Prasun Kumar · Vijay Kothari *Editors*

Wound Healing Research

Current Trends and Future Directions

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Part III
Interdisciplinary Approach to Wound Care

SilverSol® a Nano-Silver Preparation: A Multidimensional Approach to Advanced Wound Healing

A. de Souza, A. H. Vora, A. D. Mehta, K. Moeller, C. Moeller, A. J. M. Willoughby, and C. S. Godse

Abbreviations

ABL	American Biotech Labs
ATCC	American Type Culture Collection
CFU	Colony forming units
DNA	Deoxy ribose nucleic acid
DTA	Differential thermal analysis
EPA	Environmental Protection Agency
ESBL	Extended-spectrum β-lactamase
FDA	Food and Drug Administration
FHSA	Federal Hazardous Substances Act
FTIR	Fourier-transform infrared spectroscopy
ICP-MS	Inductive coupled plasma-mass spectrophotometry
IL	Interleukin
MCP	Monocyte chemoattractant protein
MDR	Multi-drug resistant
MIC	Minimum inhibitory concentration
MRI	Magnetic resonance imaging
MRSA	Methicillin resistant *Staphylococcus aureus*
NADH	Nicotinamide adenine dinucleotide hydrogen
NP(s)	NP(s)

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NQO	NADH quinone oxidoreductase
PPM (ppm)	Parts per million
RNA	Ribonucleic acid
SEM	Scanning electron microscopy
TGA	Thermogravimetric analysis
TEM	Transmission electron microscopy
USP	United States Pharmacopoeia
UV-VIS	Ultraviolet visible spectrophotometry
VRE	Vancomycin resistant enterococci
XRD	X-ray diffraction

1 Introduction: Silver in Medicine

1.1 Ancient Use: Silver Compounds

Silver, a lustrous metal, has been known globally since ancient times. Metallic silver was known to the Chaldeans since 4000 BC, especially for making valuable goods. Traditionally the royal families used silver in tableware. It is interesting to note that silver came into medicinal use during the first millennium AD. Primeval Egyptians, Romans, Phoenicians, Greeks and others used silver to preserve freshness to prevent spoilage of water, milk, food (Alexander 2009). North Americans used to drop a silver coin in water for its preservation and for a long-distance transportation prior to the discovery of refrigerators. Persian King (600 to 530 BC) would drink water only if it is brought in silver vessels.

These ancient simple practices of preservation of water and food got connected to antimicrobial properties of silver much later when microbes were discovered (1665) and their role in infections was established (1884). However, the question arises whether silver is soluble enough in the water to impart a protective effect for health? As silver is sparingly soluble in water, just a few parts per trillion, it is believed that the potential ligands of goblet forming silver salts give rise to its antimicrobial potential. Most silver salts viz. silver sulfadiazine, silver halides and silver nitrate being water soluble have antimicrobial properties and oligodynamic effects.

Silver nitrate was discovered in the thirteenth century. However, its medicinal use came into existence in 1614, when it was used internally by Angelo Sala as a counterirritant, purgative, and for the treatment of brain infections. Carl Siegmund Franz Crede, a German obstetrician, in the 1880s effectively used silver nitrate eye drops for treating gonorrhoeal ophthalmia (*ophthalmia neonatorium*) in infants. The practice of using 1% solution of silver nitrate, reduced the incidence of gonorrhoeal ophthalmia to 0.13% from 7.8% (Schneider 1984). Systematic studies of silver ions were conducted by Vonnaegele. He reported the effect of silver against 650 species of unicellular organisms, proposing its promising bactericidal effects (Searle 1920). Colloidal silver also has been shown to be effective in puerperal sepsis,

staphylococcal sepsis, tonsillitis, acute epididymitis, and other infectious diseases (Duhamel 1912; Sanderson-Wells 1916; Brown 1916).

It is only by the twentieth century, the use of metallic silver for water purification was scientifically established. Later it was expanded for the treatment of wound and eye infections and for dental hygiene including the prevention and correction of pyorrhoea, gingivitis, and bad breath. Traditionally silver was used as a blood purifier, for the prevention of palpitation of the heart and for the reatment of offensive breath has been reported in 980 AD. Swallowing silver was also found to be useful in stopping epileptic seizures. In India, the Ayurvedic system of medicine describes the use of processed metals viz. gold, silver, lead, mercury for various disease conditions. The use of silver in Ayurvedic therapeutics dates back to the period of *Charaka (300 BC)* (Galib 2011).

1.2 Modern Trends: Ionic Versus Metallic Silver

Historically silver has been used for a medical purpose in different forms— as an additive in ceramic (zeolite) or in glass matrix, as silver salts (chlorides, nitrates, sulphides) or as elemental silver, though the nomenclature emerged much later. The Microbicidal capacity of different silver forms was mainly dependent on their capacity to release silver ions (Das et al. 2005). Silver nitrate has been mentioned in the Pharmacopeia in Rome in 69 B.C. (Hill and Pillsbury 1939), whereas, record of its medicinal use has been found in 702–705 AD. Colloidal silver was first used in 1891 (vide infra). Consumption of silver by humans over millennia bespeaks its safety. In India, 275 tons of silver is consumed every year in metallic form (Silver foil) as an additive in foods and sweets (Das et al. 2005).

With the advancement of nanomedicine in the twentieth century, several products containing metallic silver in nano form (<100 nm particle size) have been developed. The advantage of nano-silver over traditional forms is mainly due to its nano size with large effective surface area. This allows it to be used at extremely low concentrations without side effects compared to silver ions or silver nitrate. In the early twentieth century, medicinal nanoscale silver colloids became available commercially under trade names Collargol, Argyrol, and Protargol and thereafter their use became widespread within 50 years (Nowack et al. 2011). Collargol, a silver preparation with 10 nm particle size was used as early as in 1897 (Nowack et al. 2011). The estimated worldwide production and use of nano-silver by 2011 was 320 tons per year (Nowack et al. 2011). Among the 1300 nanotechnology-based marketed metallic products one fourth comprise nano-silver (Munger et al. 2014). Compared to ionic silver (chemical forms), nano-silver has distinct physicochemical properties which lead to their efficacy as an antibacterial, anti-viral, and anti-inflammatory agent (Yardley 1998).

1.3 SilverSol®: Coated Nano Silver

SilverSol®, developed by American Biotech Labs (ABL), USA using a patented technology,—(US6743348B2 United States) is a uniquely engineered colloidal silver preparation having microbicidal, wound healing and several other activities (Holladay et al. 2001). A decade of efforts by ABL has resulted in the development of SilverSol®. The term "sol" specifies the chemical nature of the silver preparation as 'a pure mineral permanently suspended in the water where the mineral's charge is transferred to the entire body of the water'. The elemental form of zero-valent metallic silver particles is coated with silver oxide, with particle size ranging between 5 and 50 nm. The nano size confers multidimensional bioactivity and high stability to the product. SilverSol® is the only patented engineered product in the world, containing nano-silver particles with proven safety and multidimensional efficacy at extremely low concentrations. A wide variety of SilverSol® formulations available both in liquid and gel forms are colourless, odourless, and tasteless.

1.4 The Multidimensional SilverSol®

The nano-silver particles in SilverSol® have a unique structural arrangement, which confers a wide range of activities to SilverSol® at extremely low concentrations. SilverSol® has significant antimicrobial activity, effective against several microbes, including fungi, bacteria, protozoa and viruses, which makes it efficacious in various infectious conditions such as Malaria, Influenza, human immunodeficient viral (HIV), and Hepatitis B viral infections, vaginal infections, oral and urinary tract infections. Severe infectious conditions caused by antibiotic-resistant bacteria—MRSA and VRE can also be treated successfully with SilverSol®. Besides antimicrobial activity, SilverSol® has been shown to possess activity in cancer cell lines. Several in vitro and in vivo studies; conducted to confirm its potential, have yielded positive results with clinical evidence in Malaria, MRSA infections, HIV, pain, inflammation of various origins as well as in wound healing of various aetiology. SilverSol® oral products have also been used as immune enhancers.

The current review focuses on the promising effects of various SilverSol® products in the treatment of acute and chronic wounds. It also gives its detailed attributes regarding safety, efficacy and pharmacology. Physicochemical properties and regulatory status of the product are also described. Clinical cases with complex, infected wounds of varied aetiology and severity, treated with SilverSol® have been described in a separate section. Case histories of over 22,000 patients undergoing various dental procedures treated with SilverSol® products have been summarized. Quicker healing time with relief from post-surgical pain and swelling was found to be prominent in these patients.

2 Silversol® Technology

2.1 History of SilverSol® Discovery

Dr. William D. Moeller (Photograph 1), an entrepreneur; transitioned in the mid-1970s from a highly successful insurance career to become part owner and CEO of multiple mining companies, including American Consolidated Mining Company and Clifton Mining Company. Along with his sons, he developed property in and around Gold Hill, Utah, USA. A former mining boomtown during the turn of the twentieth century, Gold Hill is riddled with valuable mineral deposits. Its original operations ceased after World War II as the demand for metals waned (https://en.wikipedia.org/wiki/Gold_Hill_Utah). William and his team picked up where the old miners left off, assaying, drilling, and mining the veins for their famously high levels of copper, silver, gold, lead, and tungsten.

William had a great talent and tenacity when it came to solving problems. He was able to focus and engineer a way to solve complex problems. He acquired the mining property from the scattered owners with small plots, gradually building a block of mining claims that incorporated about 13 square miles of land. During the 1990s, the prices of precious metals dropped to historic lows. William overcame this hurdle by diversifying into the application of silver, which had been a mainstay in medicine prior to the advent of antibiotics. He teamed up with Robert Holladay—an electrician and chemist, and Herbert Christensen—an engineer, and together they began to research colloidal silver products and the manufacturing methods for them. Their efforts resulted in the formation of nano-silver with antimicrobial and other properties that are far better than those of the usual colloidal silver, due to their innovative tetrahedral structure coating. William was not only successful in obtaining patents to protect the invention but was also successful in getting FDA approvals for several versions of the product.

Photograph 1: Dr. William D. Moeller (1936–2014)

Today ABL, the company founded by Williams, has continued the original spirit of advancing the manufacturing and providing highly effective and very stable forms of medicinal silver. Along with universities and government labs, and in close collaboration with other research groups, such as Viridis Biopharma Pvt. Ltd. (VBPL) from Mumbai (India), they spent the next decade researching the silver particles they had created and testing them against a large number of varied microorganisms. The vast experimental data that was generated proved that SilverSol® was capable of killing most pathogenic bacteria including *Yersinia pestis* causing bubonic plague and also many yeasts, fungi and viruses (Roy et al. 2007; Pedersen et al. 2008; Pedersen and Moeller 2009; Revelli et al. 2011). They also proved it was safe for use on and in humans and animals (Munger et al. 2014). They showed that if taken orally it did not harm the probiotics and when used topically on a wound, it protected the wound from infection, expedited the healing, reduced the inflammation and pain (Revelli et al. 2011), and reduced the formation of scar tissue (Pedersen and Moeller 2009). This unmatched silver technology created by William and his team is known worldwide as SilverSol® Technology. Over 400 major studies conducted including published clinical studies demonstrate the capability of the technology. SilverSol® besides safe was found to be highly effective against pathogens that are difficult to manage. It is making waves on many disease fronts, including treatments for ailments like Malaria (Pedersen and Hedge 2010), MRSA, and HIV (Pedersen et al. 2008), to name but a few. The studies done in various government laboratories in US, showed the killing of infectious viruses viz. SARS and H5N1 by the technology (Pedersen et al. 2008).

William's diligent work led to the technology obtaining several US FDA clearances, as well as clearances and approvals by other international governments. It has garnered more than 70 US and international patents, with numerous new patents currently pending. Before he died, William's goal was to change the medicinal history of the world. Having sold more than 22,000,000 units of SilverSol Technology products worldwide and having helped improve the lives of hundreds of thousands of patients, it is safe to say he achieved his goal.

2.2 *Characterization of SilverSol®*

SilverSol®, a colloidal solution of silver is a 2-phase stable solution of metallic silver. The crystalline solid phase of ultrahomogenous silver in the liquid phase of water forms solid state epitaxy—an amazing phenomenon of metallic aquasols in which the crystalline templet imparts its structure to the amorphous solid phase, imposing the amorphous phase to crystallize as per the crystalline template. In SilverSol®, silver in Ag_4O_4 forms a thin coating around metallic silver suspended in water. This confers a unique tetrahedral structure to SilverSol®, wherein metallic nano-silver particles are surrounded by 4 AgO and 2 water molecules (Fig. 1). This structure, while being stable, also imparts multidimensional biological properties to

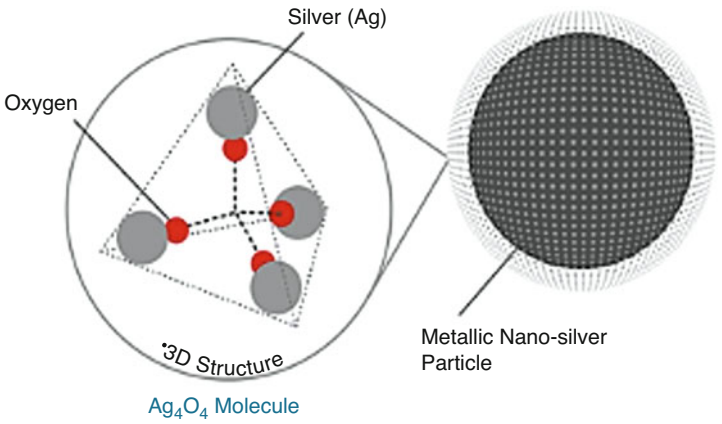

Fig. 1 Tetrahedral structure of SilverSol®

SilverSol® nanoparticles (NPs) without any side effects (Sect. 2.3 safety studies). This is of key importance, as the safety of the NPs is a major concern in nanomedicine.

The phase analysis of SilverSol®, using advanced material science has been reported by Roy et al. They conducted phase equilibrium studies of the biphasic SilverSol®. Various techniques viz. Differential thermal analysis (DTA), Thermogravimetric analysis (TGA), X-ray diffraction (XRD), Scanning electron microscopy (SEM), and Transmission electron microscopy (TEM) were used for the solid phase analysis. On the other hand by using Fourier-transform infrared spectroscopy (FTIR), UV–VIS, and Raman spectroscopy the liquid water phase was analysed. The readers may refer to the paper published by the group for the detailed methodology and the data (Roy et al. 2007). Some salient features highlighting the peculiar distinctive characteristics of SilverSol® as enlisted below:

- The water phase of the SilverSol® was reported to be pure and the purity was maintained in the presence SilverSol® nano particles.
- Transmission electron microscopy revealed that the particle size of nano-silver ranged between 10–50 nm with a median around 30 nm. It was observed that the bigger particles of 20–30 nm size comprised of a group of 5–7 nm particles, seized together by relatively weak bonds (vide infra). These 5 nm, metallic, silver-containing 'mobile' units, with oxide cover (in the form of layers or 'skins) around them, confer stability and unique bio-activity to SilverSol®.
- Roy et al. also compared SilverSol® to several silver colloids that were commercially available. Using Raman Spectroscopy, the SilverSol® could clearly be distinguished from any of the silver colloids, as well as from the deionized water and the HPLC water. This led to the conclusion that while the water was pure in the presence of metallic silver, its structural arrangement was different than that of HPLC and deionized water (Roy et al. 2007).

Later in 2011, an interesting study was conducted by Revelli and the group. They observed using TEM and photoelectron spectroscopic imaging (PSI), various microorganisms treated with SilverSol® and demonstrated the localization of silver NPs within the microbes. Using TEM, they observed the localisation of clustered silver NPs both within *Escherichia coli* and *Staphylococcus aureus* but not in several other bacterial strains studied. However, when the PSI technique was used, all treated strains showed dispersed silver particles localized within all the bacterial cells (Revelli et al. 2011).

2.3 *Safety Studies of SilverSol®*

Recently, Metallic NPs with a wide range of composition and size, have been introduced for the treatment and diagnosis of several ailments and diseases. This has, however, led to an increase in human exposure to several metals including silver. The safety of metallic NPs is an ongoing concern due to their accumulation in the body which is likely to cause some side effects (Magaye et al. 2012). It has been shown that NPs can have varied degree of cellular uptake and toxicity through their interactions (influenced by their size, shape, charge, and the constituent material) with biological systems (Albanese et al. 2012). Almost a quarter of currently available commercial nanoproducts used for the medicinal purpose are made up of nanoscale silver (Munger et al. 2014) which have extensive medicinal and surgical applications (Rai et al. 2014; Lee and Jun 2019). It is therefore the responsibility of the manufacturer to ensure the safety of their silver NP product.

2.3.1 Safety of SilverSol®: Unique Structure

The unique arrangement of metallic silver and its oxide in water (vide supra) ensures the essential non-toxicity and the safety of SilverSol®. Safety of SilverSol® has been established both for topical application and oral administration. It is widely known that excessive oral intake of ionic (soluble form) silver, may cause irreversible blackening of the skin termed as Argyria. Trop et al. reported raised liver enzymes and argyria when silver-based dressing was used in burn patients (Trop et al. 2006).

2.3.2 Safety of SilverSol®: Ultra-Low Effective Dose

SilverSol® containing 10–40 ppm silver is more efficient, about 1000 times than other forms of colloidal or ionic silver, in destroying pathogens. On the contrary, some silver products contain up to 300,000 ppm silver (Sellman 2010). Consumption of at least 900 mg of silver in a year (2.4 mg/day for 1 year) is needed to develop Argyria. The recommended dosage of 10 ppm SilverSol®—½ to 1 spoon (15–30 μg in 1.5–3 ml) once or thrice a day is 50 times less than the dose needed to develop

argyria. As per the Environmental Protection Agency (EPA) standards, 5 μg/kg body weight silver per day (350 μg/day for a 70 kg adult) can safely be taken by an adult (https://www.govinfo.gov/content/pkg/FR-1999-08-17/html/99-21253.htm). Considering this cut-off, consumption of 6 tablespoon 10 ppm ASAP solution every day by an average weight person would not cross the safety limits even if continued up to 72 years. Besides these safety calculations, a complete safety profile of SilverSol®, has been established by ABL by conducting more than 30 studies. Some of them are enlisted below.

2.3.3 Cytotoxicity Studies In Vitro

ASAP 10 ppm silver solution was studied on Murine fibroblast cell line—L929 by Nelson Laboratories USA using agar overlay test method. In brief, in a 6 well plate, L929 cells were grown to 80% confluency. Over the cell mat, a layer of 1% agar was put, on which the discs containing 100 μl of 10 ppm ASAP solution were placed. The plates were then incubated. The effect of silver that diffuses through the agar layer was studied on cell growth. The results were assessed in terms of the zone around the disc as per the criteria stated in the United States Pharmacopeia & National Formulary. The score of cytotoxicity due to ASAP was 1 as compared to the positive control—latex natural rubber, which gave a score of 4 (Nelson Laboratories Report 2013).

2.3.4 Animal Toxicity Studies

Acute oral toxicity ASAP 22 ppm solution was tested in rats as per the guidelines of the Federal Hazardous Substances Act (FHSA) regulations, 16 CFR 1500 (NAMSA, California report 1999). ASAP 22 ppm, 5 gm/kg (a dose 50 times higher than that of the human dose) was given to rats. There was no significant toxicity or mortality observed in rats monitored over 14 days. Another study was conducted at Shri CB Patel Research Centre, Mumbai, India, using 10 and 32 ppm ASAP solutions. A dose of 50 ml/kg body weight was injected into the peritoneal cavity of Swiss albino mice. There was no mortality and no organ toxicity seen at the end of 72 h, which implied that the safety criteria as per the USP requirements were met.

2.3.5 Selective Inaction on Probiotics

Colloidal silver has emerged as an effective antimicrobial agent, acting across a wide spectrum of the microbial population. Chemical compounds and antibiotics are effective over a smaller range of microbes, whereas colloidal silver can kill over 600 types of microorganisms. Interestingly, it has been reported by VBPL that SilverSol® 10 and 22 ppm does not kill bacteria used in probiotics. In an in vitro study, SilverSol® 10 and 22 ppm was studied against two marketed probiotics—

Lactisyn (containing *Lactobacillus lactis, Lactobacillus acidophilus, Streptococcus lactis and Streptococcus thermophilus*) and Kyo-Dophilus® (containing *Lactobacillus acidophilus, Bifidobacterium bifidum and Bifidobacterium longum*). There was no inhibition of bacteria in the formulation when grown in presence of ASAP (Data on file at ABL). Another more extensive study conducted at Dr. Ron W Leavitt's laboratory, Brigham Young University, in 2004 showed similar results. They showed ASAP to be bactericidal against the pathogens tested at varying degrees but not against the various bacteria used as probiotics viz. *Lactobacillus, Bifidobacterium and Streptococcus* species. They used several concentrations of ASAP 32 ppm (0.13 ppm to 16.0 ppm) in broth microdilution assay against standard ATCC bacterial cultures. Based on Minimum inhibitory concentration (MIC) results, they reported that the growth of *Bifidobacterium* was not affected at all at the highest concentration (16 ppm) and growth of *Lactobacillus* was marginally affected at 4 and 8 ppm. Whereas all pathogens tested were inhibited at MIC of 2 ppm (Data on file at the ABL). The study concluded that the consumption of probiotics in conjunction with ASAP would be beneficial.

2.3.6 Clinical Safety: Effect on Haematology and Metabolic Markers

Clinical safety of silver and other metallic NP has been a continuous concern. Several in vitro and in vivo studies have been shown the safety of SilverSol® as described in Sect. 2.1.3 to 2.1.5. However, it is important to ensure the clinical safety of SilverSol®. Munger et al. conducted a Phase 1 clinical study. Healthy volunteers were given 10 and 32 ppm SilverSol® for 14 days and the effect on biological systems was evaluated.

The first prospective double-blind placebo-controlled crossover study in the 60 healthy volunteers was conducted by Munger et al. This systematic study was conducted as par the 'International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use' Guidelines for Good Clinical Practice and the Declaration of Helsinki, with the approval from the Institutional Review Board—University of Utah (Munger et al. 2014). The study registration was done with Clinical-Trials.gov (identifier: NCT01243320). Total 36 subjects received orally placebo as 15 ml sterile water or 15 ml 10 ppm oral SilverSol® solution and 24 subjects received 32 ppm oral silver particle daily for 14 days. At the end of 14 days, there was 3 days wash out period, after which the volunteers received a crossover dose of sterile water or dose of respective silver particles. All the subjects were investigated at baseline and at the end of 3, 7 and 14 days for physical examination, medical and drug history, a panel of metabolic markers, hematology and urine analysis. There were no significant changes in clinical or physical findings and in the metabolic, hematologic or urine analysis.

In the above-mentioned study, eighteen subjects receiving 10 ppm and eleven subjects receiving 32 ppm SilverSol®, also underwent cardiac and abdominal MRIs post 3–14 days of treatment. There were no morphological or structural changes noted. The markers of oxidative damage and inflammation—hydrogen peroxide

production or peroxiredoxin protein expression and pro-inflammatory cytokine RNA expression analysed in these volunteers were unchanged. IL-8, IL-1α, IL-1β, MCP1 (Monocyte chemoattractant protein-1) and NQO1 (NADH quinone oxidoreductase-1) showed no statistical difference between the subjects treated with active silver and placebo solutions.

2.4 *Efficacy of SilverSol®*

The activity of SilverSol® has been shown in vitro and in vivo studies. Studies conducted in human suffering from several illnesses too have indicated its efficacy. ABL so far has conducted more than 400 studies at 60 different private, U.S. government, university and military labs across the world. All the data collectively confirm the activity, efficacy and safety of SilverSol® (Data on file at the ABL).

2.4.1 Microbicidal Activity In Vitro

Traditionally use of ionic silver (silver nitrate) as an antimicrobial was most popular. It was used as both bacteriostatic and bactericidal (Ricketts et al. 1970; Berger et al. 1976b; Tilton and Rosenberg 1978; Ritchie and Jones 1990), antifungal (Miller and McCallan 1957; Brown and Smith 1976; Berger et al. 1976a), protozoicidal, (Wysor and Zollinhofer 1972) and antiviral (Coleman et al. 1973) agent. However, it was not so effective against bacterial spores, cysts of *Entamoeba histolytica* and Mycobacteria (Zanger et al. 2008). In case of *Pseudomonas aeruginosa* a non-linear order of death was observed with silver ion (Brown and Anderson 1968). Whereas a rapid bactericidal action of silver ion (silver nitrate 0.5 and 1 μg/ml) was observed in water but not in broth (Ricketts et al. 1970). Several other forms of silver viz. silver citrate, lactate and proteinate, and silver sulfadiazine have also been developed.

Ravelin in 1869 (Ravelin 1869) was the first to report antimicrobial effect at extremely low concentrations of metallic silver and other metal derivatives. Von Naegeli found that metallic silver at 0.0000001% (1 ppm) concentration would kill the common fresh-water *Spirogyra* (Von Naegelli 1893). Germination of *Aspergillus niger* spores was prevented by metallic silver at 60 ppm (0.00006%) (Russell and Hugo 1994). Interestingly, SilverSol® too kills nearly all microorganisms at similar concentrations 10–50 ppm including the ones resistant to antibiotics.

In the studies conducted with standard ATCC culture and resistant strains; SilverSol® showed antimicrobial potential. An in vitro study was conducted using SilverSol®- Silver Water Dispersion™ Solution (De Souza et al. 2006). Eight microorganisms viz. *Shigella flexneri*, *Salmonella typhi*, *S. aureus* 6538 P. *Bacillus subtilis*, *Candida albicans,* MDR (multiple-drug resistant) strains of *E. coli and P. aeruginosa,* methicillin-resistant *S. aureus* were treated individually with Silver

Water Dispersion™ and nineteen commonly used antibiotics. Further synergistic activity of Silver Water Dispersion™ was studied by the group using it in a combination with individual antibiotics. MICs of Silver Water Dispersion™ and the antibiotic solutions were determined using the macro-dilution test. The agar cup method was used for zone of inhibition studies using individual antibiotics and synergistic combinations. MIC of Silver water dispersion™ Solution was found to be in the range of 2–17 ppm, the lowest inhibitory concentration was against *S. typhi*. The highest inhibitory concentration was found to be against *B. subtilis*. Total 96 tests were conducted to determine the synergistic activity of Silver Water Dispersion™ in combination with other standard antibiotics. Five combinations were found to be synergistic- with amakacin, cefoperazone, ceftizidine (against MDR—*E. coli* and *P. aeruginosa*) and kanamycine, 89 were additive, and two were antagonistic—with amoxicillin and oxacillin.

Microbicidal activity of ASAP nano-silver solution was reported by Bhat et al. against drug-resistant pathogens—bacteria and Candida. The resistant bacteria studied included Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus faecalis*, drug-resistant *Escherichia coli*, ESBL (extended-spectrum β-lactamase) producing *Klebsiella pneumoniae*, drug-resistant *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Shigella flexneri* were isolated from clinical samples. The inhibitory and microbicidal effects of ASAP were determined by broth dilution and suspension test. *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as controls. Bacteria were found to be more susceptible than Candida. It was observed that an exposure time of around 30–60 min would kill bacteria while *C. albicans* was killed after 120 min of exposure to ASAP (Bhat et al. 2009).

In another study by Revelli et al., the activity of 10 ppm SilverSol® was compared with 5 antibiotics—Erythromycin, Ofloxacin, Tetracycline, Penicillin and Cefaperazone. MIC was also determined against *Streptococcus pyogenes* (ATCC 19615), *Streptococcus gordonii* (ATCC 10558), *Escherichia coli* O157: H7 (ATCC 43895), *Streptococcus mutans* (ATCC 25175), *Streptococcus pneumoniae* (ATCC 6303), *E. coli* (S.E. 163 Luria Strain B ATCC 11303), *Klebsiella pneumoniae* (ATCC 13883), *S. typhimurium* (ATCC 14028), *Enterobacter aerogenes* (ATCC 13048), *P. aeruginosa* (ATCC 27853), *Streptococcus faecalis*, *Shigella boydii*, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Salmonella enterica* subsp. *arizonae* and, *Enterobacter cloacae*. MIC for the majority of gram-negative organisms tested was found to be 2.5 ppm and for *P. aeruginosa, Shigella boydii*, and *K. oxytoca showed* lower MICs (1.67 ppm, 2.19 ppm, 1.25 ppm, respectively) (Revelli et al. 2011).

Recent collaborative studies conducted at Texas Tech University have investigated the antibacterial activity of SilverSol® Gel (Ag-gel). The group investigated the inhibitory activity of Ag-gel against bacteria causing tooth decay and plaque formation (Tran et al. 2019). The activity of Ag-gel was first tested against individual bacteria and colony forming units (CFU) were monitored. the effect of Ag-gel on biofilm formation was determined by placing suspensions of these bacteria (approximately 4×10^2 CFU—colony forming units of each) on a 6 mm paper disc. Ag-gel

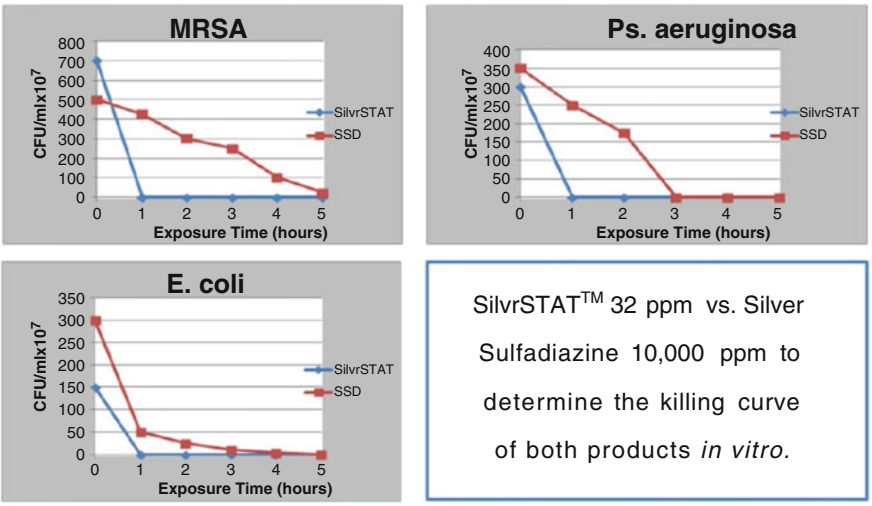

Fig. 2 Kill Time Curve for SilverSTAT® versus Silver Sulfadiazine Cream

0.5 gm was applied on the inoculated discs which were then placed on the growth medium in plates. Micro-aerobic conditions were generated using EZ GasPak in a jar in which the plates were incubated at 37 °C for 24 h. Biofilm formation was monitored by scanning electron microscopy. In the CFU assay, Ag-gel inhibited 100% growth of all the test bacteria, as against in the placebo gel and in the blank cellulose disc wherein bacterial growth was seen to be as high as 6 logs. In the biofilm formation assay, *S. salivarius*, *S. sanguis*, *S. mutans* and the mixture of all three strains developed microcolonies on the cellulose discs indicating the formation of typical biofilms. In presence of Ag-gel, no such biofilm formation was seen.

In another study done by the group, synergistic action of silver colloidal gel (Ag-gel) with 5% Betadine solutions was determined against both Gram negative and Gram-positive bacteria. The experiments were carried out using colony forming unit assay, and confocal laser scanning microscopy. Ag-gel alone inhibited the growth of all the bacteria, except *Klebsiella pneumoniae* CI strain. On the contrary, bactericidal activity was not seen when 5% Betadine was used alone. Interestingly, Ag-gel in combination with 5% Betadine solution, completely eliminated even *K. pneumoniae* (Tran et al. 2020).

SilvrSTAT® 32 PPM hydrogel—one of the products of SilverSol® used as an antibacterial wound dressing gel has shown significant bactericidal effect as seen in the comparative kill curves with currently available silver products approved for use in wound management. In-vitro comparison of kill curves for SilvrSTAT® (32 ppm silver) exemplify clinical relevance of SilvrSTAT® over silver sulfadiazine cream (10,000 ppm), for eradication of MDR microbes in infected wounds (Fig. 2)—Data on file at ABL.

2.4.2 Clinical Efficacy of SilverSol®

Multidimensional SilverSol® products have demonstrated clinical effects in numerous ailments, besides wound healing. This section gives an overview of these studies.

In 4 various hospitals, about 120 studies have been conducted in patients with various illnesses viz. eye infections, upper respiratory tract infections, retroviral infections, malaria, external cuts, abdominal pain and diarrhoea, urinary tract infections, sore throats, bronchitis, vaginal yeast infections, ear infections, gonorrhoea, pelvic inflammatory disease, various mouth problems, etc. Almost all the patients treated orally with 10 ppm SilverSol® recovered within 1 week (Data on file at ABL).

In Ghana, West Africa studies were conducted at three hospitals by Dr. Kwabiah, Dr. Sackey, Dr. Abraham at Air Force Station Hospital, Korie-Bu Teaching Hospital and at the Justab Clinic/Maternity respectively. Fifty-eight patients were treated by their attending physicians in respective hospitals using ASAP 10 ppm Solution as an alternative to antibiotics both as an oral and topical application depending upon the illness. Orally the dosages given were 5–10 ml twice or thrice a day for infections including malaria, HIV, fungal skin infections, upper respiratory and urinary tract infections, peptic ulcers, infectious abscesses, sore throat and pelvic inflammation. In case of halitosis and gingivitis it was used as a mouth wash, and in vaginal yeast infection as a douche. ASAP drops were used for eye infections such as conjunctivitis, ear infections viz. otitis media and upper respiratory tract infections such as sinusitis and rhinitis. Within 7 days all the patients showed a complete recovery. The details of all these studies are deposited as ABL's proprietary records and we are not describing these studies here in detail as the focus of the article is wound healing.

SilverSol® products, 10 ppm and 30 ppm SilverSol® liquid as well as the 32 ppm SilvrSTAT™ Hydrogel, have been used effectively in dental surgeries (e.g. extractions, bone grafts, Guided Tissue Regeneration, Periodontal, Laser, Dental Implant and Endodontic surgeries). A profound ability of these products has been demonstrated by Willoughby AJM in treating oral infections and speeding up wound healing without negatively impacting the oral microbiome (and probiotic bacteria) or gut health. The readers can visit www.ddsource.com to for specific dental protocols and learn more. Some of the data has been given in a separate Sect. 3.6.8 below.

2.5 Bioavailability and Pharmacodynamics of SilverSol®

After an oral administration of SilverSol®, its concentration in urine and blood was measured by Munger et al. in a study conducted in healthy volunteers (Munger et al. 2014). In this study, healthy volunteers were given 15 ml of 10 ppm and 32 ppm SilverSol® for 14 days. Blood and urine were collected on the 14 day at $\leq$2 h of

ingestion of the last dose. In the group receiving a 10 ppm dose, blood samples were also collected at ≥24 h. after ingestion of the last dose on the third and seventh day of administration. Silver concentration was measured by using ICP-MS. In the 10 ppm dosing group, 42% of the subjects showed peak silver concentration (mean concentration 1.6 ± 0.4 μg/L) in the serum at the end of 14 days. In the group receiving the higher dose –32 ppm, 92% of the subjects showed peak silver levels in the serum with the mean value 6.8 ± 4.5 μg/L. There was no silver detected in the urine irrespective of time and dose.

As to the drug interaction, SilverSol® does not interfere with most of the other pharmaceutical compounds but works synergistically with medications. If taken in conjunction with antibiotics, it will work synergistically to increase the antibiotic effectiveness by tenfold (Sellman 2010). Importantly, its antibacterial action does not affect the beneficial gut flora. The nano-silver particles in SilverSol® do not interfere with the hydrochloric acid production in the stomach. They do not fall out of suspension and hence do not accumulate in the tissues or the skin reducing risk of developing Argyria, (i.e., blue man syndrome) (Roy et al. 2007).

2.6 *SilverSol®: Regulatory Status and Products*

Different forms of silver nano products viz. silver citrate, lactate, proteinate and nitrate have been listed in pharmacopoeias and formularies around the world. The old British National Formulary included a silver nitrate lotion, but it was removed from the 1993 issue (BNF 1993). and only silver sulphadiazine came thereafter. Cream of silver sulphadiazine and ophthalmic solution of silver nitrate have been included in The USP XXII (USP 1990). Other silver products such as silver metal, silver protein, silver acetate, silver nitrate, and silver sulphadiazine have been mentioned in Martindale, The Extra Pharmacopoeia (Martindale 1993).

In the case of SilverSol® products, several approvals from various regulatory agencies in the US, India and Canada have been received by ABL and VBPL. Product licence for Silver Biotics Antimicrobial Wound Cream and Silver Biotics Antimicrobial Hand & Body Wound Lotion has been given in Canada. Similar products for dental applications have been available under the brand names OraSIL™ and CuraSIL®. US FDA has given marketing approvals to various SilverSol® products viz. ASAP OTC® Wound Dressing Gel, SilvrSTAT® Antibacterial Wound Dressing Gel, AGRX Wound Wash Antibacterial Silver Skin and Wound Cleanser (Prescription), and AGX Wound Wash Antibacterial Skin and Wound Cleanser (Over the Counter). The FDA has recently approved the SilverSol® gel as a prescription medicine for its use in the management of diverse wounds viz. caused by first and second degree burns, abrasions, lacerations, diabetic ulcers, skin, tears, and surgical wounds (Sellman 2010). In India, VBPL has been granted product licenses by FDA; for Colloidal Silver Solution 10 ppm, Amorphous hydrogel with 32 ppm colloidal silver, Amorphous hydrogel Wound dressing with 32 ppm colloidal silver and Colloidal Silver Solution 40 ppm. In 1991, the USEPA established an

oral reference dose of 0.005 mg/kg/day for silver. According to this recommendation, in the case of SilverSol® the daily intake limit of silver, for an average size adult, would be about an ounce/day of a 10-ppm product.

Considering the wider application of SilverSol®, a large number of products in gel or liquid form with different strengths are being produced and marketed by ABL. The main treatment categories where SilverSol® can be used, are immune support, skin and wound care, and oral care. These products are prepared without any artificial ingredients, dyes or flavours, preservatives or additives and are gluten free, 100% vegetarian and probiotic friendly.

3 Wound Healing

3.1 Global Challenge of Wound Healing

Would healing, controlled by several biological and molecular events, is a complex physiological cascaded process of damage repair involving three major phases viz. (1) cell migration and proliferation (2) extracellular matrix deposition and (3) remodeling. This normal course of healing may get impaired or delayed in certain pathophysiological and metabolic conditions viz. uncontrolled diabetes, diabetic neuropathy and vascular diseases resulting in the formation of chronic non-healing wounds (Mustoe et al. 2006). Infection of such wounds; especially with drug resistant microorganisms further complicates the management. Such situations often pose a therapeutic challenge to a medical practitioner.

Chronic wounds, due to prolonged morbidity pose a humanitarian and economic burden both at the individual and at the national level. Foot ulcers are common in diabetes (around 15–25%) (Marston 2006) which can become chronic and nonhealing due to complications of diabetes leading to a need of amputation of lower extremities in 12% of them (Greer et al. 2013). The costs involved with the healing of an ulcer can be up to US$45,000 (Paquette et al. 2002). In addition, the detrimental consequences on the patient's quality of life because of diminished mobility and significant loss of productivity will have a socioeconomic impact. In addition, acute and emergency wound care resulted through trauma, surgery and burns requires several procedures and high cost. In developed countries, the treatment cost of chronic wounds has been estimated to be 1–3% or even more of the total health care expenditure (Olsson et al. 2019). The prevalence of chronic wounds was reported to be 6% in 2016 in Wales, UK, which resulted in to about 5.5% cost to the National Health Services (NHS) (Phillips et al. 2016). Worldwide, the annual average cost for wound care has been reported to be around $2.8 billion in 2014 and it is estimated to rise up to $3.5 billion by 2021 (Settipalli 2015). Skin scarring is an additional burden implicated in wound healing, which can bring about an annual cost of US$12 billion.

Overall, wound healing is a great challenge both in case of handling acute emergencies and managing chronic non-healing wounds. Healthcare professionals

and patients look for novel medicine, medical devices, and newer treatment modalities, that can offer better treatment options to improve healing rates, minimizing complications and reduce hospital stays. This has created great attention for both the scientific fraternity and the commercial enterprises. The current market of wound healing products surpasses US$15 billion annually and the amount spent per year for handling wound scarring is about US$12 billion (Sen et al. 2009). Innovative biotechnology-based treatment procedures and medical devices are being developed for improved wound management. The SilverSol® products for wound healing, developed using advanced technology are at the top of the list, scientifically addressing various aspects of wound healing viz. safety, efficacy, faster healing and reducing morbidity.

3.2 Historical Aspect of Wound Healing

Wounds and their management have been a part of human existence since the time man first arrived on earth. It is interesting to examine the methods and preparations used to heal wounds over the centuries. One of the oldest records of wound management—the Smith Papyrus, was discovered by Dr. Edwin Smith—a well-known scholar in 1862. The writings (discovered by Smith) date back to around 2600 BC and cover many aspects of patients' care. It describes the cleaning and suturing of wounds, the use of antiseptics, adhesives such as acacia gum and resins, and bandaging. The resin coated bandages that were used to wrap mummies were used for wound dressing. Over thousands of years, a variety of materials have been used for wound healing such as spider webs, dung, various species of animal and insects, vinegar, beer, wine, honey, leaves and tree bark. The first description of 'three healing gestures"—washing the wounds, making the plasters, and bandaging the wound was found to be recorded on mud tablets during 2200 BC (Ackerknech 1982; Richard 1991; Yardley 1998). An interesting description of using a bandage for wound healing was found in Mesopotamian culture. It states that "mix in milk and beer (the bandage) in a small copper pan; spread on the skin; bind on him (on patient's wound), and he shall recover" (Farrar and Krosnick 1991). Egyptians used adhesive bandages which contained honey and grease for the protection of wounds from infection and vegetable fibres' lint to aid drainage of the wound. Honey has been used for thousands of years and is still a part of many advanced wound dressings. Even in India, a long before the birth of Christ, honey was used for wound care. Greek Physician Hippocrates practiced 3 measures for wound healing—(1) cleaning and drying the wound edges (2) bringing wound edges as close as possible to accelerate healing and (3) applying warm or cold wine as an antiseptic (Farrar and Krosnick 1991; Brown 1992; Yardley 1998). This description interestingly covers the modern concept of 'The TIME' in wound management (vide infra).

3.3 Challenges in Wound Healing: Current Practices and Novel Approaches

A wound, whether minor or major causes a lot of suffering to the patient due to pain, swelling and inflammation. It can cause temporary disability to prolong immobility and affect the overall quality of life. However, inflammation of the wound, the first response of the body to an injury, induces migration of various polymorphic mononuclear blood cells and monocytes to the site of injury to remove cell debris and bacteria by phagocytosis. This initial inflammation is needed for initiating the proliferative phase of the damage-repair cascade. Under normal physiological conditions, the body follows the damage repair cascade—(1) cell migration and proliferation (2) extracellular matrix deposition and (3) remodeling, which leads to wound healing. Under such normal physiological condition, therapeutic management of wounds includes supportive drug/non-drug modalities to enhance the natural process. Various wound dressings and treatments have been evolved considerably to handle such conditions. However, in case of impaired physiological condition as in diabetes, or for severe wounds, their management is a foremost challenge. Wounds tend to become chronic, infectious and non-healing in patients with co-morbidities (vide supra). Such complex wounds that include lacerations, diabetic-, pressure-, and venous- ulcers, infectious third-degree burns require a systematic management strategy, simultaneously addressing inflammation, infection and impaired physiological process.

The inflammation phase is a part of a wound healing process at the initial stage of an injury (vide supra). However, prolonged inflammation can lead to tissue damage and hamper the natural healing process. Infection of wounds is a major concern that complicates wound management. Especially wounds that take longer to heal are more prone to infections. Skin is a natural habitat for common bacteria such as *Staphylococcus epidermidis,* and various other species viz. *Staphylococcus* and *Corynebacterium*, *Brevibacterium*, *Proprionibacterium acnes*, *Pityrosporum,* hence, serves as a potential source of wound contamination (Bowler et al. 2001b; Broughton et al. 2006; Schreml et al. 2010). Normally wounds can heal in presence of these bacteria, but colonization of bacteria in slow healing wounds may hamper the healing process. Cell debris and local hypoxia at the site of the wound promote bacterial colonization and subsequent chronic infection. Colonization by drug-resistant bacteria viz. MRSA and VRE further complicate wound healing. Proliferating bacteria at the site of the wound penetrate deeper healthy tissue resulting in tissue damage and uncontrolled inflammation, leading to severe wound. β-haemolytic *Streptococcus pyogenes* and *Streptococcus agalactiae, Staphylococcus aureus, Proteus, Klebsiella, Pseudomonas, Escherichia coli, Stenotrophomonas, Acinetobacter, a*nd *Xanthomonas* are common pathogens that can infect wounds (Bowler et al. 2001a; Ovington 2003).

Prolonged inflammation and severe infection of wounds affect the normal wound healing process. Infections reduce the essential growth factors and degrade fibrin that required for natural healing. Moreover, prolonged, and uncontrolled inflammation

induces tissue damage, supports bacterial growth and collectively hampers the healing process. Biofilms formed by colonizing bacteria further create a hypoxic environment that damages tissues, supports bacterial growth and hampers fibroblast proliferation and collagen production required for the natural wound healing process. These factors collectively cause wound complications (Anderson and Hamm 2012; Okur et al. 2020). Besides these factors, other co-morbidities such as immune suppression and smoking have a negative influence on the wound healing process.

With the advancement of science and technology wound repair has become more organized through a holistic approach involving wound factors, local tissue factors, patient factors, and environmental factors. Increasing knowledge of the mechanisms of wound healing and advancement of technology has led to the expansion of superior wound healing modalities, such as hyperbaric oxygen therapy, bioengineered skin and tissue equivalents and negative pressure wound therapy (Wu et al. 2010). The details of such advancements are covered by others in this book. However, the authors would like to mention here that the 'The TIME' concept is now considered to be an essential Wound Care Process. It covers 4 important measures of wound healing namely **T**—Tissue, Removal of devitalized tissue, **I**—Inflammation/Infection and its prevention & control, **M**—Moisture Management and **E**—Edge Protection. SilverSol® wound healing products made from silver NPs address all these aspects of 'The TIME' concept.

3.4 Silver in Wound Healing

Hippocrates (400 BC) mentioned in his medical writing, beneficial effects of silver in healing and in disease-alleviating properties. He highlighted an ability of silver in tissue repair and wound healing and applied silver preparations for the treatment of ulcers. Marion Sims; an American physician in 1852, who was a pioneer in the field of surgery and known as the "father of modern gynaecology" used fine silver wires to close the fistulas after surgical repair of vesico-vaginal fistula. He also employed silver catheters for urinary diversion until complete healing of repairs (Sims 1884). Later, Halsted, an American surgeon, who stressed upon the strict sterile practices during surgical methods, treated wound infections using silver foil (Hill and Pillsbury 1939). In 1520 the Swiss physician Paracelsus used Silver nitrate as a medication for wounds,' both for an internal therapy and for a topical application (Alexander 2009). Later during the period of 1800–1900 silver nitrate was effectively used to treat complicated wounds like skin ulcers, compound fractures, and oozing wounds. Crusius treated burn injuries with silver nitrate in the 1890s (Alexander 2009). The first record of applying colloidal silver on the wound as an antiseptic was found in 1891 (Grier 1968). Roe in 1915, an ophthalmic surgeon used successfully colloidal silver to treat infected corneal ulcers (Roe 1915).

Though the knowledge of using silver in wound healing is age-old, efforts to develop newer forms of silver for optimizing delivery, efficacy and safety are still on

even today. SilverSol® qualifies all these aspects to be a successful medicinal product.

3.5 SilverSol® in Wound Healing

SilverSol® is a breakthrough among the currently available advanced wound-healing technologies. It offers a next generation therapy for the treatment and management of severe, chronic and infectious wounds that are difficult to manage. The clinical efficacy of SilverSol® products has been proven in wounds of varied etiology such as acute and traumatic wounds, lacerations, diabetic-, pressure-, and venous- ulcers, infectious wounds, third degree burns, MRSA and VRE infected wounds. It is among the few nano-silver technologies which have received FDA approvals for various formulations for wound healing. Various formulations manufactured using the SilverSol® technology are available under brand names such as Armor Gel™, ASAP OTC™ Wound dressing gel, AGRX Wound Wash, Antibacterial Silver Skin and Wound Cleanser (Prescription), and AGX Wound Wash, Antibacterial Skin and Wound Cleanser (Over the Counter). SilvrStat®, Megaheal, Hyrdoheal, and Silverex Heal are some of the approved prescription varieties of the SilverSol® gel available for the treatment of lacerations, first- and second-degree burns, diabetic ulcers, skin tears, abrasions, various surgical wounds, and MRSA and VRE infected wounds.

3.6 SilverSol®: Mechanisms of Action

SilverSol® consists of metalic nano-silver with a silver oxide coating. Being metallic in nature, it acts through quite a different mechanism as compared to that of ionic silver. Various silver products irrespective of the form of silver present in them, require ionization of the metallic silver for their antimicrobial activity. The highly reactive Positively charged silver ions (Ag+) are highly reactive and kill microbes by binding to proteins, DNA, RNA, and chloride ions of microbes which are negatively charged. These ionic silvers can steal 1 electron, however, the SilverSol® metallic nano-silver has the ability to steal multiple electrons.

Conventional silver products hence (ionic form) impart their effect through the direct contact with microbes—chemical reaction. SilverSol® technology works by catalytic action, which allows the silver NP to first destroy the pathogens and then instantly recharge and "kill" continually—like a rapid-fire machine gun (Sellman 2010). This makes SilverSol® incredibly powerful, destroying pathogens thousands of times more effectively than a simple colloidal or ionic silver. This explains why other silver solutions/suspensions need to be used at concentrations up to 300,000 ppm of silver, while SilverSol® performs effectively even at 5–30 ppm. In addition, ionic silver can also bind to negatively charged particles viz. proteins

and chlorides in the wound bed fluid, reducing the bioavailability of ionic silver. Hence the high concentration of silver will have to be used for maintaining prolonged activity.

It is also known that microbes develop resistance to ionic silver but not to metallic silver by sequestering silver in its more innocuous state—ionic or sulphide form and thereby detoxify the silver. However, as reported by Revelii et al., silver present in SilverSol® being metallic, accumulates in most bacterial cells in a quite different form—as small particles seen by PSI technique (vide supra) (Revelli et al. 2011). Secondly, each silver NP in SilverSol® remains always embedded with a resonant frequency, which allows the particles to have a continuous impact on things, without direct contact with them. Moreover, the particles also have an electrostatic charge that adds to its effect.

The unique wound healing action of SilverSol® has also been attributed to its stimulatory effect on stem cell regeneration (Sellman 2010). The two properties of metallic silver viz. high conductivity and bactericidal action impart the overall efficacy to SilverSol®. In addition, SilverSol® has been shown to be non-toxic to healthy cells in cytotoxicity experiments in vitro. This has an added advantage as local application of SilverSol® as the wound site will not have any damaging effect on healthy tissue.

SilverSol® is effective in wound healing. It exerts effects of silver at 3 levels namely, prevention or clearing of infections, improving healing process, and controlling inflammation. This ultimately speeds up the wound healing and reduces pain leading to a positive clinical outcome. SilverSol® has shown promising clinical efficacy in infectious wounds through its remarkable bactericidal activity even against MRSA and VRE. Several case studies demonstrating the effect of SilverSol® in the treatment of wounds of varied etiology and severity are discussed in the following section.

3.7 *Effect of SilverSol® in Wound Healing: Case Studies*

This section describes various case studies to illustrate the efficacy of various SilverSol® branded products (ARMOR GEL™, MEGAHEAL, ASAP OTC, SilvrSTAT®, Silver Biotics Pet Vet Veterinary Gel) used for the treatment of various skin infections, and wounds of varied etiology including mild cuts, lacerations, first and second degree burns, pressure ulcers, traumatic wounds, and chronic wounds. In addition, SilverSol® in a gel form, wound wash and dressing has been used in tens of thousands of patients undergoing dental procedures. The wound healing potential of these SilverSol® products viz. wash solution ASAP™ (10 ppm) and a gel SilvrSTAT™ (32 ppm) was evident through the remarkable recovery in these patients. A separate subsection is dedicated to summarizing effects in a large number

of cases undergoing dental procedures. It opens another avenue for wound healing in oral care and focal infections (vide infra).

3.7.1 Use of SilverSol® in Mild to Moderate Wounds

Case 1 A 11-year-old female got a large abrasion on the elbow and hip due to a scooter accident. After thorough washing of wounds with water, ARMOR GEL™ was applied to the affected area once for the first 2 days, and every 2 days after that. Wounds were covered with bandages, which were changed and each time the ARMOR GEL™ was reapplied. The bandages were discontinued after day 7, but the ARMOR GEL™ was still applied once daily up to day 10. A complete recovery was seen by day 12 (Figs. 3 and 4).

Case 2 A 22-month-old male child got a deep injury on the forehead. The wound was approx. 2 cm. long and 0.75 cm deep. It was cleaned and sutured at the hospital. Bacitracin was applied to the wound and was protected with a bandage. After returning home, ARMOR GEL™ was applied instead of bacitracin. The gel was then reapplied with the bandage change—1–4 times in a day (a repeated application was needed as being small, the child tended to remove the bandage). A bandage and ARMOR GEL™ were continued till day 9 after removing the sutures by a physician on day 5. Complete healing was achieved by day 9 (Fig. 5).

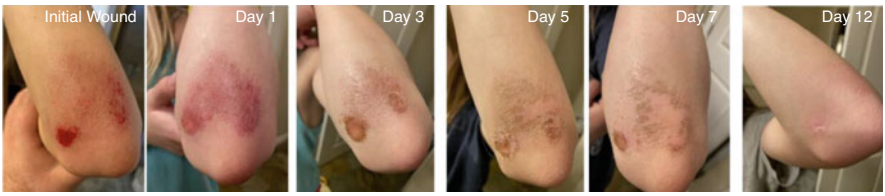

Fig. 3 CASE 1

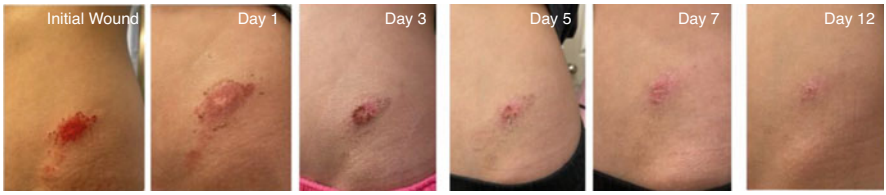

Fig. 4 CASE 1

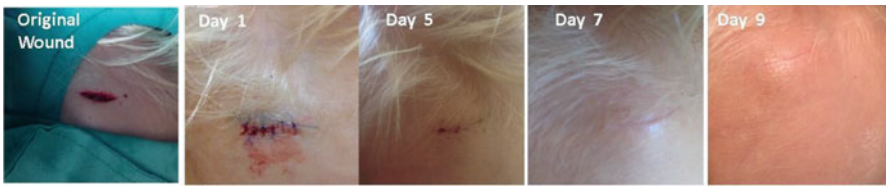

Fig. 5 CASE 2

Fig. 6 CASE 4

3.7.2 Use of SilverSol® in Superficial Pellet Wounds

Case 3 (Published) SilverSol® product Megaheal amorphous hydrogel was used for multiple pellet wounds. A 35-year-old male patient was injured accidentaly by shotgun. The wounds caused by multiple pellets were superficial and had not damaged bones and distal neurovascular status been intact. Musculoskeletal injuries were significant. Supportive treatment was given along with broad spectrum antibiotics and anti-tetanus therapy. After washing the wounds with saline, Megaheal was applied thrice a day which was followed by dressing. Patient followed every fifth day, showed gradual healing of the wounds and painless shading of remnants pellet. A complete healing was achieved by 2 weeks. The detailed case report is available as a published paper (Dharmshaktu et al. 2016).

3.7.3 Use of SilverSol® in Surgical Wounds

Case 4 A 63-year-old female had multiple surgical wounds. ARMOR GEL™ was applied to the wounds and were covered with bandages. It was reapplied 5 times daily every time with the new dressing. No other treatment or products were used for wound healing. Pictures of the wounds were taken on day 1 and day 28 (Fig. 6).

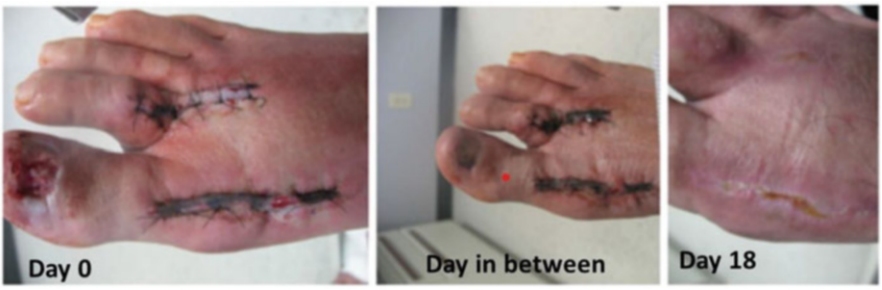

Fig. 7 CASE 5

Fig. 8 CASE 6

Case 5 A 52-year-old diabetic male undertook the voluntary corrective repair of hallux valgus and pre-dislocated second metatarsophalangeal joint (MPJ) right foot. The Silver hydrogel was applied to sutures immediately after surgery and every third day throughout the post-surgical care. The patient's sutures were removed on day 18. There was no indication of dehiscence/pull out or any sign of infection (Fig. 7).

Case 6 A 56-year-old female patient underwent plantar digital neuroplastic surgery to her third interspace right foot. The Silver hydrogel was used after surgery with dressing which was changed every third day till the 15th day. On day 15 sutures were removed. There was no pull-out, dehiscence or infection seen (Fig. 8).

Case 7 A 72-year-old male diabetic patient had to go for surgical resection of his fourth metatarsal head which was secondary to acute osteomyelitis. After surgery on the day 3 silver hydrogel was applied to the surgical wound with the change of dressing. Thereafter on every third day repeated dressing with hydrogel application was continued for 21 days. The wound healed completely by day 21 (Fig. 9).

Case 8 (Published) A 34-year-old female is a case of drug abused (IV Dilaudid injection) who developed an abscess to her right foot. Incision and drainage procedure (I&D) was urgently needed. After I&D, SilvrStat® was applied for 4 days. The dressing was changed weekly thereafter till the coaptation of tissue. Sutures were removed on day 21 without signs of infection, dehiscence or pull out (Lullove and Bernstein 2015).

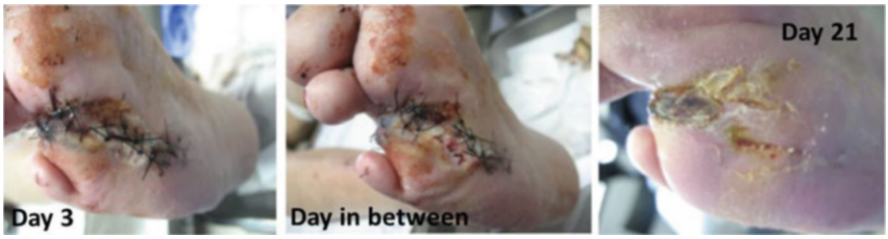

Fig. 9 CASE 7

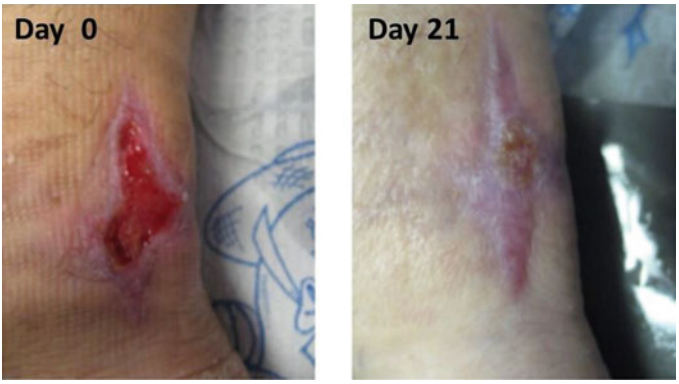

Fig. 10 CASE 9

Case 9 A 38-year-old female had a brown-recluse spider bite at the right medial lower leg. The wound so caused was irrigated and an ovine forestomach dermal template was used to obtained wound closure along with silver hydrogel application and collagen dressing. At the end of 6 weeks, there was no evidence of cytotoxicity with the collagen dressing or recurrence of infection. The wound healed completely thereafter (Fig. 10).

Case 10 Surgical wound with MRSA infection—A female patient after mastectomy got her wound infected with MRSA. Silver Sol gel was applied 4 times a day for 5 weeks. A series of photographs reveal gradual healing and complete resolution in 5 weeks (Fig. 11).

3.7.4 Use of SilverSol® for Skin Diseases

Case 11 A 5-year-old child suffering from eczema involving the bottom sides, and toes of the right foot. He was treated with the repeated application ARMOR GEL™ 24 ppm 2–3 times a day. On each application of ARMOR gel, after it could absorb, the Silver Biotics Skin Cream was applied over. After the open cracks and wounds

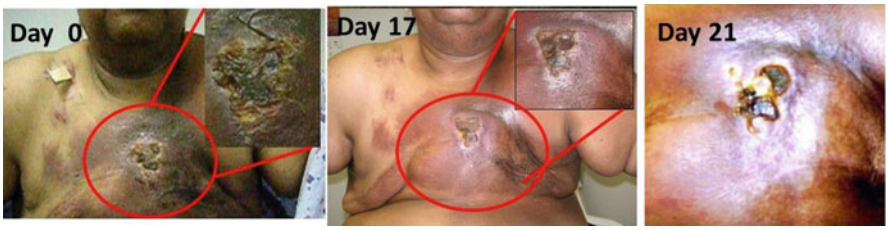

Fig. 11 CASE 10

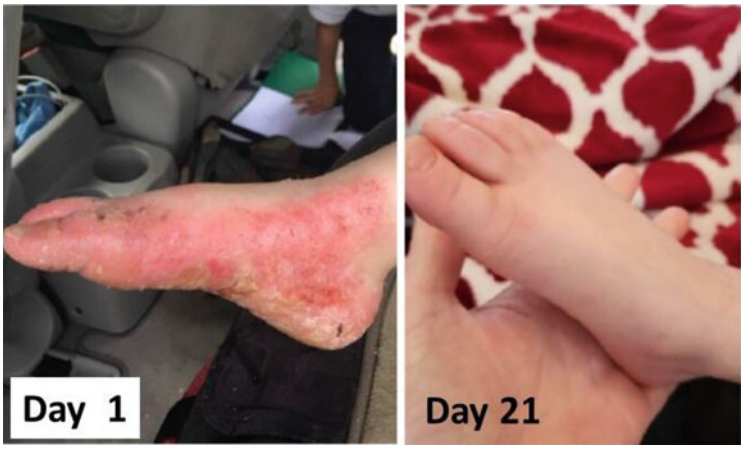

Fig. 12 CASE 11

healed, only the skin cream was used daily. There was complete healing of eczema without any sacring by day 21 (Fig. 12).

Case 12 A 10-year-old female suffering from ringworm infection with inflamed and painful skin was treated with ARMOR GEL™ local application twice a day and the affected area was left uncovered. The infection got completely cured by day 10 (Fig. 13).

3.7.5 Use of SilverSol® in Burns

Case 13 A 62-year-old male got a wound because of the spillage of hot grease onto the foot. The patient had diabetes and had undergone a liver and kidney transplant. The wound was covered with a bandage after the application of ASAP OTC and ARMOR GEL™ (24 ppm) hydrogel-wound dressing at the hospital. The ASAP OTC was then reapplied every day at the time of bandage change for 30 days. Thereafter only ASAP OTC was used on the wound which was completely healed by day 128 (Fig. 14).

Fig. 13 CASE 12

Case 14 A 1.5-year-old female child burnt her hand on a stove burner. The patient was hospitalized for 3 days and treated by a physician for severe second degree burns. After being discharged, ARMOR GEL™ (24 ppm hydrogel wound dressing) was used topically whenever the dressings were changed. Skin grafts were suggested by the physician but were put on hold. At the physician's direction, the product used was switched from ARMOR GEL™ to the prescription version SilvrSTAT® from around day 17. The wounds were healed completely by day 105 (Fig. 15).

Case 15 A 62-year-old male underwent a laser procedure that was done for hyperpigmentation. His hand got burnt during the procedure. The patient's wound was cleaned after the examination by a physician. The wound was covered with a bandage after the application of ASAP OTC/ARMOR GEL™ (24 ppm hydrogel wound dressing). The bandage was changed once a day with the application of ASAP every time until day 8. After day 8, only ASAP OTC was used on the wound, and it was applied 3–4 times per day. Complete healing was seen by day 27 (Fig. 16).

Case 16 An 88-year-old woman had complex wounds due to burns which was needed skin implantation. But the skin grafting was not successful due to her age and

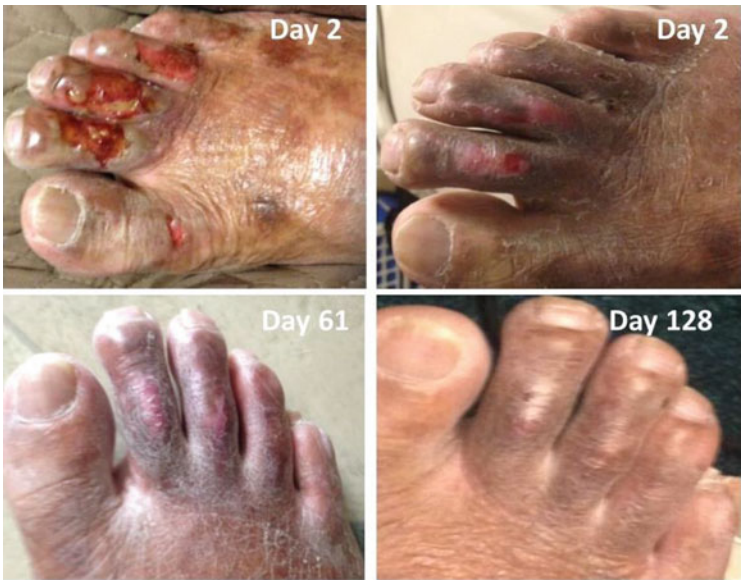

Fig. 14 CASE 13

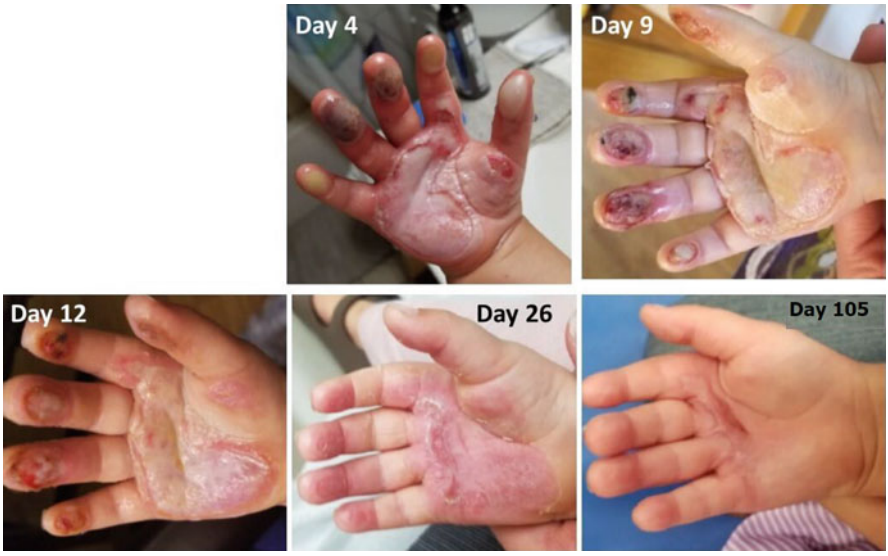

Fig. 15 CASE 14

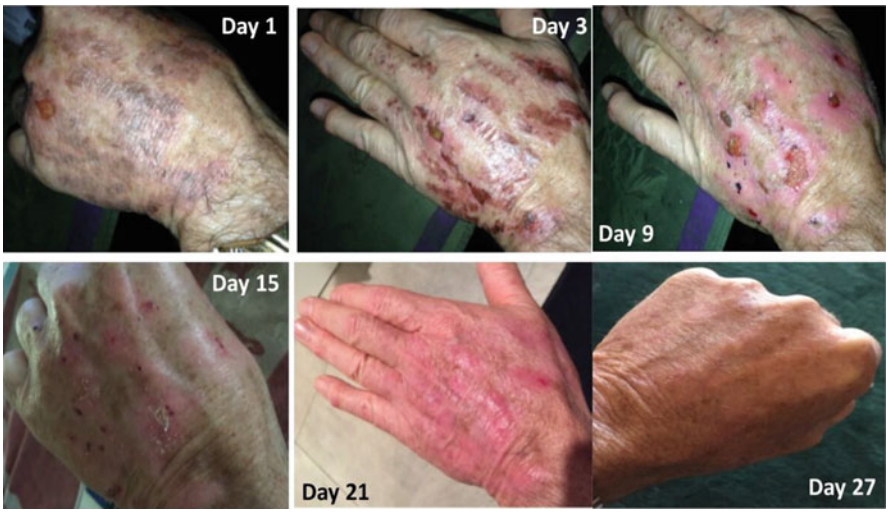

Fig. 16 CASE 15

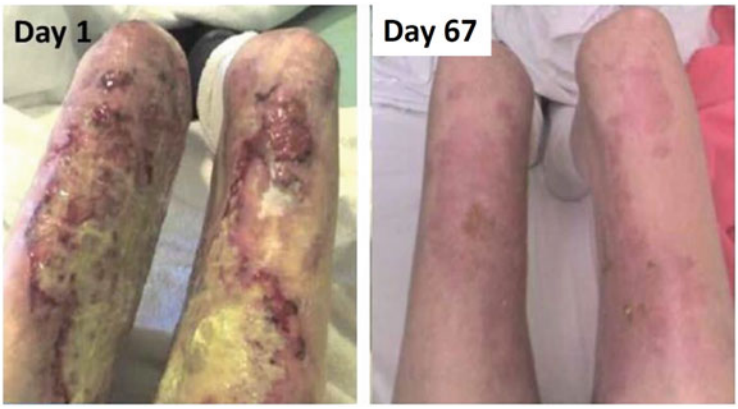

Fig. 17 CASE 16

compromised immunity. The patient recovered completely as the wound healed by 67 days of treatment with SivrSTAT® (Fig. 17).

3.7.6 Use of SilverSol® for Complicated Wounds

This section covers several complicated cases with traumatic laceration, diabetic wounds, infectious wounds (MRSA), various ulcers due to diabetes or vasculopathies, pressure ulcers (bed sores) etc. Several cases have shown recovery

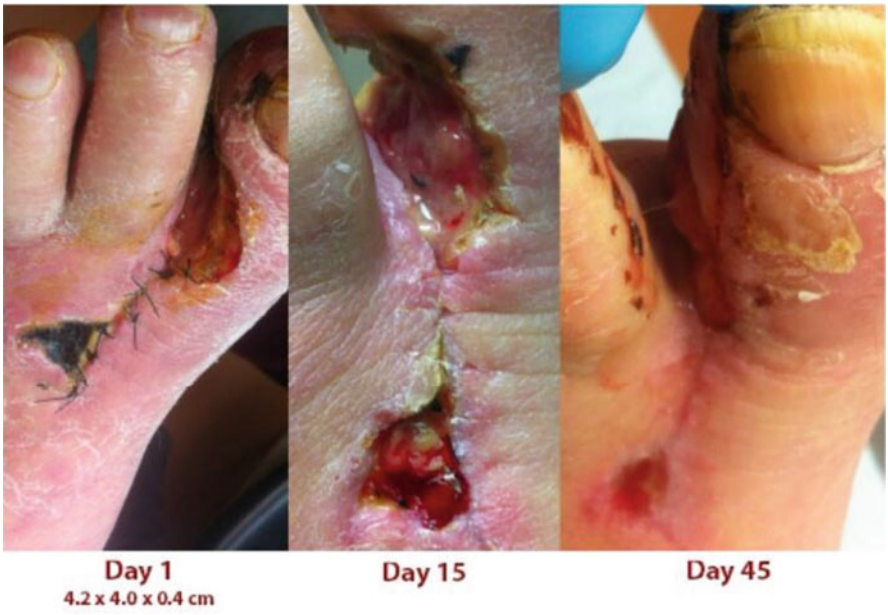

Fig. 18 CASE 17

from ulcers with various severity when treated with SilverSol® products. Some representative cases have been shown below.

Case 17 Diabetic wound infected with MRSA—A 71-year-old male diabetic patient with peripheral artery disease and past medical record of having sensory neuropathy, hypertension and dyslipidaemia. He was suffering from a complex wound located at the lateral left hallux extending to the dorsal left foot, secondary to his co-morbid conditions. Wound cultures were done which showed intense growth of MRSA along with *Proteus vulgaris* and *Enterobacter cloacae.* The wound was cleaned with debridement weekly and SilverSTAT® was applied daily with Adaptec dressing. A complete wound resolution was seen at the final evaluation on day 45 (Fig. 18).

Case 18 Diabetic wound infected with MRSA—A 70-year-old diabetic patient with an amputated limb suffered from a chronic wound. After amputation, the prosthetic device could not be fitted for 1 year due to the non-healing wound that further became complicated with MRSA infection. On the use of SilvrSTAT®, the wound healed completely within 4 months, and the patient became well enough for prosthetic fitting (Fig. 19).

Case 19 Traumatic serious laceration—A 47-year-old healthy male got a traumatic laceration about 2.5-inch-long to the eye and forehead. The orbital bone was broken, and a serious hematoma developed on both eyelids and the bridge of the nose. The wound was cleaned and closed by suturing that required eighteen stitches over the

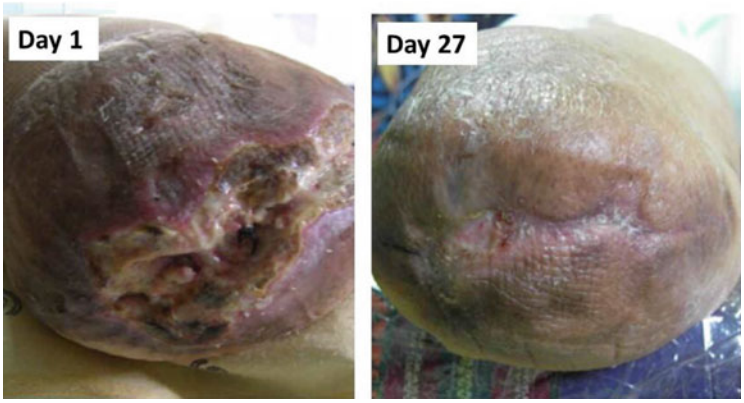

Fig. 19 CASE 18

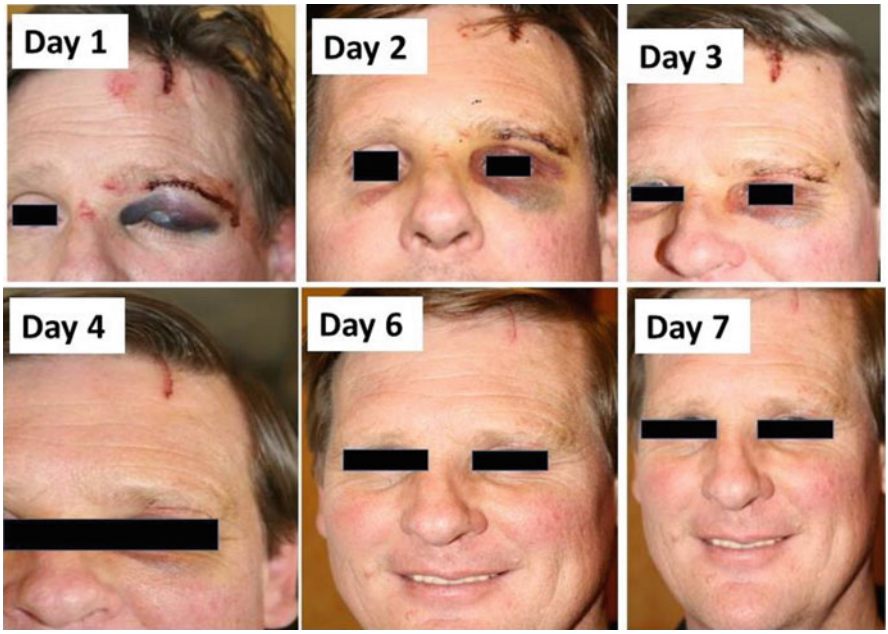

Fig. 20 CASE 19

eye and ten at the forehead. SilverSol® liquid was given orally two teaspoons twice a day and SilverSol® gel was applied topically 4 times a day. Pictures were taken at the hospital immediately after suturing and every day thereafter. Complete healing occurred by day 7 (Fig. 20).

Fig. 21 CASE 20

Case 20 Chronic Diabetic Ulcer A 70-year-old female patient presented with a wound that had not healed for 13 months. The wound was located at plantar medial right foot hallux. Medical history included diabetes; rheumatoid arthritis; hypertension and end stage renal disease. Treatment by debridement every 2 weeks and daily application of SilverSTAT® with Adaptic dressing brought about complete resolution of the wound by the final evaluation on day 52 (Fig. 21).

Case 21 and 22: Chronic Diabetic and Pressure Ulcers
ASAP wound dressing was used in 2 cases one having diabetic foot ulcers and the other having chronic pressure ulcer for 6 months. Both the cases were treated with SilverSol® and they showed recovery by 65 days and 3½ months of treatment respectively (Fig. 22).

Case 23: Bullous Pemphigoid MRSA Infected Wound
A 48-year-old woman having MRSA infection with Bollous Pemphigoid (autoimmune) complication was treated with SilverSol®. Oral administration of two table spoons twice a day of SilverSol® liquid was prescribed. The SilverSol® gel was given for topical application once a day at the time of bandage change. The treatment led to a reduction in MRSA infection and autoimmune attacks on tissues. Wound epithelialization was seen after 10 days of treatment (Fig. 23).

Case 24: Diabetic Ulcer A 70-year-old diabetic female patient with sensory neuropathy developed ulceration. Earlier history indicated suffering from rigid bunion deformity, end stage renal disease, hypertension, and dyslipidaemia.

The patient was diagnosed having chronic neuropathic clinical infection Wagner grade 2, IDSA-no (Infectious Diseases Society of America) associated with the ulcer located at plantar medial right foot first metatarsophalangeal joint. Wound cultures report indicated moderate infection with Oxacillin-susceptible *S. aureus* and light

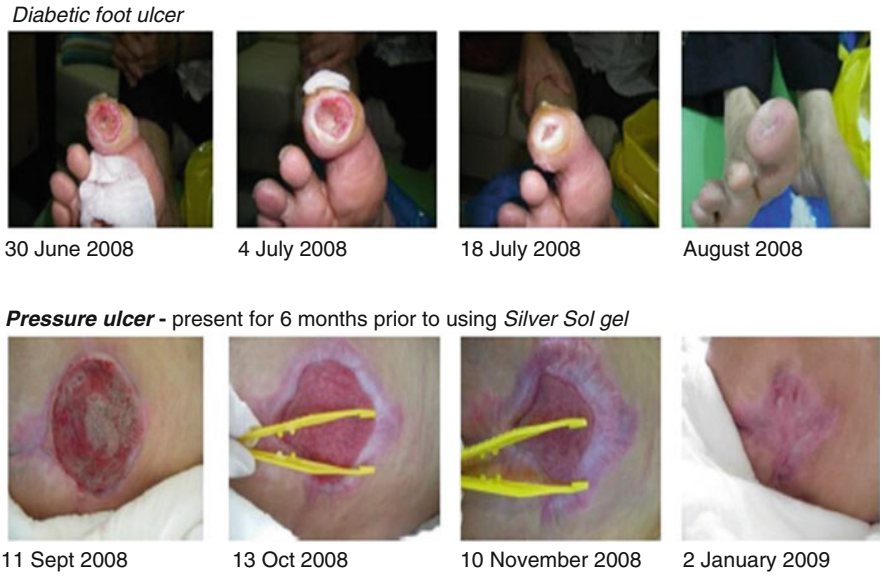

Fig. 22 CASE 21, 22

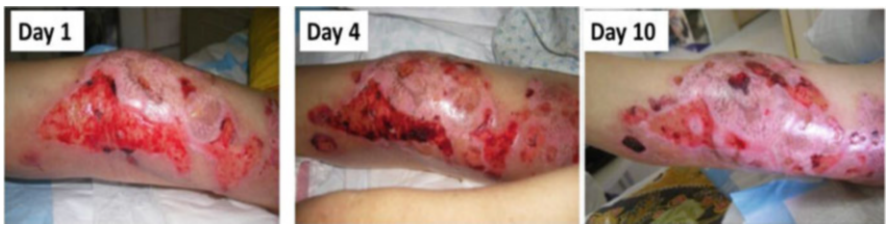

Fig. 23 CASE 23

growth of gram-negative rods. The patient's treatment included debridement and cleaning of the wound every 2 weeks. SilverSTAT® application with adaptic dressing was done daily. A Complete wound resolution occurred by the final evaluation on day 55 (Fig. 24).

Case 25 (published) A 33-year-old patient was seen in the hospital for an infected right great toe ulceration. The patient had the previous history of diabetic foot infections and this was the third occurrence on the same foot. The Patient encountered extreme pain of his right foot when he was brought to the hospital. Investigations revealed a 2.0 cm diameter ulcer to the medial aspect of the right great toe and a 4 cm tunnel from the proximal plantar first MPJ to distal plantar right great toe. No probing to the bone was identified. Past medical history included IDDM (for 26 years); depression; asthma; left great toe amputation (in 2012) The patient was allergic to Erythromycin and iodine. The patient was given operative treatment

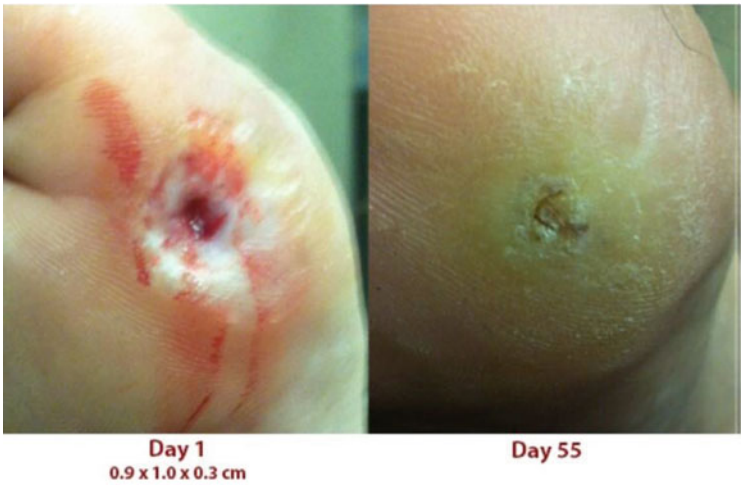

Fig. 24 CASE 24

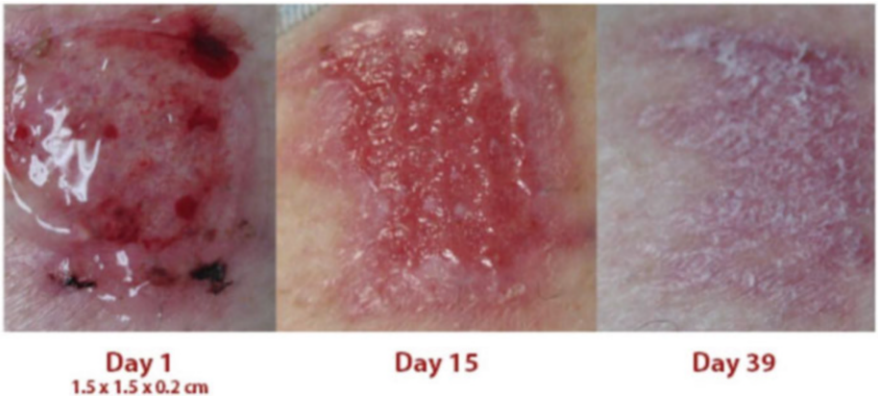

Fig. 25 CASE 26

which included debridement and incision/drainage of the abscess. Post-operative treatment included the application of SilverSTAT® every 3 days (Lullove and Bernstein 2015).

Case 26 A 58-year-old patient presented with an open wound to the left foot. The patient underwent a scheduled split thickness skin graft from the left thigh as a donor site. Past medical history included NIDDM and hypertension. Post-surgery, the patient was treated with SilverSTAT® to protect the donor site, viz. the left anterior thigh, and the application was changed every 3 days (Fig. 25).

Case 27 A 68-year-old non-diabetic patient came to the hospital for an initial consultation for a non-healing wound to the right foot. The patient was unable to

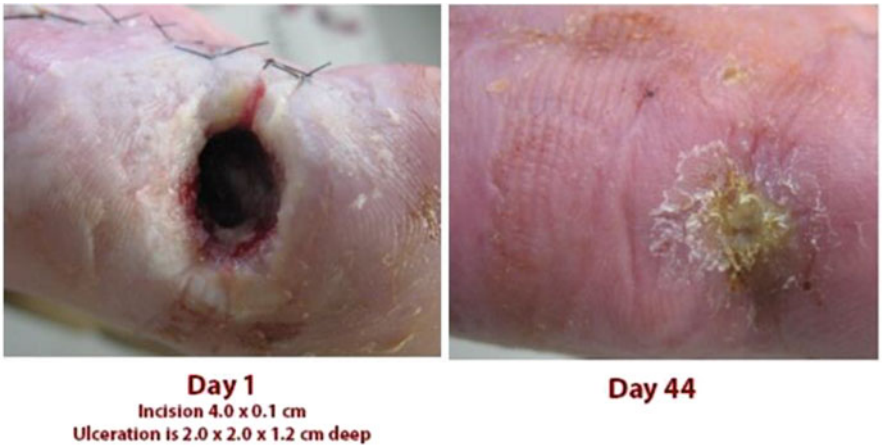

Fig. 26 CASE 27

relate further information regarding the wound, however, he stated that he had problems walking and was also complaining of pain. The patient underwent interventional angioplasty, which showed occlusion of the peroneal artery of the right leg.

The patient was operated for osteomyelitis of the fifth metatarsal. The patient was placed on negative pressure wound therapy for 2 weeks. Also, SilverSTAT® was placed into the wound and the incision on the foot was protected with a collagen dermal template with ECM. The wound completely epithelialized by post op day 44. The integrity of the incision dorsally without large evidence of adhesion scarring or fibrosis was easily seen (Fig. 26).

3.7.7 Horse Leg Wound Healing Using PetVet Gel

Case 28 The cause of the wound was unknown. The horse was kept in an outdoor corral, and the owner thought it may have been caused by a cougar or possibly by another horse. There was a large flap of skin missing from the wound and it was infected. The attending vet suggested that putting the horse down was probably the best option due to the infection, the wound's large surface area, and the inability to cover the wound due to its location. The owner was familiar with the Silver Biotics Pet Vet gel and opted to try and heal the wound first. The Silver Biotics Pet Vet gel was applied to the wound once daily by the owner and left uncovered. No other wound healing products were used. The tissue regrew and the wound completely closed after about 6 months. The infection quickly subsided and did not reoccur. It continued to heal to almost unnoticeable conditions, with both the skin and hair growing back, and almost no scar tissue formation (Fig. 27).

Fig. 27 CASE 28

3.7.8 SilverSol® in Dental Pain Infection and Inflammation: Case Studies

Andrew Willoughby, Reconstructive General Dentist from Canada and one of the co-authors of this chapter, used over 14 years SilverSol® products on his patients as a topical application during various dental surgeries *viz.* extractions, bone grafts, Guided Tissue Regeneration, Periodontal, Laser, Dental Implant and Endodontic surgeries. He has developed dozens of novel clinical protocols for specific surgical, endodontic and periodontal treatments utilizing these nano-silver products for various dental procedures. He has evaluated and assessed the efficacy and performance of the 10 ppm and 30 ppm SilverSol® liquid as well as the 32 ppm SilvrSTAT™ Hydrogel. Willoughby has confirmed the effect of these products in reducing the twelve most common oral pathogens and the formation of biofilms by them. He monitored DNA of these pathogens in saliva applying the polymerase chain reaction (PCR) test.

His exhaustive dental research with SilverSol® has demonstrated its profound ability to treat oral infections and speed up wound healing without negatively impacting the oral microbiome (and probiotic bacteria) or gut health. When utilized as a part of an integrated clinical protocol, Willoughby found that these Nano-silver products effectively eradicated bacterial infections associated with gum disease, tooth decay and dental infections, as well as accelerated wound healing and reduced inflammation. The fact that these products prevented post-operative/surgical infections and ultimately contributed to better patient outcomes far more effectively than other commonly used chemical disinfectants and antiseptics. He reported his findings and experiences using a combination of direct visual observations, intra-oral pictures, digital x-rays, computed tomography scans, ortho-pantographs, oral DNA tests and periodontal probing on over 22,000 patients having undergone almost 39,000 procedures for 20 different dental conditions (Fig. 28). He used different

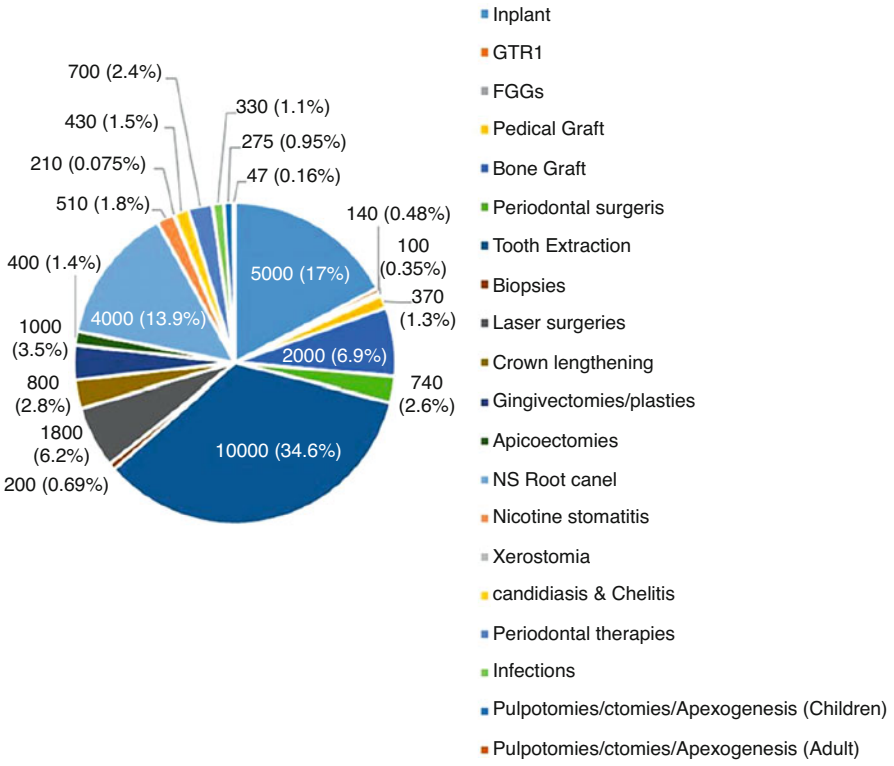

Fig. 28 Various dental procedures for which SilverSol® products were used

Table 1 SilverSol® products used for dental procedures

1	Silverstat® Antimicrobial gel during the procedure followed by a 10-day regimen of ASAP 10 ppm + 3% diluted H_2O_2 mouth rinse (3×daily)
2	ASAP 10 ppm + 3% diluted H_2O_2 rinsing followed by ASAP 10PPM for final rinse during the procedure
3	ASAP 10 ppm during the procedure
4	ASAP 10 ppm + 3% diluted H_2O_2 rinsing followed by SilverStat ®

Nano-silver products alone or in combination with 1–3% dilute H_2O_2 (Table 1). Willoughby states his experience in his report *"...After the use of these Nano-silver products (both during and after surgery) on thousands of my dental patients, the antimicrobial/wound healing benefits of this technology are truly impressive....the products help create a noticeable reduction in post-operative infection rates, accelerate healing times along with a clinically significant reduction in pain and swelling ..."* Corroborating Dr. Willoughby's clinical research, other leading Dental Implant surgeons have experienced very similar clinical outcomes with these Nano-silver products.

3.7.9 Oral Care and Focal Infection: (Proposed?) Role of SilverSol®

The oral cavity and teeth are one of the major sites that provide favourable environment for the growth of microbes. Poor oral care can lead to biofilm formation. Adental plaque containing such biofilms can have higher than 10^{11} microbes/mg (Li et al. 2000). Such oral microflora comprisings of mainly anaerobic gram-negative rods of diverse species. Apical periodontitic teeth can harbour around 200 species and marginal periodontitic teeth can harbour more than 500 species (Moore and Moore 1994; Tronstad 1992). It is now well accepted that such a focal infection in the oral cavity can spread to distant sites in the body—'Focal infection theory' (Miller 1891). The theory proposed by W Miller in 1891 speculated diffusion of microbes and their toxins from the focal infection to a distant body site. Immunocompromised hosts suffering from chronic diseases viz. cancer, diabetes arthritis or patients receiving immunosuppressive treatment are more prone to focal infection. Moreover, marginal and apical periodontitis can be a potential risk factor for the development of systemic diseases. Various dental conditions viz. alveolar abscesses, pyorrhoea alveolaris (periodontitis), and apical periodontitis, cellulitis, general oral sepsis and endodontically treated teeth, pulppless teeth, with the infection caused by viridans group streptococci are the major cause of focal infection (Easlick 1951; Pallasch and Wahl 2000; Murray and Saunders 2000). Various dental procedures including endodontic treatment, periodontal surgery or even tooth extraction and root scaling to treat these conditions facilitate the dissemination of bacteria into the systemic circulation. This dissemination leading to bacteraemia may occur within a minute after the oral procedure. The displaced microbes can reach the peripheral blood capillary system, lungs and heart causing injury through local infection, microbial toxins and inflammation (Kilian 1982).

The SilverSol® product—Ag-gel has been shown to have remarkable activity by Tran et al. against bacteria contributing to tooth decay and plaque formation—Sect. 2.4.1 (Tran et al. 2019). It was found to be bactericidal and was able to prevent biofilm formation. The above-mentioned clinical experience of Andrew Willoughby during dental procedures enables authors to extrapolate that the effect of SilverSol® may have the potential to control focal infections and the resulting systemic consequences described above. However, experimental evidence have to be generated through further studies.

Conflict of Interest The authors, A de Souza, Managing Director; Vora AH, Director; Mehta AD, director; and Godse CS Assistant Medical Director are from Viridis BioPharma Pvt. Ltd., whereas, Moeller K, is the Managing Director and Chief Executive Officer and Moeller C is the Director of Communication at ABL, LLC. Both, Viridis Biopharma and American Biotech manufacture and market SilverSol® products. Willoughby AJM is associated with DDSource, which is promoting SilverSol® products OraSIL™ and CuraSIL for dental applications.

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