, the extent of adherence of protein on epithelial surfaces post complexing was more than double that of each component alone and in the inventors experience synergistic in nature or at least well beyond that anticipated suggesting a synergistic effect was possible but that could not have been previously expected or predicted.

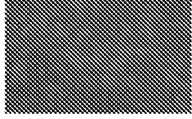
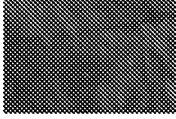
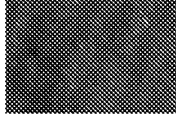
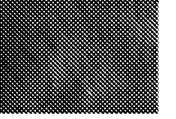
It was observed that the selective bactericidal activity of the protein described herein can be adversely affected if the temperature of any step of the process exceeds 37°C, and the pH drops below 2.0 or greater than 9.0.

Adherence of the EAPC to epithelial surfaces

- 5 To determine if the complex was adherent to epithelial layers a portion of pig’s skin was soaked in a solution of 50mg/mL of the complex (containing 50mg/mL protein), washed twice in phosphate buffer at pH 7.4 and stained with Coomassie Blue, which stains for protein. This gave an intensely stained skin compared to pig’s skin that had not been soaked in the complex (Figure 1). Treatment of pig skin with 50mg/mL modified whey protein isolate fraction (containing 50mg/mL protein) or the
- 10 microbiome regulating proteins from bovine milk (containing 50mg/mL protein) alone, then washed, showed some binding of these components to the skin, but not as much as with 50mg/mL of the complex of both components.

Portion of pigskin treated without or with the EAPC, and the components of the EAPC, washed twice and stained for protein attached to the epidermal layer of the pig’s skin.

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Treatment	Pig skin	Pig skin soaked in 50mg/ml of microbiome regulating proteins for 60 secs, then washed.	Pig skin soaked in 50mg/ml modified whey protein isolate for 60 secs then washed.	Pig skin soaked in 50mg/ml of the EAPC for 60 secs then washed.
Result				
Relative increase in protein binding	1.0	1.13	1.63	2.35

- The epithelial binding activity of the complex was much greater (more than double) than the modified whey protein isolate alone or the microbiome regulating proteins alone. The modified whey protein isolate has been specifically prepared to attach to epithelial surfaces. Thus, it would be expected that
- 20 the amount of binding to the pig’s skin of this component would be similar to the amount of binding of the complex. However, the complex binding was 44% greater than the modified whey protein isolate alone, suggesting a synergistic effect of the components, or that the process used to generate the complex was increasing the adhering ability of the protein complex.

Retention of the enzyme bioactivity

The enzyme lactoperoxidase is a protein that has been implicated in the microbiome regulating proteins isolated from bovine milk (NZ547859 and PCT/NZ2017/050043). Lactoperoxidase activity was measured by the addition of the complex, or the components of the complex separately, to a solution of hydrogen peroxide in 100 mM Phosphate buffer, pH 5.5, and monitoring the oxidation of 2,2-Azino-bis(3-Ethylbenzthiazoline-6-sulphonic acid) (ABTS) spectrophotometrically at 436nm.

Lactoperoxidase activity of the combination of the microbiome regulating proteins from bovine milk was retained when the microbiome regulating proteins were complexed with the modified whey protein isolate to form the EAPC. As would be expected, the modified whey protein isolate had no peroxidase activity.

Lactoperoxidase activity of the EAPC compared to the components of the complex:

Component	Lactoperoxidase activity (Units)
Modified whey protein isolate (50mg/ml)	0
Microbiome regulating proteins (25mg/ml)	205005
EAPC (50 mg/ml) of whey protein isolate (25 mg/ml) and microbiome regulating protein (25 mg/ml)	223339

Specific bactericidal activity

Commensal bacteria

Microbiome regulating proteins isolated from bovine milk have been shown to have no effect on the growth of commensal bacteria such as those from the *Lactobacillus* family PCT/NZ2017/050043. In order to determine the effect of the EAPC containing these microbiome regulating proteins on a commensal bacteria *Lactobacillus acidophilus* bacteria were cultured anaerobically, and the complex was added to the cultures and numbers of bacteria after 24 hours were determined as the optical density at 650nm. The complex of the modified whey proteins and the microbiome regulating protein isolated from bovine milk had no effect on the growth of the commensal bacteria *Lactobacillus acidophilus* (Figure 1)

Pathogenic bacteria

Microbiome regulating proteins isolated from bovine milk have been shown to be bactericidal to pathogenic bacteria such as *E. coli* PCT/NZ2017/050043. To determine the effect of the EAPC containing microbiome regulating proteins on *E. coli* these bacteria were cultured and the EAPC was added to the cultures and numbers of bacteria after 24 hours were determined as the optical density at 650nm. The EAPC inhibited growth of the pathogenic bacteria *E. coli* to a similar extent as the microbiome regulating protein alone from bovine milk (Figure 2). Note that the square data points in Figure 2 correspond to results seen for addition of the microbiome regulating proteins and the circular data points correspond to results seen for addition of the EAPC.

Effect of the EAPC bound to skin on growth of pathogenic bacteria

- 5 *S. aureus* is a pathogenic bacteria often associated with skin disease. The microbiome regulating proteins isolated from bovine milk has been shown to be bactericidal to *S. aureus* grown in culture PCT/NZ2017/050043. Samples of pig skin treated with the microbiome regulating proteins from bovine milk then washed showed a modest inhibition of *S. aureus* growth. However, samples of pig skin treated with the EAPC, then washed showed significantly greater inhibition of *S. aureus* growth (Figure 3). This confirms the results seen in Table 1 confirming that the EAPC binds well to epithelial surfaces such as skin and retains the bactericidal activity of the microbiome regulating proteins from cow's milk.

10 Dental paste - Formulation and Method of manufacture

The method for making a dental paste or gel according to embodiments of the invention, comprises the steps of at least:

heating glycerol 62 to 95% w/w, optionally at around 95°C;

blending 2 to 4 % w/w thickening agent, optionally agar, into the glycerol;

- 15 optionally adding one or more excipients in the range 0 to 45%, optionally 0 to 32% w/w to the glycerol and thickening agent combination and cooling, optionally to 40°C or below;

adding and dissolving 0.03 to 3 % w/w epithelial-adhering protein complex (EAPC); and

cooling, optionally to approximately 18°C or below, to form the dental paste or gel.

- 20 In examples, blending thickening agent is performed in a step wise manner, preferably at approximately 90 – 100°C. In examples, the cooling is undertaken in a stepwise manner from 40°C to 18°C.

Working ranges

During further formulation studies, prior to conducting further *in vitro* and *in vivo* activity studies, it was clarified that some components and component ranges could be modified slightly.

- 25 The following working ranges were further determined in that testing:

Composition component	Working range
Glycerol	50 - 95%
1 st Thickening agent (e.g. Agar, Xanthan gum, carrageenan or other gum)	2 to 3%

Textural/ 2 nd Thickening agent (different to 1 st) (e.g. rice flour or chitosan and/or silica)	0 - 45%
Epithelial-adhering protein complex (EAPC)	0.1 – 1.5%

Thus, in addition to the specific examples provided below, the agar component may be replaced with at least one substitute thickener where the same function is achieved. Other further textural agents, maybe included to provide a higher total of total thickener, to bolster the function of modifying viscosity of the glycerol.

The table below exemplifies the composition of the dental paste provided in some possible embodiments:

Composition component	Working range	Example #1	Example #2	Example #3	Example #4	Example #5
	w/w %	w/w %	w/w %	w/w/ %	w/w%	w/w%
Agar (or xanthan gum or carrageenan for example)	2 to 3	2.6	2.6	2.6	2	3
Glycerol	50 to 95	86.4	86.4	94.5	90.5	79
Further Thickening agent (Rice flour or chitosan/silica etc)	0 to 45	10.5	10	0	5	15
Epithelial-adhering protein complex (EAPC)	0.03 to 3	0.5	1	2	2.5	3

Dental Paste production and effect/application

The applicant has therefore generated a novel process and new dental gel or paste composition with the view that such a composition, when applied to the teeth of a companion animal, such as a canine, would for the first time enable selective bactericidal activity to be delivered specifically in and be retained at the oral mucosa sufficiently long enough to consistently deliver an effective amount of the EAPC.

In doing so, oral health may be restored, and the symptomatic signs of oral disease reduced in canines and companion animals. However, to ensure such effect, it was crucial to determine that the relevant level of bioactivity is maintained in the product as claimed and produced by the process described herein. The following testing was undertaken to confirm those assertions.

Bioactivity - preliminary study

The paste/gel of the invention was tested using lactoperoxidase as an indicator of microbiome regulating activity. As discussed previously, the enzyme lactoperoxidase is a protein implicated in the microbiome regulating proteins isolated from bovine milk.

Lactoperoxidase activity was therefore measured by the addition of the dental paste as provided in Example 3 of the table above, to a solution of hydrogen peroxide in 100 mM Phosphate buffer, pH 5.5, and monitoring the oxidation of 2,2-Azino-bis(3-Ethylbenzthiazoline-6-sulphonicacid) (ABTS) spectrophotometrically at 436nm.

The level of lactoperoxidase activity was 56 U/g immediately after manufacture and 75 U/g 10 days after manufacture, as also shown in graphical form of **Figure 4**.

Lactoperoxidase activity of the combination of the microbiome regulating proteins formulated from the complex within the dental paste was retained and these levels are approximately what would be expected in the original complexed form indicating that formulating the EAPC further into the new dental paste does not reduce the biological activity of the antibacterial component.

Bioactivity - Confirmatory studies

Further testing to confirm bioactivity following formulation of the bioactive component i.e. the microbiome regulating protein within the epithelial-adhering protein complex when combined with the recited components and provided within the final formulation of the dental paste of the invention was undertaken.

Preparation of EAPC (Epithelial-adhering protein complex)

Two samples of the EAPC were prepared to enable comparison between expected bioactivity of two pastes using two microbiome regulating protein batches having either good activity or low activity:

1. 50mg of the EAPC (including 25mg microbiome regulating protein batch 18713 – good activity)
2. 50mg of the EAPC (including 25mg microbiome regulating protein batch 18317 – very low activity)

Preparation of comparison pastes

A formulation to exemplify the functional features of the composition was provided by the PLACEBO composition below.

- 5 Pastes (3g) were made from each of the comparative bioactive forms using the placebo formulation in accordance with the table detail below, namely: Trial Dental paste (Placebo paste with 50mg EAPC 1 containing 25mg of active microbiome regulating protein batch 18713), active microbiome regulating paste (placebo paste with 25mg microbiome regulating protein batch 18713 only, no EAPC) and low activity microbiome regulating protein paste (placebo paste with 50mg EAPC 2 containing 25 mg/ml of low activity microbiome regulating protein batch 18317).

10 Paste Formulation Table

PLACEBO formulation	Trial Dental PASTE 3g	Active microbiome regulating protein PASTE 3g	Low-Activity microbiome regulating protein PASTE 3g
Glycerol	Glycerol	Glycerol	Glycerol
Water (dilution only)	Water (dilution only)	Water (dilution only)	Water (dilution only)
Fumed Silica	Fumed Silica	Fumed Silica	Fumed silica
Xanthan Gum	Xanthan Gum	Xanthan Gum	Xanthan Gum
Carrageenan	Carrageenan	Carrageenan	Carrageenan
NONE	50mg EAPC (sample 1 above) 25mg microbiome regulating protein batch 18713	Milk regulating protein (only) no complex 25mg microbiome regulating protein batch 18713	50mg EAPC (sample 2 above) 25mg microbiome regulating protein batch 18317

Where relevant, the paste components of the formulations were within the working ranges specified previously. For the trial dental paste the components were in accordance with the various embodiments of the invention described above.

- 15 The water component was included only for consistency in formulation, and in particular to avoid heating the glycerol, to ensure no potential denaturing of the bioactive in the process of formulation and testing.

In vitro confirmation of comparative activity in pastes

Initial indication that activity could be maintained in the formulated dental paste was then required to be confirmed by additional *in vitro* testing.

Two linked indicators were tested to confirm that activity. Lactoperoxidase is one of the enzyme proteins that is selectively fractionated into the microbiome regulating component of the epithelial-adhering protein complex in the formulation. Re-confirming activity of lactoperoxidase in the final formulated paste would therefore be indicative that necessary activity was present for the intended applications. Further, the ability to kill pathogenic bacteria can be determined from antibacterial activity shown against the commonly cultured bacteria *Bacillus subtilis*. The effect the dental paste on *B. subtilis* growth was therefore also tested.

The table below shows the lactoperoxidase activity of the three comparative pastes:

Sample Name	Microbiome regulating protein batch	Lactoperoxidase activity
		U/g
Trial dental paste	18713	93
Active (microbiome regulating protein - only) paste	18713	215
Low activity paste	19317	2

The paste expected to have little activity, having been formulated with the batch of microbiome regulating protein known to have low activity confirmed immaterial lactoperoxidase activity in formulation. The enzyme activity of the paste with the batch of microbiome regulating protein that was active gave a paste that was active, as expected. The trial dental paste was much greater than the low activity paste, but compared to the active paste with only the microbiome regulating protein of the same batch some activity was lost. The decrease in lactoperoxidase activity may have occurred during the manufacture of the EAPC.

Next, the effect of the three pastes on the growth of *B. subtilis* over a 12-hour 40 min period (760 min) in lab conditions was determined. The dose of each paste delivered the same amount (0.08 mg/ml) of the microbiome regulating protein component into the bacteria culture solution sample.

Growth rate of *B. subtilis* with no paste (not shown) was determined to be akin to the growth rate in the presence of the low activity paste, growing to a maximum OD of around 0.25, after 760 mins as seen in **Figure 5**. As expected, the active microbiome regulating protein only paste inhibited the growth rate of *B. subtilis* more than the low activity paste.

As regards the trial paste, one might expect inhibition of *B. subtilis* to be slightly reduced based on the *in vitro* activity shown from the previous comparable lactoperoxidase activity. However, the dental paste of the invention completely inhibited *B. subtilis* growth over the period investigated. This suggests when presented with a biological challenge the activity of a comparable concentration of the bioactive component when provided in a form in accordance with the invention may be enhanced.

To establish the concentration of microbiome regulating protein that was required to fully inhibit *B. subtilis* growth the three pastes were increasingly diluted into the bacteria culture solutions and the

bacterial growth was measured after 500 minutes, while the bacteria were still in a state of rapid growth.

The results are visually illustrated in the graph of **Figure 6**, and the minimum inhibitory concentration (MIC) of the pastes is as follows:

Sample Name	Microbiome regulating protein batch	Minimum inhibitory concentration (Microbiome regulating protein content mg/ml)
Trial Dental paste	18713	0.02
Active (microbiome regulating protein only) paste	18713	0.93
Low activity paste	19317	>0.93

5

As expected from the previous data, the microbiome regulating protein was effective at reducing the growth rate of *B. subtilis* quickly from 0.15 mg/ml but did not fully inhibit growth until concentration was at 0.93 mg/ml. The low activity paste was required to be in a much higher concentration to inhibit growth and did not inhibit relative growth to a level to lower than 70% (i.e. did not fully inhibit).

10 However, the trial dental paste was significantly more effective at preventing the growth of *B. subtilis* than either of the two other pastes, despite having been prepared from the same amount and from the same batch (18713) as the active microbiome regulating protein only paste.

In conclusion, the trial dental paste appears to demonstrate a significant level of antimicrobial activity, much higher than expected from its microbiome regulating protein content.

15 The applicant proposes that a positive interaction between formulated components in the composition of the present invention yields something more than a mere additive effect. When the required components are complexed and formulated as per the composition of the invention and embodiments thereof described herein, a synergy occurs in which bioactivity is enhanced compared to that which is expected from a relative composition with equivalent amount of bioactive.

20 Dental Paste Dog Trial

Following confirmation that a useful bioactive effect is maintained and likely enhanced by embodiment compositions of the invention, evidence was required to elucidate that the bioactivity would translate to practical veterinary application. Specifically, a comparison between the new active formulation including a combination of two main functional components within a biocomplex (EAPC),
25 as compared to the placebo paste in which no active was present. The trial dental paste was manufactured comprising the following components of Glycerol, Thickening agent (s) and EAPC 1, in accordance with the table shown previously and having the working tolerances and ranges described. The placebo paste was identical, without the EAPC.

30 The trial dental paste and relative comparative placebo paste described herein were supplied for a 14-day dog trial designed to ascertain if practical use of the dental paste resulted in a benefit to the canine microbiome *in vivo*.

Animal sample pool and dental condition:

A total of 20 dogs were recruited to the trial and divided between trial paste and placebo paste groups. Dogs were of a variety of ages and breeds.

Dogs were required to have a significant dental condition at the start of the trial in accordance with the following grading authority:

- 5 Wisconsin Department of Agriculture, Trade and Consumer Protection and Division of Animal - Grading Canine Dental Condition: <https://datcp.wi.gov/Documents2/CanineDentalGradeChart.pdf>.

To be included in the trial all dogs were required to have a dental condition of at least grade 1 but below grade 3 as below:

MINOR / GRADE 1

- 10 Soft plaque has built up on the surface of the teeth. A small amount of tartar is also present. Tartar was formed when mineral deposits from saliva precipitated in plaque. Bacteria have grown, multiplied and invaded the gum tissues around the tooth causing early gingivitis, and red, inflamed gums.

MODERATE / GRADE 2

- 15 Moderate tartar buildup is present. It has acted as a wedge, pushing the gums away from the teeth, causing food, bacteria, and debris to accumulate there. The gums are red, swollen, and painful and bleed when touched. At this stage, proper dental care is crucial to reverse the progress of dental disease.

MAJOR / GRADE 3

- 20 Plaque and tartar formation is heavy. Pockets of bacteria and debris have accumulated to form further pockets of infection along the teeth. The infection has destroyed tissues around the teeth that hold them in place, and some have become loose. The gums appear bright red and bleed freely.

SEVERE / Grade 4

- 25 Severe tartar formation and gum disease is present. Toxic debris and inflammation have caused extensive tissue death. Roots are infected, abscessed, and rotten. The thin wall of bone surrounding teeth has deteriorated, and many teeth are loose. Bacteria may be spreading through the entire body in the bloodstream, potentially causing harm to vital organs. Pain can also be severe at this stage.

Trial Protocol

Participants received one tube of dental paste (trial dental paste or placebo paste) and two kits for taking oral swabs. All were instructed to follow the trial protocol:

- 30 Day 1

Collect baseline oral swab: wipe the swab tip in a rolling motion along the upper right gum line for 15 seconds. Insert the swab into the collection tube and follow the instructions supplied with the swabbing kit to prepare the sample for transport. Label the swab with dog name, date and number (1) for the first (baseline) swab taken.

Day 2 - 13

Apply the paste to dog's mouth once daily between meals (i.e. not less than an hour before or after feeding dog). Use finger to apply 3ml of paste to the gumline (i.e. the tooth/gum margin) of the upper arcade of dog's mouth.

5 Day 14

Apply the paste to dog's mouth for the final application. Do this at least one hour after dog has eaten anything. Do not feed dog between the final paste application and taking the second swab.

30 - 45 minutes after the final paste application, take the second (final swab): wipe the swab tip in a rolling motion along the upper right gum line for 15 seconds. Insert the swab into the collection tube and follow the standard instructions supplied with the swabbing kit to prepare the sample for transport. Label the swab with dog name, date, and number (2) for the second (final) swab taken.

Results and discussion

Upon completion sixteen dogs successfully completed the trial and the swabs for analysis were received in the lab for long-read 16S mRNA sequencing.

15 Four dogs were in the placebo paste group and twelve dogs in the trial paste group.

The abundance of bacteria in the oral cavity of the dogs was determined from long-read 16S mRNA sequencing of the material on swabs taken from the oral cavity of dogs in the treatment group (given trial paste made in accordance with an example of the invention, containing the before described EAPC (epithelial-adhering protein complex) and dogs in the placebo paste group (the paste not containing the EAPC). The species of bacteria were determined from the 16S mRNA sequences.

Key pathogenic species typically associated with canine oral health were identified from the literature. For example, the species described in "Assessment of changes in the oral microbiome that occurs in dogs with periodontal disease (2021) Santibanez R *et al. Veterinary Sciences*, 8, 291.", "The bacteriome of the oral cavity in healthy dogs and dogs with periodontal disease (2021). Niemiec B *et al. American Journal of Veterinary Science*, 83, 1." and "Molecular identification of bacteria associated with canine periodontal disease (2011). Riggio MP *et al. Veterinary Microbiology*, 150, 3-4.", (25 *Porphyromonas gulae*, *Pasteurella dagmatis*, *Actinomyces sp.*, *Tannerella forsythia*, *Treponema denticola* and *Corynebacterium sp.*) were measured to make a comparison and discern any statistically significant difference in the levels of these six pathogenic bacteria in the canine microbiome after treatment with the trial paste relative to the placebo paste group.

As may be illustrated by the direct comparison made in **Figure 7**, the abundance of six species of the pathogenic bacteria associated with canine dental disease was significantly reduced using the trial paste comprising the EAPC. As shown in the graphic, *Porphyromonas gulae*, *Pasteurella dagmatis*, *Actinomyces sp.*, *Tannerella forsythia*, *Treponema denticola* and *Corynebacterium sp.* all decreased in the test group utilising the trial dental paste of the invention comprising EAPC.

In contrast, in the placebo paste group the abundance of four of those species increased, and while the abundance of two of those species decreased it was to a lesser extent than the group given the trial paste.

As illustrated clearly in **Figure 8**, treatment of the canine microbiome, using the trial paste of the invention, decreased the total abundance of six key species of pathogenic bacteria by 6.63%, while the placebo paste had no statistically relevant effect on the total abundance of those same species.

5 This strongly suggests the trial dental paste or gel according to the above-described embodiments is likely useful as a veterinary medicament. Moreover, this maybe in the treatment protocol wherein the paste is effective by daily application.

Such a dental paste is therefore likely to help prevent oral disease mediated by an excess of pathogenic bacteria in mammals and/or animals, particularly canines. The dental paste of the invention may be particularly useful when oral disease includes one or more of the following symptoms: tooth decay,
10 gum inflammation, calculus, periodontal disease, halitosis.

Moreover, a chew or animal toy at least partially coated with or comprising the dental paste or gel as before described may be beneficial in the maintenance of oral health in an animal.

While certain representative embodiments and details have been shown for purposes of illustrating the invention, it will be apparent to those skilled in the art that various changes may be made without
15 departing from the scope of the disclosure, which is further described in the following appended claims.

The embodiments described above may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, and any or all combinations of any two or more said parts, elements, or features.

20

Claims:

1. A dental paste or gel composition, comprising:
at least 2 to 4 % w/w thickening agent, optionally agar;
5 at least 50 to 95 % w/w glycerol; and
at least 0.03 to 3% w/w epithelial-adhering protein complex.
2. The composition of claim 1, wherein the epithelial-adhering protein complex comprises:
microbiome regulating proteins and a modified whey protein isolate from milk.
3. The composition of claim 2, wherein the microbiome regulating protein (s) is selected
10 from lactoperoxidase, lactoferrin, lysosomal alpha-mannosidase, immunoglobulin G,
angiogenin, ribonuclease 4 or quiescin sulfhydryl oxidase and/or combinations thereof and
or the modified WPI is formed from soluble native protein, protein aggregates and protein
fibrils produced from whey protein isolates.
4. The composition according to any preceding claim, wherein the thickening agent, optionally
15 agar, is in the range of at least 2 to 3% w/w, preferably in the range 2.6 to 3% w/w.
5. The composition according to any preceding claim, wherein the epithelial-adhering protein
complex component of the composition is at least 0.03%, 0.5%, 2.5%, 2% or 1% w/w, and/or
at no more than 3%.
6. The composition of any preceding claim, wherein the glycerol is in the range 80 to 95% w/w
20 optionally, in the range 90 to 95% w/w.
7. The composition according to any preceding claim, wherein the composition of the may
further comprise up to 45% w/w, optionally up to 32% w/w, of one or more excipients selected
from one or more of arable gum, xanthan gum, alginates, chitin, aloe extract, guar gum,
25 hydroxypropyl cellulose, sodium casein, corn starch, carboxymethylcellulose, gelatine, agar,
dextrin, methylcellulose, polyvinyl alcohol, microfiber cellulose, microcrystalline cellulose,
seaweed cellulose, sodium polyacrylate, sodium polyphosphate, carrageenan or yeast cell
wall.
8. The composition according to any previous claim, for use as a medicament, optionally for use
as a veterinary medicament.
- 30 9. The composition according to Claim 8, wherein the use as a veterinary medicament
comprises a daily topical application of the composition to the oral mucosa.
10. The composition according to claim 8 or 9, for use in the treatment or prevention of oral
disease in mammals and/or animals, preferably canines, mediated by an excess of pathogenic
bacteria.

11. The composition according to claim 10, wherein the oral disease includes and/or is evidenced by one or more of the following conditions: tooth decay, gum inflammation, calculus, periodontal disease, halitosis.
- 5 12. A food supplement comprising the composition according to any previous claim, for use in the maintenance of canine or feline health.
13. A chew or animal toy comprising the composition of any preceding claim, optionally wherein the chew or toy is at least partially coated with the composition.
- 10 14. A method of preventing or treating oral or dental disease and/or improving or maintaining oral health, comprising administering to a mammal or animal, preferably a canine, in need thereof the composition according to any of claims 1 to 7.
15. A method of making a dental paste or gel composition, comprising the steps of at least:
- 15 providing 50 to 95% w/w glycerol and optionally heating, preferably up to 95°C;
- adding and dissolving 2 to 4 % w/w thickening agent, optionally agar into the glycerol;
- optionally adding 0 to 45% w/w of one or more excipients to the glycerol and thickening agent combination;
- 15 cooling the combination, optionally to 40°C or below;
- adding and dissolving 0.03 to 3 % w/w epithelial-adhering protein complex, optionally wherein the epithelial-adhering protein complex comprises: microbiome regulating proteins or a "protein fraction" and a modified whey protein isolate (WPI) from milk and optionally
- 20 wherein the microbiome regulating protein (s) is selected from lactoperoxidase, lactoferrin, lysosomal alpha-mannosidase, immunoglobulin G, angiogenin, ribonuclease 4 or quiescin sulfhydryl oxidase and/or combinations thereof and or the modified WPI is formed from soluble native protein, protein aggregates and protein fibrils produced from whey protein isolates; and
- 25 cooling, optionally to 18°C or below, to form the dental paste or gel composition.