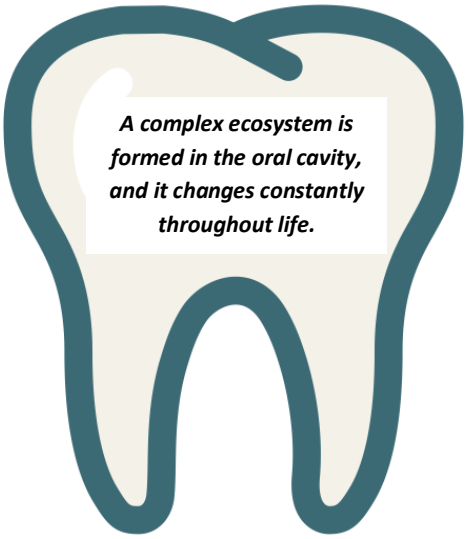
SilverSol: Paradigm Shifting Applications in Clinical Dentistry

The Oral Flora in Dental Infections

The oral cavity is colonized by a diverse range of microorganisms. These comprise ~300–500 species of bacteria, fungi and protozoa, of which only ~10% are regularly isolated using conventional culture techniques. In a recent study, ~40% of the bacteria identified using 16S rRNA amplification methods were of novel phylotypes, and presumably many of these represent bacteria that cannot be isolated using conventional culture methods.

Of the bacteria that are easily recovered upon routine culture, the α-haemolytic streptococci are among the most frequent isolates. Other bacteria found in the oral commensal flora include coagulase-negative staphylococci, Gram-negative cocci belonging to the families Neisseriaceae and Veillonellaceae, Lactobacilli, Spirochaetes, Corynebacteria and Mycoplasmas. A complex ecosystem is formed in the oral cavity, and it changes constantly throughout life. Many commensal microorganisms will cause disease if provided with appropriate conditions. Gram-positive aerobic cocci, α-haemolytic streptococci, Peptostreptococci and Gram-negative anaerobes are frequently isolated from oral infections.



During the last decade approximately 10 species, mainly Gram-negative anaerobes, have been noted as putative pathogens in periodontal disease. The Gram-positive and facultatively anaerobic mutans streptococci are aetiologically the most important bacteria in dental caries. Data have rapidly increased on the association of these bacteria with certain periodontal diseases or caries, on phenotypic and genotypic characteristics, pathogenic mechanisms, antibiotic susceptibility patterns and transmission among family members. Oral bacteria may spread into the blood stream through ulcerated epithelium in diseased periodontal pockets and cause transient bacteraemia, which are regarded as increased risk, especially for immunocompromised patients or persons with endoprostheses. In these patients, routine antibiotic prophylaxis is recommended for invasive dental care procedures. Also, the new association between dental infections and myocardial/cerebral infarction have offered new challenges for cooperation between dental and medical researchers.[1]

Streptococcus mutans is considered to be the primary bacterium involved in plaque formation and the initiation of dental caries and the most cariogenic of all the oral *Streptococci* responsible for dental caries. *Streptococcus sanguinis*, a strain of the "Viridans Streptococcus group" mostly found in dental plaque and cavities within the healthy oral mouth, may come to inhabit the heart valves through the bloodstream following invasive (surgical) dental procedures, leading to severe subacute bacterial endocarditis.[2]

S. mutans can adhere the enamel surface, produce acid metabolites, build glycogen reserves and to synthesize extracellular polysaccharides. Mutans streptococci create acidic environment creating a risk for formation of cavity. During the formation of dental plaque, *S. mutans* adhere to primary colonizers by cell to cell interaction which forms biofilm on the teeth which induces bacterial growth.

Streptococcus sanguis is normally found in the human oral cavity. Due to low cariogenicity, it forms a colony on tooth surface which gets aggregated by other oral bacteria and leads to maturation of dental plaque. Another organism, *Streptococcus salivarius* belonging to the *salivarius* subspecies is found in oral cavity in humans a few hours after birth and remain there as the predominant inhabitant. All these organisms enhance caries formation and thus the progression of periodontal disease.

Candidal organisms are commensals of the oral cavity and their opportunistic infection depends on the interplay of local and systemic factors. Endodontic treatment failure has been associated with the persistence of microbial flora following therapy in the root canal system, and this includes fungi like Candida, which are resistant to conventional root canal irrigants.[3] *Candida albicans* and *Candida glabrata* cause infections including oral thrush and dental stomatitis, a painful infection affecting around seven out of ten denture wearers. Infections like these are particularly difficult to treat because the microorganisms involved form biofilms.[4]

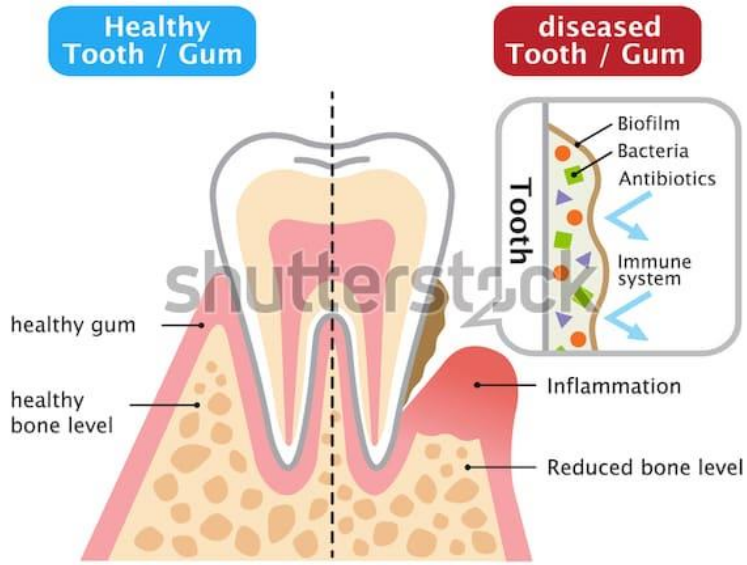
Microbial Biofilms in dental infections

Biofilms represent bacterial communities embedded in self-produced extracellular polymeric matrix that is attached to a surface. The microbial population comprising a biofilm can be made up of single or multiple bacterial species. The extracellular matrix is an intermediate environment for biofilm bacteria that stabilizes the three-dimensional biofilm structure and mediates bacterial adhesion.

In the early stage of biofilm formation, the attached bacteria proliferate, aggregate and form characteristic microcolonies. Cells from the surrounding area are recruited, bind to existing structures and become embedded in extracellular matrix.

It has been recognized for considerable period that the organisms growing in the biofilms are more resistant to antibiotics than the same species growing in a planktonic (unattached)

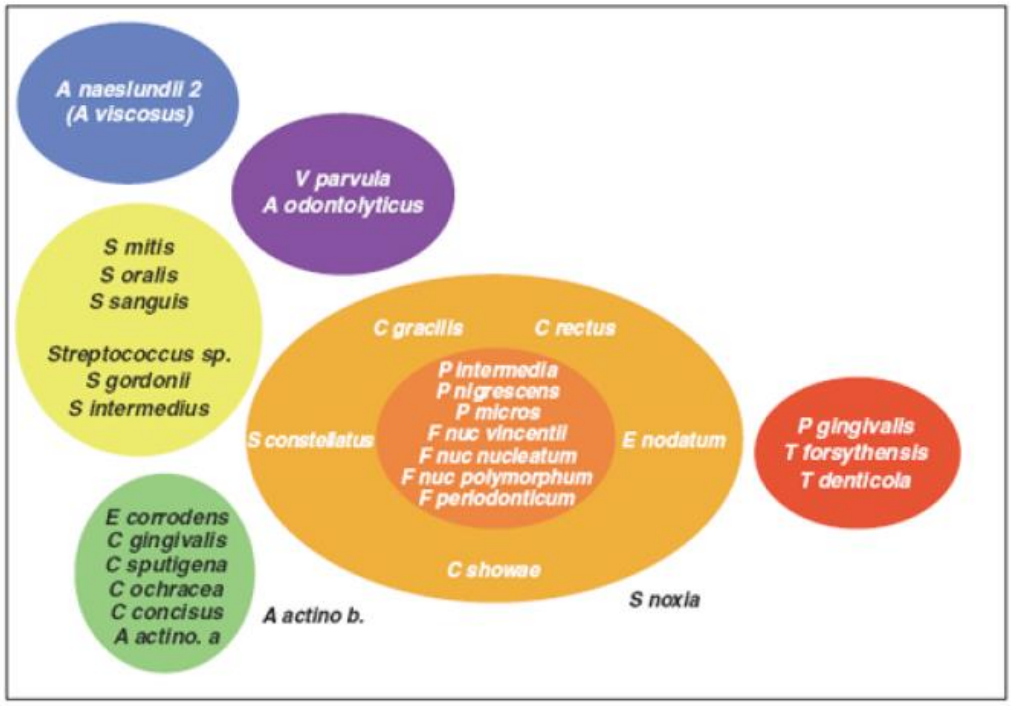
state. Given the decreased sensitivity of an organism on a surface to antimicrobial agents, it has been argued that it would be more appropriate to determine the biofilm inhibitory concentration and biofilm killing concentration. Thus, it is important to understand the factors leading to antimicrobial resistance in biofilms such as dental plaque. Common biofilm forming microorganisms associated with dental plaque include *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Peptostreptococcus micros* etc.[5]



Teeth are colonized by oral bacteria from saliva containing more than 700 different bacterial species. If removed regularly, the dental biofilm mainly comprises oral streptococci and is regarded as resident microflora. But if left undisturbed, a complex biofilm containing up to 100 bacterial species at a site will build up and may eventually cause development of disease. Depending on local ecological factors, the composition of the dental biofilm may vary considerably. With access to excess carbohydrates, the dental biofilm will be dominated by mainly gram-positive carbohydrate-fermenting bacteria causing demineralization of teeth, dental caries, which may further lead to inflammation and necrosis in the pulp and periapical region, i.e., pulpitis and periapical periodontitis. In supra- and subgingival biofilms, predominantly gram-negative, anaerobic proteolytic bacteria will colonize and cause gingival inflammation and breakdown of supporting periodontal fibres and bone and ultimately tooth loss, i.e., gingivitis, chronic or aggressive periodontitis, and around dental implants, peri-implantitis. Furthermore, bacteria from the dental biofilm may spread to other parts of the body by bacteraemia and cause systemic disease. Basically, prevention and treatment of dental biofilm infections are achieved by regular personal and professional removal of the dental biofilm.[6]

Candida-associated denture stomatitis is a form of oral Candida infection, covering areas of the mucosa which support the upper denture. It is associated with biofilm formation by Candida species, of which Candida albicans and Candida glabrata are the most abundant in

the oral cavity. Biofilms are associated with antimicrobial resistance and, therefore, infections related to them are recalcitrant and tricky to treat.[7]



Microbial complexes in subgingival biofilm

Colour designations were used to denote the association of particular bacterial complexes with periodontal infections. The *blue*, *yellow*, *green*, and *purple* complexes designate early colonizers of the subgingival flora. *Orange* and *red* complexes reflect late colonizers associated with mature subgingival plaque.

Bacterial species contained in the yellow, green, and purple complexes appear to colonize the subgingival sulcus first and predominate in gingival health. In contrast, orange complex

bacteria are associated with gingivitis and gingival bleeding. Interestingly, bacteria of the orange complex may also be associated with red complex microorganisms including *Porphyromonas gingivalis*, *Tannerella forsythensis*, and *Treponema denticola*, organisms found in greater numbers in diseased sites and in more advanced periodontal disease.

Dental Antiseptics and Antimicrobials: Limitations of currently available agents

An increase in antibiotic-resistant bacteria has created interest in using inexpensive, safe, and highly bactericidal/virucidal antiseptics in periodontal therapy. Antiseptics attack multiple components of infectious agents, practically eliminating the risk for development of resistance and are particularly valuable in the treatment of biofilm infections, which may be unresponsive to even high concentrations of antibiotics. Moreover, because the contents of inflamed periodontal pockets are emptied into the oral cavity every 90 seconds, and relatively small amounts of antimicrobial agents are applied sub-gingivally, the risk for antiseptics entering the gingival tissue and causing systemic damage is minimal. This high degree of safety allows frequent and broad use of antiseptics in periodontal treatment.

Povidone Iodine

The most common commercial form of povidone-iodine is a 10% solution in water, yielding 1% (10,000 ppm) available iodine. Povidone-iodine is active in vitro against all major periodontopathic bacteria and exhibits a wide virucidal spectrum, covering both enveloped (e.g., herpes viruses) and non-enveloped viruses. Organic material present on any surface can lead to neutralization of disinfectant capability of iodine. [20]

Povidone-iodine can give rise to allergic reactions, including itching, burning, and reddening and blistering in the area of application, so a patient's history of allergy to iodine or shellfish must be evaluated. Prolonged iodide intake can inhibit thyroid hormone synthesis and cause goitre, myxoedema, or hyperthyroidism; therefore, povidone-iodine should not be used in patients with thyroid dysfunction, pregnant woman, infants, or in routine patient self-care. Iodophors like Povidone iodine can have irritating effect on mucous membranes.[19]

The varied studies provide evidence that in most instances, povidone-iodine did not effectively promote good wound healing; in fact, most studies showed either impaired wound healing, reduced wound strength, or infection.[16]

Chlorhexidine

Chlorhexidine, a bisbiguanide, has been an important oral antiseptic for more than 40 years, and numerous studies and meta-reviews have confirmed its antiplaque and antigingivitic

effects. Though it has a broad-spectrum antimicrobial activity, no antifungal activity was seen

Chlorhexidine is inactivated by organic serum compounds in the gingival crevice fluid. As the antimicrobial action of the cationic chlorhexidine is neutralized by anionic compound surfactants in toothpastes, chlorhexidine should not be used in conjunction with tooth brushing. A major disadvantage of chlorhexidine is its propensity to dark stain tooth surfaces. Dark staining gaps along the margin of tooth-coloured restorations may reflect into the filling material and necessitate replacement of affected restorations. Moreover, activity of chlorhexidine declines in the presence of organic matter because its activity depends on a specific pH.[18]

Reports of *S. aureus* and *Streptococcus sanguis* using *in vitro* studies suggest problems with potential use of chlorhexidine owing to the emergence of resistance, which may be plasmid mediated. Low level exposure to chlorhexidine (as potentially occurring in deeper layers of oral biofilms) may result in development of cross-resistances toward antibiotics.[8]

Chlorhexidine is a popular antimicrobial agent in dental treatments. It has a cationic molecular component that attaches to the areas of cell membrane with a negative charge and causes cell lyses. However, it is not capable of dissolving debris or pulp tissue.[17]

Sodium Hypochlorite

Sodium hypochlorite (NaOCl) is a highly active cytotoxic oxidant recognized to be among the most potent antiseptic and disinfectant agents against bacteria, fungi, and viruses.

Patients are also advised to rinse orally with 0.2% sodium hypochlorite for 30 seconds, 2 or 3 times per week. This is equivalent to 8 mL (2 reduced teaspoonfuls) of 6% household bleach in 250 mL (a full glass) of water. More frequent rinsing may produce a brown-black extrinsic discoloration of the teeth. Diluted hypochlorite solutions gradually lose strength, so fresh solutions should be prepared for each use.

It also has disadvantages such as toxicity and risk of tissue destruction, bad taste, inability to eliminate all the microorganisms present in infectious canals (8) and risk of physically changing the structure of dentinal canal walls.[17]

Metronidazole

Dentists are the most frequent prescribers of metronidazole. Metronidazole is a narrow spectrum antibiotic that has a unique bactericidal effect against anaerobic bacteria.

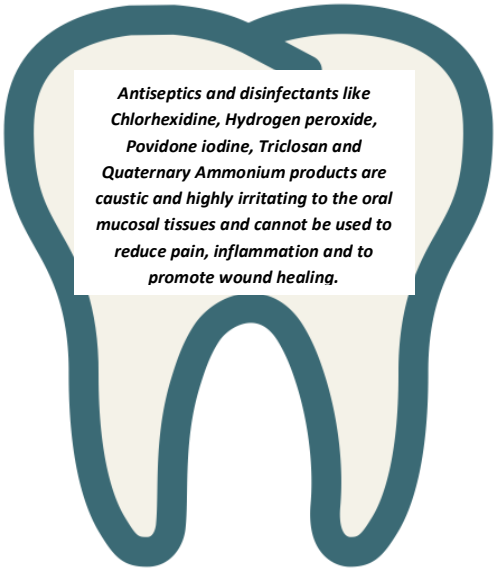
However, the emergence of resistance to this drug may be slower than if it were used alone, because in order to target both aerobic and anaerobic organisms, metronidazole is used empirically in combination with one or more antibiotics, although resistance to the drug may be associated with mobile genetic elements, aiding spread.

Triclosan

Triclosan is a non – ionic antiseptic used in diverse consumer products in order to reduce and prevent contamination by bacteria and fungi. As an ingredient in dentistry products such as toothpastes and mouth washes, it has shown to reduce tooth cavities, dental plaque and gingival related diseases.

Despite its antibacterial activity there has been controversy regarding the use of Triclosan. In 2009 Denmark suggested that Triclosan be banned due to being linked to antibiotic and bacterial resistance, endocrine disrupting effects and environmental effects. Since then the use of Triclosan has much reduced.

Antiseptic/Antimicrobial agent	Type	Recommended concentration	Primary mechanism of antimicrobial action	Drawbacks
Povidone Iodine	Iodophors - oxidizing agents	1 - 2 %	Inactivation of enzymes & proteins	Allergic reactions on site of application, impaired wound healing
Chlorhexidine gluconate	Non oxidizing bisbiguanide	2 - 4 %	Cell membrane damage and cell lysis, coagulation of intracellular proteins	Stains tooth surfaces, resistant strains prevalent, inactivated by organic serum compounds in gingival fluid. No action against *P. aeruginosa*
Sodium Hypochlorite	Oxidizing agent	0.5% or 200 - 5000 PPM	Highly active cytotoxic oxidant, disrupts cell membrane transport chain	Irritates oral mucous membranes, black-brown discoloration of the teeth, less effective in presence of organic environment
Metronidazole	Antibacterial	1.5 - 25%	Inhibits nucleic acid synthesis by disrupting the DNA of microbial cells	Narrow antimicrobial coverage since it is active against oral anaerobic bacteria only, bacterial resistance development
Triclosan	Antibacterial & antifungal	0.30%	Triclosan is bacteriostatic, and it targets bacteria primarily by inhibiting fatty acid synthesis and Interferes with enzymatic activities in microbes	May disrupt the endocrine system, Environmental concerns, use surrounded by controversy



Anti-infective periodontal treatment includes mechanical pocket debridement (scaling and root planing) to remove dental calculus, periodontal pocket irrigation with potent antiseptics, systemic antibiotics for advanced disease, and proper patient self-care. Pharmacotherapeutics targeting subgingival microorganisms can significantly enhance treatment outcomes. However, because porous subgingival calculus comprises a protective reservoir for bacterial survival during anti-infective therapy, meticulous scaling and root planing must precede antiseptic and antibiotic periodontal treatment.

One of the biggest challenges faced by dentists is treating and managing pain, inflammation, healing and infection. Antiseptics and disinfectants like Chlorhexidine, Hydrogen peroxide, Povidone iodine, Triclosan and Quaternary Ammonium products are caustic and highly irritating to the oral mucosal tissues and cannot be used to reduce pain, inflammation and to promote wound healing.

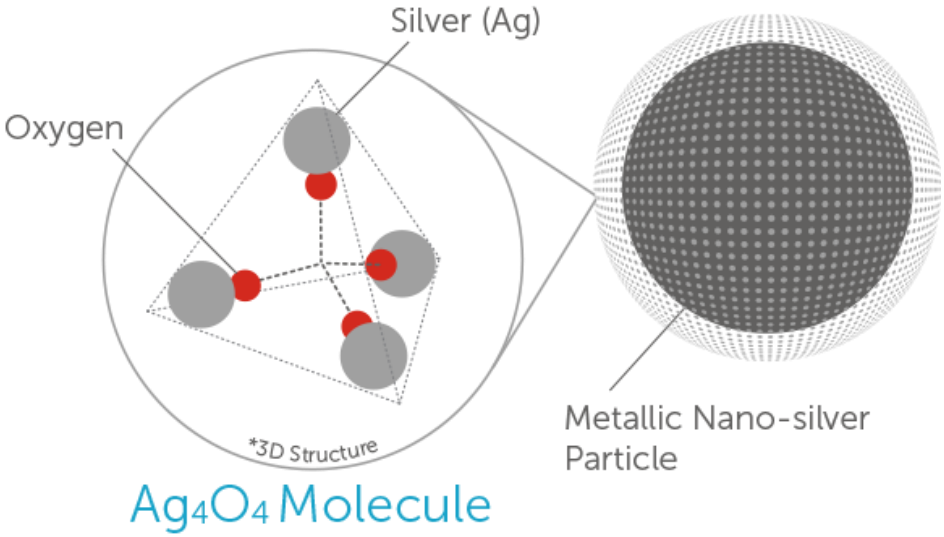
SILVERSOL® COLLOIDAL NANO SILVER: A Unique Bio-disruptive Nanotechnology

Silver has been associated with human medicine and healthcare for over two millennia. The 'father of modern medicine,' Hippocrates, wrote of using silver to improve wound care around 400 BC, and during the intervening years silver has featured in a wide range of writings, most of which highlight its capabilities particularly with regards to limiting inflammation and infection. For thousands of years in various countries, especially India, silver has been used for what was empirically known to be its bactericidal properties – to keep water, and often foods, safer.

In recent years, the advent of new technology, especially nanotechnology, has led to a resurgence of interest in silver. The number of papers and patents on silver has been rising exponentially, and with better research and technology, silver has been made safe and more effective. There are now many silver containing products on the market which are used as disinfectants, wound dressings and so on.

Colloidal silver is a broad term for a solution that can contain various concentrations of ionic silver compounds, silver colloids, or silver compounds bound to proteins in water. Colloidal silvers have been around for hundreds if not thousands of years and have been used by many ancient civilizations to maintain health.
Through a new manufacturing technology, patented under multiple patents, an advanced Nano-Silver solution discovered by American Biotech Labs, USA, has become the new standard by which all other silver products are measured. This new generation of colloidal silver is called SilverSol® or SilverSol® Technology. Not only is SilverSol® Technology more advanced than the original colloidal silvers, but through years of research at leading universities, ABL has compiled thousands of pages of test work documenting many key advances.

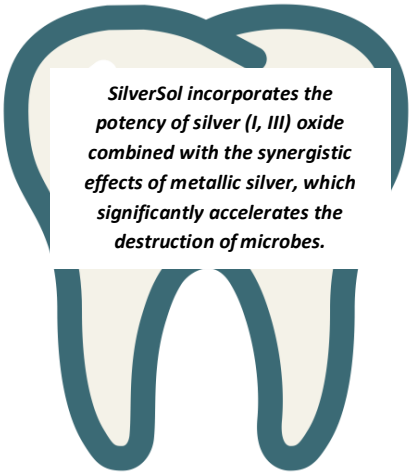
SilverSol contains nanoparticles of size 5-15 nm which comprise an interior of metallic silver and an exterior of silver oxide[9] including multivalent silver (I, III) oxide, Ag_4O_4. SilverSol incorporates the patented nanotechnology as well as various patented technologies pertaining to the manufacture of SilverSol, which render SilverSol unique, efficacious at low dosage and safe.
Unlike conventional silver compositions, Silver Sol is completely colourless and stable to light and chemical changes without use of any additives. This means that Silver Sol remains in solution and is not deposited in the skin and thus will not cause argyria.[10]



Research has uncovered multiple modes of action by which the Metallic Nano-silver Particle functions. First, it has an ability to steal multiple electrons (compared to ionic silvers which can only steal one). Second, each particle is permanently embedded with a resonant frequency, which allows the particles to have a positive effect on things, without needing direct contact with them. Lastly, the particles also utilize a very useful electrostatic charge.

Once the microbe is bound to the SilverSol particle, silver (I, III) oxide (Ag_4O_4) begins to exert its effect. The mechanism of Ag_4O_4 involves the acceptance of an electron by trivalent silver ions and ejection of an electron from monovalent silver ions through the aqueous media. The pathogen becomes “electrocuted” by electrons emanating from all Ag_4O_4 in the vicinity.[11]

SilverSol resonates at 890-910 THz, *i.e.* 330-337 nm, which is in the UVA range. This frequency allows Silver Sol to kill bacteria and viruses by destroying proteins. UVA light is abundant in the environment. Resonance of Silver Sol facilitates absorption of an electron from a microbial protein. However, once the electron is accepted, a very high energy situation arises, and this energy is transferred to an electron which is fired at the microbe, causing electrocution[9] in the presence of oxygen.



SilverSol is a highly effective antimicrobial product which leverages on the latest nanotechnology. SilverSol incorporates the potency of silver (I, III) oxide combined with the synergistic effects of metallic silver, which significantly accelerates the destruction of microbes. The multiple modes of action guarantee that the microbes are relentlessly bombarded and thus annihilated.

The SilverSol technology does not kill bacteria via chemical cell lysis as do most other disinfectant technologies. SilverSol attacks microbes via a series of pathways including protein inactivation, DNA denaturing and disassociation, binding affinity and an oligodynamic effect. These various modes of action are referred to as “bio-disruptive” and “catalytic”. Also, the inherent plasmonic resonance of the solid metallic nanoparticle can also be described as “catalytic”. In the sense that the resonance confers a vibrational effect to surrounding cells which affect only pathogens and not normal cells or cell function. And lastly, rapid destruction of pathogens in wounds and around diseased and inflamed tissue leads to the down regulation of Matrix Metalloproteinase (MMPs). These multiple modes of action all confer upon SilverSol profound anti-pathogenic properties as well as pro-healing activities.

The unique molecular structure of SilverSol confers a constant bioavailability thus allowing for highly effective activities at ultra-dilute concentrations relative to any other silver molecular structure. Even after the silver oxide coating has been stripped away, the nano-catalytic action inherent to the pure metallic silver particle continues to kill bacteria - therefore the bioavailability and bacteriostatic nature of the SilverSol technology is unparalleled.

SILVERSOL: What makes it so unique?

SilverSol nano silver, also termed as “Nano Metallic Silver Tetrahedal Tetraoxide” (NMSTTO) has FDA clearance for use as a broad spectrum antibacterial agent, surgical wound wash and irrigant and wound healing hydrogel as well as a disinfectant in medical device insertion sites, surgical incisive wounds, donor and recipient graft sites, burns and other topical lacerations and abrasions.

SilverSol technology (NMSTTO) based products in dentistry offers the advantage of superior antimicrobial with wound healing properties and helps better management of post-operative pain, inflammation and infection. The value-added features and benefits of these NMSTTO products revolve around its powerful, clinically proven antimicrobial, wound healing, anti-inflammatory and pain reducing properties.

The key clinical benefits of NMSTTO technology in dental procedures are as follows:

1. **Profound antimicrobial efficacy** - SilverSol Gel was tested against numerous bacterial and yeast strains (Candida albicans, Staphylococcus aureus, MRSA, Pseudomonas aeruginosa, Escherichia coli). Results indicate that SilverSol Gel completely destroyed all forms of bacteria tested to a LOG 5 reduction. Silver Sol Gel far surpassed FDA

required kill percentages by killing all yeast and bacteria tested at a significant level of 99.99900 %.

Nano silver has the capacity to potentiate the bactericidal effect of antibiotics through a common mechanism of action involving the overproduction of ROS. The combined treatment of antibiotic and Nano Silver showed a significantly higher production of ROS than either agent alone.

SilverSol kills MRSA and other bacterial strains tested and prohibits any mutation. This means SilverSol can be used safely and effectively every day without causing the bacteria to mutate and become resistant. *Porphyromonas gingivalis* is a gram-negative oral anaerobe involved in periodontitis, an inflammatory gum disease. *P. gingivalis* is promptly killed by SilverSol concentrations as low as 10 PPM.

2. **Increased rate of wound / tissue healing** – NMSTTO based products help rapid re-epithelialization and closure of wounds in the oral cavity. Also, SilverSol reduces swelling, inflammation and pain to speed up the healing process. Silver Sol destroys bacteria, viruses and fungus, thus reducing the burden on the immune system and allowing an already overworked immune system to prioritize its actions towards healing and rebuilding functions. Stem cells are activated and work to improve healing outcomes.
3. **Decreased inflammation, redness, oedema and pain** – When SilverSol is regularly applied by the patients post surgically, by 7-10 days post operatively the surgical site tissues are usually firm and pink and there is a noticeable lack of redness, exudates and puffiness at the surgical site. NMSTTO product has a profound pain reducing effect thus reduces the need for NSAID/analgesic medications.
4. **Biofilm removal** - Among metal nanoparticles with proven antimicrobial activity, those made of silver are particularly effective bactericidal agents. Nano-silver affects bacterial membrane permeability by attaching to the cell membrane surface and modifying the cell potential. They also interact with bacterial membrane proteins, intracellular proteins, phosphate residues in DNA, and to interfere with cell division, leading to bacterial cell death. The anti-biofilm activity of silver nanoparticles has been demonstrated in several studies. Several studies have demonstrated the inhibition of *in vitro* biofilm formation by a variety of bacterial species at specific nanoparticle concentrations.[12] As per a recent study, over 6 logs of inhibition (100%) were found for *S. mutans*, *S. sanguis*, and *S. salivarius* for the SilverSol gel-treated bacteria when compared with the control gel. In addition, the SilverSol gel also inhibited biofilm formation by these three bacteria mixed together.[13]
5. **Decreased scar tissue formation** – Increased healing via primary intention due to topical application of SilverSol hydrogel introduced into the surgical site and onto the sutures after the flaps had been re-approximated was observed. Rapid re-epithelialization also occurred with a dramatic lack of scar tissue formation. It appears that stem cell activation with SilverSol, could be responsible for the rapid adhesion of the laceration and assist in the removal of the scab and reduction of the scarring.
6. **Decreased infection and post-surgical complications** – This is primarily due to increased healing via primary intention due to rapid re-epithelialization of the overlying gingival tissues. SilverSol gel has proven to be effective in the treatment of chronic wounds by resolving clinical or subclinical infection, relieving persistent pain,

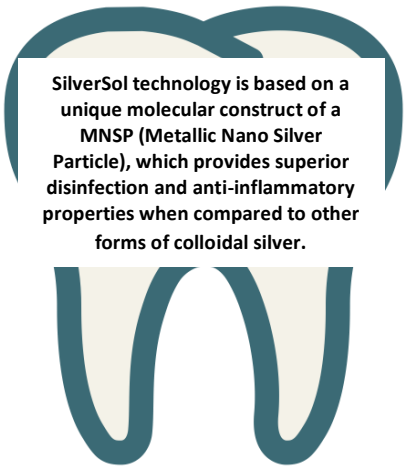
improving the appearance of unhealthy tissue, promoting healthy granulation tissue and decreasing recurrent wound breakdown.

SilverSol vs Ionic Silver & other Silver compounds

Almost all silver products today are based on an ionic or silver ion construct and the problem with ionic silver is that “in vivo”, it has very limited efficacy and bioavailability. Most ionic forms of silver are very easy to make – usually a chemical form of silver (i.e. Silver Nitrate) is diluted down to a certain ppm level and then a protein is added to the silver for stability. Ionic silver by definition, lacks a single electron and works very hard to reach a state of equilibrium by stealing back an electron from a cell or pathogen.

In in-vitro studies, ionic silver tends to kill bacteria slightly faster than metallic silver but, in human studies – once inside the body - the silver ions are much less effective because their limited ionic charge is quickly neutralized by other cells and thus, rendered ineffective as they are excreted in their first pass through the liver. Ionic or chemical forms of silver are highly unstable and because they require such high concentrations to be effective, they tend to react poorly when formulated into or with other compounds.

On the other hand, SilverSol technology is based on a unique molecular construct of a MNSP (Metallic Nano Silver Particle), which provides superior disinfection and anti-inflammatory properties when compared to other forms of colloidal silver. These MNSPs range in size from 5-7 nanometres and are surrounded by a multivalent silver oxide coating comprised of thousands of Ag4O4 molecules. In dry form, each MNSP will cluster, but not touch each other due to the electric charge of its silver tetric oxide coating. In a solution of pure water, SilverSol molecules will uniformly disperse via attachment to the water molecules. What truly differentiates SilverSol from all the others and makes it so much more effective is that each pure SilverSol molecule has this very thin, unusual silver oxide coating, which emits a unique electrical charge barrier.



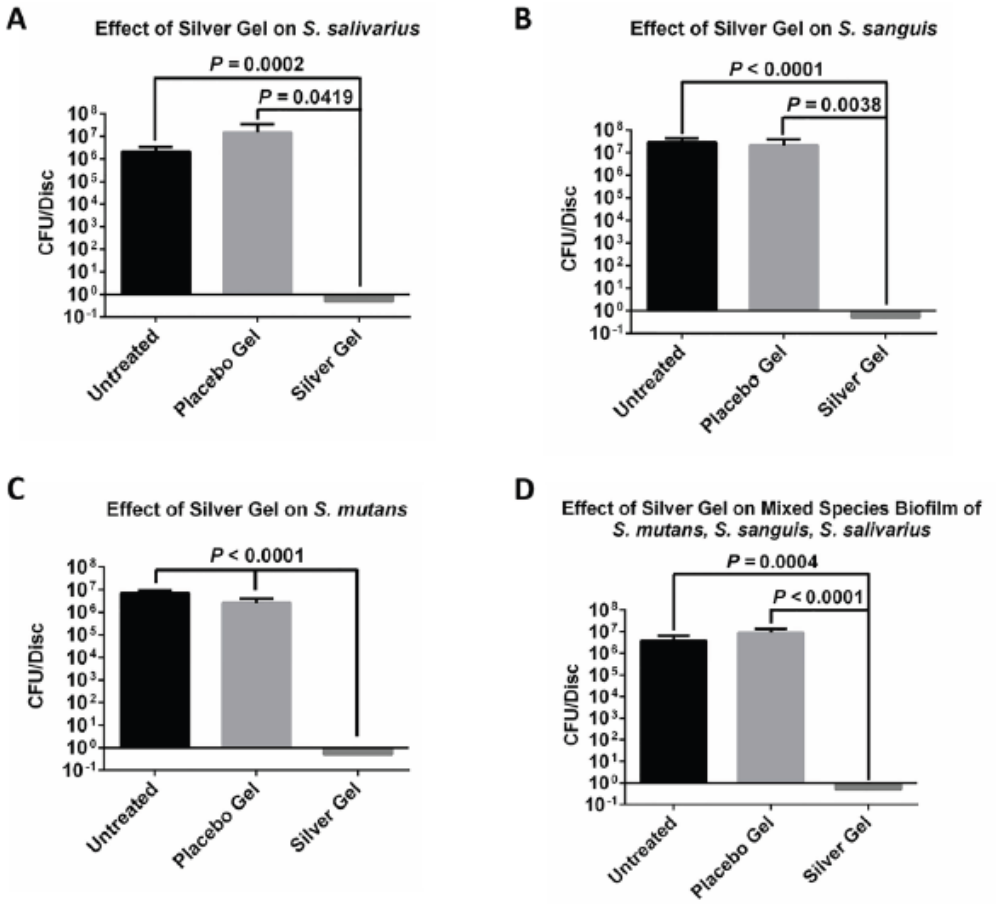
Nanoparticles less than 10 nanometers in diameter have an inherent vibrational frequency or “flux” which can be quantified - in the case of SilverSol, this resonance flux or vibration occurs at between 890- 910 terahertz (200-300 nanometers) which is the same resonance frequency at which germicidal ultraviolet light causes bacteria, viruses and yeast to be destroyed. This vibrational frequency is unique to SilverSol and is passed from the metallic silver particle to the outer Ag4O4 coating and then to the surrounding water molecules because of the strong molecular bond between the silver tetric oxide coating and the water molecules.[14]

SilverSol: In-vitro evaluation of antimicrobial activity against oral pathogens

The incidence of periodontal diseases (including dental caries and bleeding gums) is estimated to be about 20–50% of the global population. The pathological organisms responsible for these caries/ periodontal diseases are *Streptococcus mutans*, *Streptococcus sanguis* and *Streptococcus salivarius.*

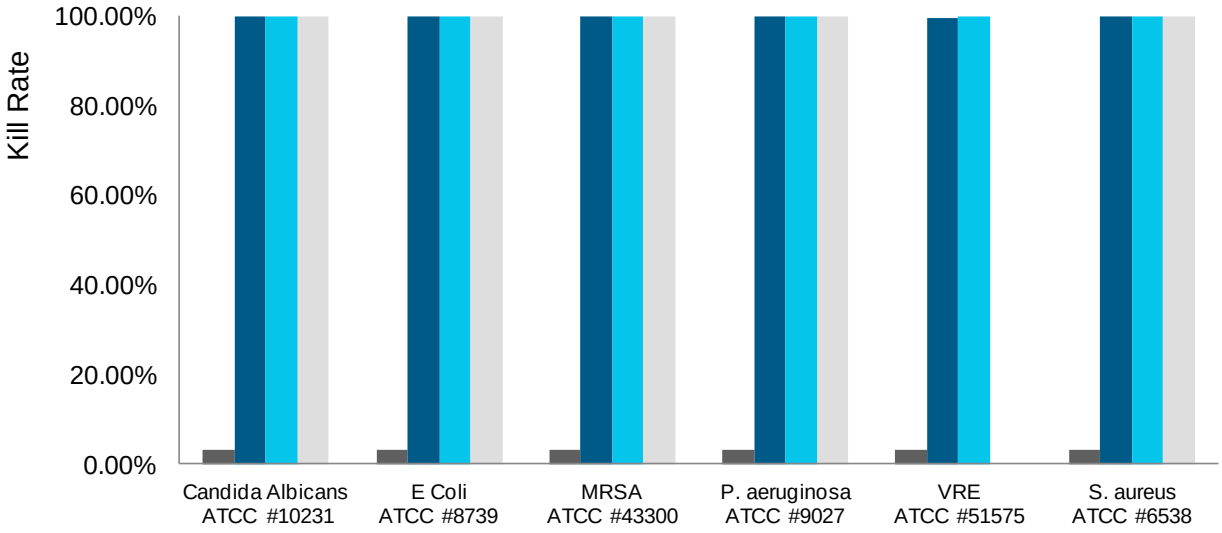
Mutans streptococci create acidic environment creating a risk for formation of cavity. *Streptococcus mutans*, *Strep. sanguis* and *Strep. salivarius* enhance caries formation and thus the progression of periodontal disease. It is the interaction of *S.mutans* with other streptococci that is thought to be important in dental plaque formation. During the formation of dental plaque, *S. mutans* adhere to primary colonizers by cell to cell interaction which forms biofilm on the teeth which induces bacterial growth.

The effect of SilverSol gel on viability of *S. mutans*, *S. sanguis*, *and S. salivarius* was assessed by quantifying their colony forming units (CFU) in presence or absence of the test gel. The effect of this formulation on biofilm-forming ability of these bacteria was studied through scanning electron microscopy. Using the CFU assays, over 6 logs of inhibition (100%) were found for *S. mutans*, *S. sanguis*, and *S. salivarius* for the SilverSol gel-treated bacteria when compared with the control gel. In addition, the SilverSol gel also inhibited biofilm formation by these three bacteria mixed together.[21]



Colony forming unit analysis of biofilm formation. (**A**) *S. salivarius*, (**B**) *S. sanguis,* (**C**) *S. mutans* or (**D**) all three on untreated discs, discs treated with placebo gel or discs treated with colloidal SilverSol gel.

SilverSol Gel was tested against numerous bacterial and yeast strains to determine how well it could kill a wide range of disease-causing pathogens. This was measured by percent reduction in pathogens or Log reduction.[22]



Results indicated that SilverSol gel quickly and completely destroys Yeasts (Candida albicans) and bacteria (Staphylococcus aureus, MRSA, Pseudomonas aeruginosa, E. coli) within one hour. SilverSol gel kills quickly and completely to a Log 5 reduction. This is significant because an FDA Category 1 wound care approval requires the approved product to be able to achieve a 1 Log reduction (90% kill rate) in 7 days of contact and a 3 Log reduction (99.9% kill rate) within 14 days. Remarkably, SilverSol achieved a 4-5 Log reduction (99.999% kill rate) in the first hour of contact.

In addition to bacteria, nano silver solution has cidal effects on a wide range of fungi, protozoa, and even viruses.[23]

Sadeghi *et al*. assessed the effects of silver nanoparticles against *Actinomyces viscosus* and *Streptococcus sanguinis* present in microbial plaque and revealed that silver nanoparticle solution had good antibacterial activity against these strains and this effect was achieved in lower concentrations of the solution compared to chlorhexidine.[24]

Porphyromonas gingivalis is a gram-negative oral anaerobe, which is involved in periodontitis, an inflammatory gum disease. As per an in vitro study, Colloidal Nano silver solution has an excellent activity against this pathogen achieving 99.99% kill rate as shown below[25]:

Time Intervals	Inoculation CFU	Recovered CFU	Log Reduction	Reduction %
3 Minutes:	1,200,000	<15	4.9	99.99
10 Minutes:	1,200,000	<15	4.9	99.99
30 Minutes:	1,200,000	<15	4.9	99.99

SilverSol – Mega Clinical experience in 17500+ invasive dental surgeries

A continuing problem in dental Surgery and other dental procedures is the presence of an extensive bacterial flora in the human mouth. This has been dealt with by administering antibiotics systemically prior to and after dental procedures. Such antibiotic treatment may prevent systemic infections, but circulating antibiotics are not always able to prevent local infections at the site of the dental procedure. Even where obvious infections are avoided there is often considerable pain and swelling post operation.

The remarkable ability of SilverSol products to kill microorganisms and prevent their multiplication allows improved dental procedures that reduce pain and swelling and promote healing. SilverSol is particularly effective in disinfecting dental surfaces during dental procedures. SilverSol hydrogel is advantageously applied to flaps of periodontal tissue and around the bone prior to suturing the tissue. Similarly, it is effective when applied to tooth extraction sockets and to any gingival tissue wounded or exposed by a dental procedure. The disinfectant SilverSol rinse is also effective in disinfecting internal tooth

surfaces exposed, for example, in the removal of decay or the remodelling of a tooth prior to the application of a crown or other type of dental restoration.

SilverSol is non-toxic to human tissue and highly effective against a broad spectrum of both Gram positive and Gram-negative bacteria, viruses, yeast and fungi and has been proven to provide a continuous dynamic antimicrobial bacteriostatic environment capable of reducing bacterial bioburden and thus post-operative infection, inflammation and pain. It has also been established as a powerful wound healing agent capable of significantly accelerating the healing process. No bacteria or virus has ever been shown to develop a resistance to silver hydrosol.

Dr. Andrew Willoughby DMD, FICCMO, LVIF, FAGD, FICOI, FAACFP, MICCMO has earned and established an excellent reputation within the dental and healthcare communities as a progressive cosmetic and neuromuscular aesthetic dentist. He is the Chairman and Founder of Hartfell Technologies Inc., which he has worked tirelessly to build with his friend and co-Founder Dr. Mark Duncan. HTI is a leader in the utilization of nanotechnology in dentistry. His latest patent application entitled “Dental Uses of Nanometallic Silver Hydrosol” establishes new paradigms for the use of nanotechnology in dentistry and is a continuance-in-part application of a US patent issued to American BioTech Labs of Alpine Utah in 2003.

SilverSol wound healing hydrogel is now an integral part of every surgical technique and protocol employed by Dr. Willoughby as a general, reconstructive and implant-based dentist.

Dr. Andrew Willoughby was frustrated with the inability of antibiotics to prevent post procedure infection in his dental implant patients. It seems that over 50% of his patients were contracting difficult to treat bacterial infections after the procedures. He had heard of American Biotech's studies and decided to acquire and apply this technology, for experimental purposes, to his practice. He injected the ASAP SilverSol gel directly into the cavity into which the screw or rod would be affixed to the bone to hold the new replacement tooth. He was amazed that of the first 300 cases not one developed an infection. He and a group of colleagues decided to form a company, VeraSIL Therapeutics, Inc., to explore other possible novel applications. They referred to this American Biotech technology as Nanometallic Silver Tetrahedal Tetraoxide (“NMSTTO”).

Over the past 14 years, Dr. Willoughby has performed more 17,500 invasive peri-osseous surgical dental procedures and many thousands more non-surgical procedures all with SilverSol wound healing hydrogel and surgical wound wash irrigant solutions. The vast majority of these procedures have involved both hard (osseous) and soft (connective and gingival) tissue grafts where bone and its overlying soft tissues have been either cut, curetted, removed, reshaped, lavaged, lasered, irrigated, grafted and or regenerated with the help of GTR and collagen membranes – all with no reported material adverse effects. According to Dr. Willoughby, the use of SilverSol products is associated with improvement in wound healing and decrease rates of pain, inflammation and post-operative complications.[15]

Dental Implant Placement – More than 3100 patients
SilverSol surgical wound wash irrigant was introduced into the IV bag that is hooked to the surgical water driven hand piece for creating the osteotomy and then application of SilverSol hydrogel into the osteotomy site prior to ratcheting down the dental implant (DI) fixture. This prevents migration of mucosal bacteria into the alveolus and speeds up wound healing and integration of the DI.

Dental Implant removal with Bone Allograft and GTR (Guided Tissue Regeneration) – More than 140 patients
The DI removal process generates a lot of heat due to the Trephine burs, so copious amount of SilverSol wound wash irrigant is used and any bone damage is counteracted by the anti-inflammatory wound healing properties of SilverSol wound wash irrigant. Antibiotic coverage is typically employed here.

Free Gingival Grafts (GTTs) – More than 100 patients
In FGGs, the donor site is coated with SilverSol hydrogel as is the piece of connective tissue and the split thickness recipient site. Post operatively there is significantly lesser swelling, inflammation and discomfort (pain) with the use of SilverSol hydrogel. Antibiotic coverage is typically employed here.

Connective Tissue Grafting (CTG) and Sliding Pedicle Grafts – More than 370 patients
In both cases, a combination of SilverSol wound wash irrigant and SilverSol hydrogel is used to thoroughly disinfect and cover the surgical site. Postoperatively there is significantly less swelling, inflammation and discomfort (pain) with the use of SilverSol products and tissue at the recipient site does not appear to slough off in the traditional fashion.

Bone Grafts – More than 2000 patients
Most of these grafts are done after extraction of the teeth/tooth and curettage of the sockets to remove any residual granulation tissue or infection and then flushing them out with SilverSol wound wash irrigant to disinfect the sockets. Then the bone allograft is rehydrated in SilverSol wound wash irrigant. After packing the alveolar defect with bone material and butterflying the membrane to place and suturing the flap, SilverSol hydrogel is applied over top of the sutures. This significantly reduces post op surgical complications and pain and inflammation as well.

Periodontal surgeries – More than 740 patients
These procedures cause considerable pain, inflammation and swelling. When SilverSol surgical wound wash irrigant is used to clean and lavage the surgical site, and SilverSol hydrogel is used below the flaps and then placed topically on top of the sutured tissues, patients experience significantly less pain, inflammation and swelling as well as less chance of post-surgical infection and faster wound healing. Antibiotic coverage is typically employed here.

Dental Extractions – More than 10,000 patients
In all dental extraction procedures, SilverSol hydrogel is applied into the extraction socket, under the flaps and top of the sutures. In badly infected or periodontally diseased teeth, the pockets are flushed using SilverSol wound wash irrigant + 3% H2O2 prior to tooth/teeth extraction. This helps to significantly reduce post-surgical infection and inflammation and the risk of dry socket.

Biopsies – More than 200 patients
Excisional biopsies are performed using SilverSol surgical wound wash irrigant and followed by application of SilverSol wound healing gel to the tissue. These tissues heal faster with less post op pain.

Laser surgeries – More than 1800 patients
After laser surgeries such as Hard & soft tissue "crown lengthening" and Gingivectomies/Gingivoplasties, SilverSol hydrogel is applied on top of the surgical /sutured tissues as part of post op care. Patients are asked to apply the gel 3-4 times daily for 7-10 days post operatively. This leads to less post op pain & discomfort and quicker healing time.

Endodontic Surgeries (Apicoectomies/retrofills and perforation repairs) – More than 400 patients
The typical retrofill material is MTA Pro Root Repair and it is mixed with equal amounts of SilverSol wound wash irrigant which has a proven antimicrobial wound healing benefit. After the retrofill is completed, apply SilverSol hydrogel into the osteotomy site and suture the flap closed. Antibiotic coverage is always employed in such procedures. SilverSol products significantly accelerate healing, reduce post op complications and infections as well as reduce pain and inflammation.

Non-Surgical Root Canal Therapy (NSRCTs) – More than 4000 NSRCTs on a combination of single and multi-rooted Mx & Md teeth using following products/protocols:

- **Endo Irrigant Solution for RCTs:** Combination of SilverSol wound was irrigant solution and 3% H2O2 is used to flush out root canals to help remove biofilm layers.
- **RC Lubricants:** A combination of aqueous 17% EDTA solution and SilverSol hydrogel is used as a wound healing lubricant. This helps eradicate bacteria and 100% of their associated biofilms.
- **RC Sealer:** Zinc oxide, calcium phosphate and barium sulphate-based root canal sealing material is usually used. SilverSol hydrogel is mixed with the liquid

component of this sealer containing Eugenol (an essential oil) into a paste which offers improved disinfectant and wound healing properties.

Patients with Chair-side Soft denture relines and immediate denture insertions after full arch clearances – More than 765 patients

Patients with loose fitting dentures typically suffer from oral fungal infections and ulcerated lesions which commonly occur at the peripheral border of the denture. One common solution is to perform a chair side denture reline and after this material has cured and trimmed and the denture adjusted, SilverSol hydrogel is applied to the inside of the newly relined denture and the patients asked to reapply twice daily. This helps to resolve fungal infections and quickly heal the ulcerations.

After full upper or full lower arch clearances, SilverSol hydrogel is applied inside the denture and patients are instructed to reapply the hydrogel twice daily for 10-14 days. At the post op follow up, the surgical tissues are firmer, healthier without formation of necrotic pseudo-membrane. Pain and swelling also are noticeably reduced. These patients are also prescribed oral antibiotics for 7-10 days.

Treating Patients with Nicotine Stomatitis – More than 510 patients

Nicotine stomatitis is an inflammatory process caused by heat from smoking and the chemicals in the smoke. It usually presents on either the tongue, palate cheeks or residual alveolar ridges as dry, raised, cracked, scaly lesions with white or red dots. Treatment includes cutting back on smoking, applying SilverSol hydrogel 4-6 times daily to the affected areas and rinsing with SilverSol wound wash irrigant + 3% H_2O_2 solution 4-6 times daily for 10 days.

Treatment of Oral Candidiasis and Angular Cheilitis – More than 430 patients

These are very common in denture wearers mainly due to ill-fitting dentures, change in antibiotics (and fungal overgrowth) or an underlying immunodeficiency. After cleaning their denture and disinfecting with SilverSol wound wash irrigant solution+ 3% H_2O_2, the patients are placed on the following regimen – 1. Apply SilverSol hydrogel inside the denture 4-6 times daily for 10 days. 2. Rinse with SilverSol solution + 3% H_2O_2 solution 4-6 times daily for 10 days. 3. Soak the denture in a solution of SilverSol + 3% H_2O_2 overnight.
With this regimen patients can eliminate their oral candidiasis within 5-7 days.

Periodontal therapies – More than 700 patients

For non-surgical treatment of refractive periodontitis, a combination of scaling and root planing is used with sub gingival irrigation with SilverSol wound wash irrigant solution + 3% H_2O_2, Laser curettage with SilverSol wound wash irrigant solution + 3% H_2O_2 irrigant wound wash and/or CAD/CAM custom trays filled with SilverSol hydrogel and adjunctive use of SilverSol oral rinse + 3% H_2O_2.
Central to this treatment protocol is the use of CAD/CAM custom fabricated trays capable of delivering SilverSol hydrogel to the teeth and surrounding gums. This ensures a significant reduction in both pocketing depths and the total number of CFUs of the pathogenic bacteria responsible for periodontal disease.

Acute / Chronic Infections – More than 330 patients
In patients who present with large intra oral or extra oral swellings as a result of alveolar abscess or facial cellulitis, when good drainage in the surrounding periodontal tissues cannot established, patients are placed on antibiotics and SilverSol solution (2-5 ml) is injected into and around the swollen tissue subcutaneously. This alleviates pain and swelling rapidly and eliminates the source and spread of infections.

As Dr. Andrew Willoughby puts it "The dental applications of SilverSol proprietary wound healing, disinfectant nanotechnology has in my opinion, created a new sub-category of dental products which I refer to simply as **Nano Silver based Wound Healing, Disinfectant Technologies** and this new class of dental products have had a noticeable impact on improved outcomes for literally thousands of my patients."

Indications

- Gingivitis & Periodontitis
- Dental Caries
- Endodontic infections (Pulpitis, Root canal infections, Apical Periodontitis)
- Surgical Prophylaxis in various dental surgical procedures, implant placement and guided tissue regeneration using bone allografts, membranes and general restorative procedures
- Orthodontic procedures
- Prophylaxis of infections in Non-surgical Laser therapy procedures

Conclusion

The broad-based application of SilverSol technology in dentistry is virtually unlimited since almost everything done by dentists has to do with killing pathogenic microorganisms, while healing tissue, and you can do both contemporaneously without harming the normal oral flora. With the advent of this technology, dentists have been able to modify and improve on a whole host of clinical protocols for everything from non-surgical treatment of refractory periodontitis, to implant placement and guided tissue regeneration using bone allografts, membranes and general restorative procedures, to products and clinical protocols.

The SilverSol technology based products can be used in a modified nonsurgical periodontal protocol to a) prevent the dental bio-film and bacterial populations from "maturing" and transforming into more damaging anaerobic populations or b) eliminate these aggressive pathogens in the mouth once they have taken hold and utilize an Oral DNA test as part of a confirmatory diagnosis.

Thus, the SilverSol technology is pathogen specific, wound healing, anti-inflammatory and reduces pain - it could be a paradigm shifting technology for dentistry.

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