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(54) Title: DENTAL COMPOSITIONS

(57) Abstract: An oral care composition comprising an effective amount of a therapeutic agent adsorbed onto nanospheres, together with orally acceptable diluents or carriers.

Dental Compositions

This invention discloses dental compositions which provide enhanced delivery of an antimicrobial agent to teeth and gums.

Dental plaque is a general term for the complex microbial community found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin. Plaque that becomes calcified is referred to as calculus. Plaque has been implicated as the cause of caries, gingivitis and periodontal disease.

The control of plaque is very important since it has been implicated as the main cause of dental diseases. The main approaches to the control of plaque have been mechanical plaque removal eg tooth brushing or flossing or the use of chemical anti-microbial agents in oral care products such as toothpaste and mouthwash. Whilst toothbrushing easily removes plaque this is only a short term measure as the plaque rapidly recolonises the tooth surfaces and indeed may not be entirely removed from the more inaccessible areas such as fissures, interproximal spaces or the gingival crevice.

So however efficiently the teeth are cleaned, plaque build up starts almost from cessation of brushing. Bacteria rarely come into contact with clean enamel. As soon as the tooth surface is cleaned, salivary glycoproteins are adsorbed onto the surface of the tooth enamel forming the acquired salivary pellicle. Large numbers of bacteria are found in saliva (up to 10^8 CFU ml^{-1}) which, unless swallowed, are likely to come into contact with a tooth surface and can initiate colonisation of that surface.

Coccal bacterial species such as *S. sanguis, S. oralis and S. mitis* are adsorbed onto the acquired salivary pellicle within about 2 hours of cleaning. Other pioneer species such as *Actinomyces* are also found but obligate anaerobic bacterial species are rarely detected at this stage. These primary colonising populations multiply, forming micro-colonies which become embedded in bacterial extracellular slimes and polysaccharides together with additional layers of adsorbed salivary proteins and glycoproteins. Growth of individual micro-colonies eventually results in the development of a confluent film of micro-organisms. The growth rates of the bacteria are fastest during this early period with doubling times from 1 - 3 hours having been calculated. As

the plaque develops into a biofilm so metabolism by the pioneer species creates conditions suitable for colonisation by species with more demanding atmospheric requirements. Also additional nutrients become available and the diversity of the microflora increases both in terms of the morphological types and in the numbers of species.

Any method of oral hygiene needs to inhibit this rapid build-up of plaque. Plaque control using antimicrobial agents has been a successful approach for many years, but to be effective, the antimicrobial must be retained in the oral cavity for sufficient time to take effect. Dental preparations are used infrequently, perhaps once or twice a day, for a short period of time, usually for less than three minutes. These short exposure times means that there are long periods in which the oral cavity has no protection from the harmful bacteria implicated in the formation of plaque.

It is not possible to simply increase the amount of the antimicrobial to ensure that all the harmful plaque bacteria are killed. Aside from general health considerations, it would disrupt the ecology of the oral microflora which may lead to the development of resistant strains of organisms, or the opportunistic overgrowth of more pathogenic species.

To offer better protection, it would be desirable if any new dental composition could provide an increased residence time for the antimicrobial agent in the oral cavity. Retention of the antimicrobial agent on the surfaces of the mouth would ensure that the agent has sufficient time to take effect. Further protection would be afforded if the composition provided a reservoir of the antimicrobial, allowing sustained release of the agent between the use of dental preparations. This would prevent the build-up of plaque between treatments.

The present invention provides an oral care composition comprising an effective amount of a therapeutic agent, preferably an antimicrobial agent, adsorbed onto nanospheres together with orally acceptable diluents or carriers. The nanospheres should be capable of penetrating the oral mucosa and releasing the active material therefrom. This achieves increased residence time in the oral cavity and provides sustained release of the therapeutic agent (antimicrobial agent) into the oral cavity. Due to improved epithelial penetration, more of the therapeutic agent would be retained in the upper

layers of the gingiva. Any antimicrobial agent would then be retained in the oral cavity long enough to act against the harmful bacteria. The therapeutic agent is released over a sustained period providing continuing protection until subsequent use of a dental cleaning preparation. A further advantage is that some therapeutic agents may be stabilised by adsorption into the nanospheres.

Nanospheres are very small polymer particles which are composed of a porous polymer having a large surface area giving them the capability to adsorb active materials and to release them over an extended time period. They have previously been used in a number of skincare compositions. By choosing for the nanosphere material a polymer or copolymer which is capable of being absorbed into the mucosa of the oral cavity, but not metabolised, nanospheres provide an effective manner of delivering an active to the oral cavity. The nanospheres may be manufactured to form the so-called "latex"-type nanospheres (for example nanospheres manufactured from styrene/acrylic copolymers) or the so-called "polysiloxane"-type nanospheres (for example nanospheres manufactured from silicone polymers manufactured by the controlled polymerisation of alkoxy-silanes to give polymers with reactive silanol-type functions on the outer surface of the nanospheres which must be deactivated to prevent aggregation of the nanospheres). The surface modifying compounds may contain quaternary ammonium groups such as those found in the modified latex-type nanospheres sold as Nanospheres 100 NH. The surface of the polysiloxane-type nanospheres may be modified by using compounds which have an alkylthiosulphate-type function (Bunte salts) which can form covalent bonds with free-SH groups on proteins, for example to give bio-adhesive nanospheres such as those sold as Nanospheres 100 NCK

Preferred nanospheres are those commercially available from Exsymol under the trade names Nanospheres 100 Triclosan NH (MPG), Nanospheres 100 Vitamin E Acetate, Nanospheres 100 Silanol, Nanospheres 100 "LIPO plus", Nanospheres 100 Menthol, Nanospheres 100 concentrated in OMC and Nanospheres 100 DSH C, Nanospheres 100 ACIDE SALICYLIQUE (Sio), Nanospheres 100 GLYCYRRHIZINATE (NH) and Nanospheres 100 ACIDE ASCORBIQUE (NCK). "Nanospheres 100" is a registered trade mark.

The nanospheres used in this invention have a diameter in the range of 50 nm to 1000 nm, preferably between 60 and 140 nm. The therapeutic agent may

comprise 0.05 to 20% of the nanospheres by weight.

The nanospheres may comprise 0.01% to 10% of the oral care composition, preferably 0.05% to 5%, most preferably 0.1% to 3%.

The amount of an antimicrobial agent in the oral composition both 'free' and incorporated in the nanospheres, preferably comprises 0.00001% to 0.1% by weight, more preferably 0.00005 to 0.05%, most preferably 0.001 to 0.03%.

Suitable therapeutic agents include vitamins and glycyrrhizinate.

Suitable antimicrobial agents include halogenated diphenyl ethers such as triclosan (2',4,4'-trichloro-2-hydroxy-diphenyl ether) or halogenated bis-phenol methanes such as hexachlorophene [bis(3,4,6-trichloro-2-hydroxyphenyl)methane] or bromochlorophene [bis(4-chloro-6-bromo-2-hydroxyphenyl)methane].

Compositions containing an antimicrobial agent adsorbed onto a nanosphere provide sustained release of the antimicrobial agent. When teeth are cleaned using such a composition the effect of this is to slow the re-colonisation of the surface of cleaned teeth by bacteria when compared to conventional dental compositions. Without being bound by any theory it is believed that the nanospheres are absorbed into the upper layers of the gingiva. From there the active is slowly released over time. The effect of this sustained release is to provide a consistent presence of an antimicrobial agent in the oral cavity. In conventional dental compositions, the active may not be present long enough to come into contact with all the harmful bacteria and to kill them. This is particularly true of bacteria in the more inaccessible cavities, where decay is most likely to start. The present invention provides more sustained and constant concentrations of antimicrobial agent in the oral cavity so that bacteria are more likely to be eradicated. Further, after use with conventional dental compositions, the concentration of an antimicrobial agent in the oral cavity quickly falls. Once the concentration of agent falls there is nothing to prevent harmful bacteria recolonising the teeth. The more constant level of the antimicrobial agent provided by the present invention prevents this happening, stopping the build up of plaque.

The present invention also provides for the use of nanospheres adsorbing an antimicrobial agent in an oral care composition in the treatment of tooth decay and gum disease. The words "treat" and "treatment" used herein are intended to embrace the alleviation, cure and reduction of symptoms of established disease states and the prevention or prophylaxis of disease. The oral care compositions of the present invention also give sustained release of the antimicrobial agent. The release profile of the composition depends on the concentration of the agent in the composition and nanosphere, and the type of nanospheres used. Compositions may provide sustained release over an extended period, preferably one to twelve hours.

The effect of the sustained release of the antimicrobial agent in the oral cavity is to prevent the build up of plaque. Plaque is implicated as a source of tooth decay and gum disease. Accordingly the present invention teaches the use of an antimicrobial agent adsorbed onto a nanosphere in the treatment of tooth decay and gum disease. The best mode is as an oral composition; this invention teaches the use of an antimicrobial agent adsorbed onto a nanosphere in the preparation of a medicament for the treatment of tooth decay and gum disease.

The dental composition may be formulated as a toothpaste, mouthwash, tooth gel, toothpowder, dental tablet or a dental gel and may be formulated in a manner known to those skilled in the art.

Such compositions may, as appropriate, contain materials which are well known to those skilled in the art of dental preparations such as, for example, humectants, surfactants, abrasives, fluoride sources, desensitising agents, flavourings, colourings, sweeteners, antimicrobial agents or bacteriocides to act as preservatives, anti-tartar or anti-calculus agents, structuring agents, chelating agents, whitening agents, vitamins, other anti-plaque agents not absorbed into nanospheres and any other therapeutic actives.

Suitable humectants for use in dentifrice compositions include polyhydric alcohols such as xylitol, sorbitol, glycerol, propylene glycol and polyethylene glycols. Mixtures of glycerol and sorbitol or sorbitol and xylitol are particularly effective. A humectant helps to prevent dentifrice compositions from hardening on exposure to air, and may also provide a moist feel, smooth texture,

flowability, and a desirable sweetness in the mouth. Suitably, such humectants may comprise from about 0-85%, preferably from about 0-60% by weight of the oral hygiene composition.

Suitable surfactants for use in dentifrices, mouthwashes etc. are usually water-soluble organic compounds, and may be anionic, nonionic, cationic or amphoteric species. The surfactant used should preferably be reasonably stable, and able to produce a foam in use.

Anionic surfactants include the water-soluble salts of C10-C18 alkyl sulphates (e.g. sodium lauryl sulfates), water soluble salts of C10-C18 ethoxylated alkyl sulphates, water soluble salts of C10-18 alkyl sarcosinates, water-soluble salts of sulfonated monoglycerides of C10-18 fatty acids (e.g. sodium coconut monoglyceride sulfonates), alkyl aryl sulfonates (e.g. sodium dodecyl benzene sulfonate), sodium salts of the coconut fatty acid amide of N-methyltaurine and sodium salts of long chain olefin sulfonates (eg sodium C14-C16 olefin sulfonate.

Nonionic surfactants suitable for use in oral compositions include the products of the condensation of alkylene oxide groups with aliphatic or alkylaromatic species, and may be for example, polyethylene oxide condensates of alkyl phenols, ethylene oxide/propylene oxide copolymers (available from BASF Wyandotte Chemical Corporation under the trade name `Pluronic'), ethylene oxide/ethylene diamine copolymers, ethylene oxide condensates of aliphatic alcohols, long chain tertiary amine oxides, long chain tertiary phosphine oxides, long chain dialkyl sulfoxides and mixtures thereof. Alternatives include ethoxylated sorbitan esters such as those available from ICI under the trade name "Tween".

Cationic surfactants are generally quaternary ammonium compounds having one C8-18 alkyl chain and include, for example, lauryl trimethylammonium chloride, cetyl trimethylammonium bromide, cetyl pyridinium chloride, di-isobutylphenoxyethoxyethyldimethylbenzylammonium chloride, coconut alkyl trimethylammonium nitrite and cetyl pyridinium fluoride. Also useful are benzyl ammonium chloride, benzyl dimethyl stearylammonium chloride, and salts of tertiary amines having one C1-18 hydrocarbon group and two (poly)oxyethylene groups.

Amphoteric surfactants may be aliphatic secondary and tertiary amines comprising aliphatic species which may be branched or unbranched, and in which one of the aliphatic species is a C8-18 species and the other contains an anionic hydrophilic group, for example, sulfonate, carboxylate, sulfate, phosphonate or phosphate. Examples of quaternary ammonium compounds are the quaternized imidazole derivatives available under the trade name `Miranol' from the Miranol Chemical Company. Other amphoteric surfactants that may be employed are fatty acid amido alkyl betaines where one alkyl group is commonly C10-C12 such as cocamido propyl betaine, for example Tego Betain supplied by T H Goldschmitt.

Suitably, the surfactant is included in an amount of from 0-20%, preferably 0-10%, most preferably 1-4% by weight of the oral hygiene composition.

Suitable abrasives include particulate cellulose, silica, alumina, insoluble metaphosphates, calcium carbonate, dicalcium phosphate (in dihydrate and anhydrous forms), calcium pyrophosphate, natural and synthetic clays, and particulate thermosetting polymerised resins selected from melamine-ureas, melamine-formaldehydes, urea-formaldehydes, melamine-urea-formaldehydes, cross-linked epoxides, melamines, phenolics and cross-linked polyesters. Suitable silica abrasives include the hydrated silicas, particularly those available under the trade names `Sident' from Degussa AG, `Zeodent' from J M Huber Corporation and `Sorbosil' from Crosfield UK. Suitably, the particulate cellulose is highly purified cellulose such as that available under the trade names `Elcema' from Degussa A.

Fluoride sources suitable for use in oral hygiene compositions of the present invention include sodium fluoride, zinc fluoride, potassium fluoride, aluminium fluoride, lithium fluoride, sodium monofluorophosphate, stannous fluoride, ammonium fluoride, ammonium bifluoride and amine fluoride. Preferably, the fluoride source is present in an amount sufficient to provide from about 50 ppm to about 4,000 ppm fluoride ions in the composition. Inclusion of a fluoride source is beneficial, since fluoride ions are known to become incorporated into the hydroxyapatite of tooth enamel, thereby increasing the resistance of the enamel to decay. Fluoride is also now thought to act locally on the tooth enamel, altering the remineralisation-demineralisation balance in favour of remineralisation. Inclusion of a fluoride source is also desirable when a

polyphosphate anti-calculus agent is included, in order to inhibit the enzymic hydrolysis of such polyphosphates by salivary phosphatase enzymes.

Suitable desensitising agents include, for example, formaldehyde, potassium salts such as potassium nitrate, tripotassium citrate, potassium chloride, potassium bicarbonate and strontium salts such as strontium chloride (suitably as hexahydrate), strontium acetate (suitably as hemihydrate) and also sodium citrate.

Flavouring agents may be added to increase palatability and may include, for example, menthol, oils of peppermint, spearmint, wintergreen, sassafras and clove. Sweetening agents may also be used, and these include D-tryptophan, saccharin, dextrose, aspartame, levulose, acesulfam, dihydrochalcones and sodium cyclamate. Typically, such flavouring agents are included in amounts of from 0-5%, preferably from 0-2% by weight of the oral hygiene composition. Colouring agents and pigments may be added to improve the visual appeal of the composition. Suitable colourants include dyes and pigments. A suitable and commonly used pigment is pigment grade titanium dioxide, which provides a strong white colour.

Suitably, as described above, the compositions of the invention may include a further antimicrobial agent as a preservative, antibacterial and/or anti-plaque agent. Suitable antimicrobial agents include water soluble sources of certain metal ions such as zinc, copper and silver such as zinc citrate and silver chloride, cetyl pyridinium chloride, the bis-biguanides (such as chlorhexidine), aliphatic amines, phenolics such as bromochlorophene and triclosan, salicylanilides and quaternary ammonium compounds. Optionally, the formulations may also contain enzymes that will disrupt the pellicle or interfere with bacterial intercellular polysaccharides. Examples would include proteases such as papain and bromelain or dextranases. Natural enzymatic biocidal systems such as a system comprising lactoperoxidase and glucose oxidase may also be employed.

The composition may also comprise an anti-calculus agent. Suitable anti-calculus agents include zinc salts such as zinc citrate and zinc chloride, polyphosphates and pyrophosphates. Suitable pyrophosphates include the sodium and potassium pyrophosphates, preferably disodium pyrophosphate,

dipotassium pyrophosphate, tetrasodium pyrophosphate and tetrapotassium pyrophosphate and mixtures thereof. A preferred source of pyrophosphate is a mixture of tetrasodium pyrophosphate and tetrapotassium pyrophosphate. Suitable polyphosphates include sodium tripolyphosphate.

Structuring (gelling) agents may be required in, for example, dentifrices and gums to provide desirable textural properties and "mouthfeel". Suitable agents include natural gum binders such as gum tragacanth, xanthan gum, gum karaya and gum arabic, seaweed derivatives such as Irish moss and alginates, smectite clays such as bentonite or hectorite, carboxyvinyl polymers and water-soluble cellulose derivatives such as hydroxyethyl cellulose and sodium carboxymethyl cellulose. Improved texture may also be achieved, for example, by including colloidal magnesium aluminium silicate. Suitably, the structuring agent is included in an amount of from 0-5%, preferably 0-3% by weight of the oral hygiene composition.

Suitable vitamins for inclusion in the dental preparations of the present invention include vitamins A, B5, B6, C and E.

The invention is illustrated by the following non-limiting examples.

Example 1 - Anti-plaque Toothpaste

	% w/w
Sorbitol (70% soln)	45.00
Hydrated Silica Abrasive	6.94
Hydrated Silica Thickener	11.1
Sodium Saccharin	0.26
Sodium Fluoride	0.24
Sodium Carboxymethylcellulose	0.9
Titanium Dioxide	0.5
Sodium Lauryl Sulphate	1.5
Sodium Hydroxide	0.1
Flavour	0.91
Nanospheres (with 5% triclosan)	2.00
Water	qs

Method of preparation

Sodium saccharin, sodium fluoride, and sodium hydroxide were added to the bulk of the water. Sorbitol was added to the mixture and stirred until dissolved. The mixture was then added to sodium lauryl sulphate dissolved in water. Hydrated silica abrasive, hydrated silica thickener, sodium carboxymethylcellulose and titanium dioxide were then added and the bulk mixed under vacuum until homogeneous. To this was then added the flavour and the nanospheres and the bulk mixed under vacuum until homogeneous.

Example 2 - Anti-plaque Toothpaste

	% w/w
Sorbitol (70% soln)	45.00
Hydrated Silica Abrasive	6.94
Hydrated Silica Thickener	11.1
Sodium Saccharin	0.26
Sodium Fluoride	0.24
Sodium Carboxymethylcellulose	0.9
Titanium Dioxide	0.5
Sodium Lauryl Sulphate	1.5
Sodium Hydroxide	0.1
Flavour	0.91
Nanospheres (with 5% triclosan)	1.00
Water	qs

Method of preparation

Sodium saccharin, sodium fluoride, and sodium hydroxide were added to the bulk of the water. Sorbitol was added to the mixture and stirred until dissolved. The mixture was then added to sodium lauryl sulphate dissolved in water. Hydrated silica abrasive, hydrated silica thickener, sodium carboxymethylcellulose and titanium dioxide were then added and the bulk mixed under vacuum until homogeneous. To this was then added the flavour and the nanospheres and the bulk mixed under vacuum until homogeneous.

Example 3 - Anti-plaque Mouthwash

	% w/w
Sorbitol (70% soln)	5.00
Sodium Fluoride	0.05
Sodium Saccharin	0.04
Ethanol 96%	8.00
Polysorbate 20	0.15
PEG-40 Hydrogenated Castor Oil	0.15
Flavour	0.10
Colour	qs
Nanospheres (with 5% triclosan)	0.03
Water	qs

Method of preparation

To a stirred solution of water and sorbitol was added sodium fluoride, sodium saccharin and colour. A premix of PEG-40 hydrogenated castor oil, polysorbate 20, flavour and ethanol was then added to the mixture. To this bulk was then added the nanospheres.

The experiment described below demonstrated that nanospheres were able to adsorb to oral epithelial cells and that they were able to deliver triclosan through the oral epithelium in an in-vitro cell model. The model used in these experiments was a skin sample onto which layers composed of keratinocytes and fibroblasts were deposited. The amount of triclosan in these layers was determined using UV spectroscopy. The results showed that the nanospheres were effective in delivering triclosan to the keratinocytes and to the fibroblast layers.

A) Preparation of oral epithelial cells

All procedures were carried out in the laminar flow cabinet. Tissues from gingival biopsies taken during the course of oral surgery were washed three times in Phosphate Buffered Saline (PBS) and cut into pieces of ca. 0.5cm^2.

The cut-up tissues were incubated in 10% trypsin at 4°C for 1 hour and then for 2 hours at 37°C. A culture medium for gingival fibroblasts (based on Dulbecco's Modification of Eagle's Medium (DMEM)) (5ml) was added to inactivate the trypsin. The oral epithelial cell layer was separated and made into a single cell suspension. The cells were incubated in Keratinocyte-SFM medium on collagen I coated tissue culture plastic in a humidified atmosphere of 5% CO_2/95% air at 37°C to give a suspension of oral epithelial cells which were used after the first passage at around 70% confluence.

B) Preparation of the fibroblast culture

The mucosa remaining from the biopsy after removal of the epithelium was placed in a petri dish containing a culture medium for gingival fibroblasts (based on Dulbecco's Modification of Eagle's Medium (DMEM)) and minced finely using a scalpel. The minced tissue was incubated in 10ml 0.05% (w/v) collagenase type I [dissolved in a culture medium for gingival fibroblasts (based on Dulbecco's Modification of Eagle's Medium (DMEM))] at 37°C overnight to disaggregate the tissue. The cells were resuspended in a culture medium for gingival fibroblasts (based on Dulbecco's Modification of Eagle's Medium (DMEM)) and plated out cells in a T75 flask. The cells were fed every 2-3 days until confluent to give a culture of fibroblast cells which was used before the end of the 4th passage

C) Preparation of experimental skin model

Samples of human skin were sterilised by storage in 98% glycerol for 4 weeks. The samples were then incubated in 1M NaCl solution for 48-72 hours at 37°C. The epidermis was peeled away from the dermis and discarded. The dermis was acellularised by incubating it in a large volume of sterile distilled water (approx. 6 x volume of skin) for 4 weeks. Spent water was replaced twice a week. Discs (12mm) of de-epidermised human dermis were punched out and sterilised by irradiation with gamma radiation. The sterile discs were placed

into 24-well inserts with the reticular (rough) surface uppermost. PBS (1ml) was added to each well to keep the tissue moist. The culture of fibroblast cells (1ml - 5 x 10^5 cells/ml) from B above was added to each insert containing the de-epidermised dermis and incubated at 37°C for 48 hours (5% CO_2, 100% humidity). This deposited fibroblast cells onto the dermis. The medium was removed and the dermis placed into 24-well inserts with the epithelial surface uppermost (fibroblasts towards base of wells). The inserts were placed individually into 24-well plates with 500µl of PBS in the wells to keep the dermis moist. The PBS was removed from the inserts/wells and 500µl Greens medium was added to the wells. The cell epithelial suspension (500µl) from A above was added to the centre of the insert. After 24-48 hours the composite was raised to the air/liquid interface. The spent medium was removed and Greens medium (500 µl) from A above was added to the well so that the reticular layer (bottom) was bathed in medium. Greens medium (100µl) was added to the centre of the insert so that the epithelial layer was moist but not fully submerged. The cells were fed every 2-3 days by removing the spent medium and replacing it with fresh Greens medium. This deposited epithelial cells on to the dermis. The resulting experimental skin model was left for approximately 2 weeks to differentiate before carrying out the experimental procedure.

D) Determination of triclosan delivery

Test samples were prepared by suspending nanospheres containing 5% triclosan in a 50% solution of sorbitol in water to give a concentration of 200ppm triclosan. The medium was removed from the centre of the inserts containing the experimental skin model and the test solution (500 µl) was placed into the centre of the insert and left for 2 minutes. The agent was removed and the model washed with PBS (500 µl).

The model was removed from the insert using a pair of forceps and placed in a clean well of a 24-well plate. Absolute ethanol (500 µl) was added and left for 10 minutes. The ethanol was agitated to remove the top layer of cells and the

ethanol extract transferred to a microcentrifuge tube. Distilled water (500 μl) was added to each tube. The tube is then centrifuged. The absorbance of the supernatant at 281 nm was determined spectrophotometrically and the concentration of triclosan that had been released into the epithelial layer was determined spectrophotometrically at 281nm by comparison with a standard curve for known concentrations of triclosan.

A 0.1% solution of sodium lauryl sulphate (500 μl) was added to each well to solubilise the mucosa and left for 30 minutes prior to the addition of ethanol (500 μl) for 1 minute. The extract was placed in a microcentrifuge tube and centrifuged at 1300rpm to remove dead cells from the extract. The absorbance of the supernatant at 281nm was determined spectrophotometrically and the concentration of triclosan which had been released into the fibroblast layer determined by comparison with a standard curve for known concentrations of triclosan.

A control solution of 50% sorbitol in water was used during the spectrophotometric determinations. The absorbance observed with this control was subtracted from the observed absorbance figures in the above experiments to give the absorbance due to the triclosan present .

E) Positive Control

An emulsion containing triclosan to use as a positive control was prepared from the following components:

Triclosan	1.00
Capric/caprylic triglyceride	10.00
Sorbitol 70% solution	10.00
Polyglyceryl-3-oleate	5.00
PEG-40 Hydrogenated castor oil	2.00
Xanthan gum	1.00
Deionised water	to 100%

The above emulsion was diluted with a 50% aqueous sorbitol solution to give a triclosan concentration of 10,000 ppm. The diluted emulsion was then used in the above experimental procedures to determine the amount of non-adsorbed triclosan in the epithelial layer and the mucosal (fibroblast) layer.

Preparation of reagents

Greens media

	Amount Added	Final Conc.
Hams F12 medium	21ml	21%
DMEM	60ml	60%
Glutamine	1ml	2mM
Pen / Strep	1ml	50U:50 μg/ml
Fungizone	250 μl	2.5 μg/ml
Foetal Calf Serum	10ml	10%
Adenine	400 μl	$1x10^{-4}$ M
Insulin	500 μl	5 μg/ml
Transferrin/Triiodthyronine	100 μl	5 μg/ml:$2x10^{-7}$ M
Hydrocortisone	16 μl	0.4 μM
Epidermal Growth Factor	10 μl	10 ng/ml

A culture medium for gingival fibroblast (based on Dulbecco's Modification of Eagle's Medium (DMEM))

	Amount Added	Final Conc.
DMEM (without phenol red)	500ml	
Foetal Calf Serum	50ml	10%
Glutamine	5ml	2mM
Pen / Strep	5ml	50U:50 μg/ml
Fungizone	1.25ml	625 ng/ml

Results

The amount of triclosan derived from the nanospheres present in the epithelial layer was 6.99 ± 3.10 μg/ml and the amount present in the mucosal (fibroblast) layer was 4.74 ± 2.68 μg/ml. The amount of triclosan derived from the positive control present in these two layers was 142 ± 153μg/ml respectively. The positive controls contained 50 times the amount of triclosan present in the test samples containing nanospheres so the expected amount which would have been present in similar amounts had been used would have been 2.8 ± 3.1 μg/ml and 1.2 ± 0.5 μg/ml respectively. Thus these data demonstrate that nanospheres would be absorbed into the oral mucosa and that the amount of triclosan present in the mucosa would be increased by the use of nanospheres.

CLAIMS

1. An oral care composition comprising an effective amount of a therapeutic agent adsorbed onto nanospheres, together with orally acceptable diluents or carriers.

2. A composition as claimed in claim 1 wherein the therapeutic agent is an antimicrobial agent

3. A composition as claimed in claim 2 wherein the antimicrobial agent is triclosan, hexachlorophene or bromochlorophene.

4. A composition as claimed in any preceding claim wherein the composition contains 0.01-10% w/w of the nanospheres.

5. A composition as claimed in claim 2 or claim 3 wherein the antimicrobial agent is present as 0.00001 to 0.1% w/w of the composition.

6. A composition as claimed in any preceding claim wherein the composition is formulated as a paste, a gel or a mouthwash.

7. A composition as claimed in claim 2 where the antimicrobial agent comprises 0.05-20% of the nanosphere.

8. The use of a composition as claimed in claim 2 to provide sustained release of an antimicrobial agent in the oral cavity.

9. The use of an antimicrobial agent adsorbed onto nanospheres in the treatment of tooth decay and gum disease.

10. The use of an antimicrobial agent adsorbed onto nanospheres in the preparation of a medicament for the treatment of gum disease and tooth decay.