CHAPTER II
THEORITICAL BACKGROUND AND LITERATURE SURVEYS

2.1 Burn Wound

There can be few conditions for which a greater number of different methods of treatment have been suggested than the wound produced by a burn. The very number of different treatments available is a sure indication that no one method has any clear advantage over the others or is universally applicable, although the claims of some authors suggest that they would have us think otherwise.

The theoretical basis of the treatment of burn wounds is simple and all methods of treatment must conform with certain well-established principles of the natural history of burns.

It is therefore proposed firstly to state briefly these basic principles, and then to discuss in more detail the pathological processes and the choice and practical application of the different methods of treatment.

2.1.1 Depth of Burning

For all practical purposes the fate of an area of burned skin depends upon the depth of skin destruction at the time of the injury. The important distinction must be made between:

2.1.1.1 Partial Skin Thickness Burn

If infection can be prevented these will heal spontaneously. There is no known treatment which will improve this spontaneous healing, but bacterial infection can seriously interfere with healing and can convert a partial thickness burn into a full thickness burn. The main aim of treatment is therefore to prevent infection. An important distinction must be made between:

2.1.1.1.1 Superficial Partial Thickness Burns

The depth of these types of burns in relation to the layers of the skin is shown in Figure 2.1. In all but the most superficial burns, the epidermis is completely destroyed but the hair follicles and sebaceous glands as well as the sweat glands are spared.

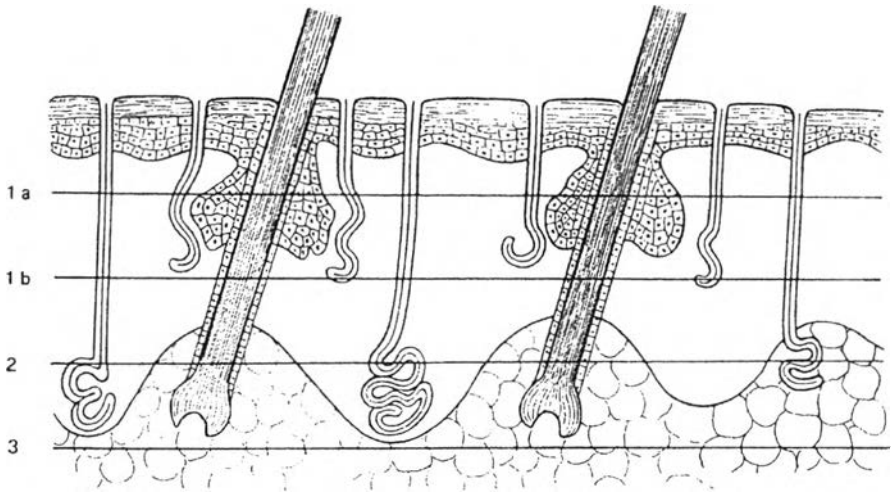

Figure 2.1 Diagram showing the microstructure of the skin and its relationship to the different depths of burning. Level 1A: superficial partial thickness burn, but deep to the sebaceous glands. It passes through hair roots and sweat glands. The dermis-fat interface is not breached. Level 2: deep partial thickness burn passes through hair roots and sweat glands. But breaches the dermis-fat interface and cuts across the fat domes. Level 3: full thickness burn passes deep to all epithelial structures.

At no place does the damage extend through the dermis-subcutaneous tissue interface. From the surviving epithelial structures, epithelium rapidly spreads to provide an intact epithelial surface. From which the superficial dead layers flake off revealing a skin which is elastic, supple and of excellent quality, and which in time may be indistinguishable from normal.

2.1.1.1.2 Deep Partial Thickness Burns

A substantial part of the dermis and all sebaceous glands are destroyed and only the deeper parts of the hair follicles or the sweat glands survive, the relative numbers and depth of these structures varying in different parts of the body. A critical factor is that, because the dermis-subcutaneous interface is not flat, the margin of tissue destruction extends into the subcutaneous tissue at the places where this pushes up into the dermis (the so-called 'fat domes'). This depth of burning can often be recognized at the stage of separation of slough and is sometimes referred to by the alternative name of 'deep dermal burn'.

2.1.1.2 Whole Thickness Burns

When the full thickness of the skin has been destroyed and there are no surviving epithelial elements, the sequence of events is quite different and the position is much more serious. In the absence of infection, the area of destroyed skin becomes dry, hard and black – the characteristic slough of a full thickness burn. In the surviving tissue immediately underneath the slough, cellular and capillary activity produces a layer of granulation tissue, and the enzymatic activity of this layer loosens the slough which finally comes away, exposing the red surface of the granulations. Since the granulations contain no epithelial cells, healing of the area can only occur by in growth of cells from the surviving epithelial edge. Initially this is rapid, but as the epithelium grows further and further away from its original site, it grows more and more slowly until finally it stops altogether and occasionally an ungrafted burn may be seen still unhealed even after 15 or 20 years. While these events are taking place on the surface, changes are also taking place in the granulation tissue. In the deeper layers collagen is laid down. There is reduction in vascularity, and finally the tissue becomes a scar tissue. At the same time, the superficial layers of granulation tissue ooze serum which clots and is itself invaded by cells and capillaries to form fresh granulation tissue. As long as the surface remains unepithelialized there is thus a continuous process of laying down of fresh granulation tissue on the surface, while the deeper layers progressively mature into scar tissue. As soon as the scar tissue is formed, it begins to contract with tremendous force, shrinking to only a fraction of its original size. It is this contraction of scar tissue which it the cause of the deformities of severe burns, and it is the aim of the surgeon to epithelialize these raw areas by skin grafting, and thus to limit and cut short the formation of scar. So long as the area remains raw it is liable to invasion by bacteria with their harmful local and general effects, and finally the raw area is a constant source of loss of protein and red cells, which places a severe drain on the patient's resources.

2.2 Bacterial Infection

In spite of recent improvements in antibacterial substances, bacterial infection of the wound is still the single most important problem of the treatment of these patients. The large raw area with its exudates of serum is like a huge culture plate on which organisms can multiply, little affected by the body defensive mechanisms. Some degree of bacterial contamination of the surface is almost inevitable, but is not necessarily incompatible with satisfactory healing of the burn if the body defenses can match the virulence of the organisms. In other instances, however, either because of great virulence of the organisms, or lowered body resistance, the balance may be upset, and local and general damage may occur.
Infection can cause trouble in the following ways:

(1) Local healing may be delayed.
(2) Viable epithelial cells may be killed, and a partial thickness defect may be converted into a full thickness defect.
(3) The 'take' of grafts may be jeopardized.
(4) Bacterial toxins may be absorbed causing general symptoms.
(5) Bacteria may invade deeper tissues causing cellulitis.
(6) Bacteria may gain entry to the blood stream causing septicaemia.

The following organisms have to be considered such as *Streptococcus β-haemolyticus, Staphylococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa (pyocyanea), Proteus vulgaris, Clostridium tetani, Eschericia coli, Klebsiella* sp.

Fungal pathogens, particularly *Candida albicans, Candida spp.* and other yeasts, become much more frequent in the bacteriological reports of cultures from burns wounds. Tetanus occasionally appears in burns but in our experience this infection has occurred only in patients injured on farms.

2.2.1 Sources and Modes of Infection

Starting with the classical work of Lister, a great deal of information has been accumulated about the modes of conveyance of pathogenic bacteria, and in recent years the work of Colebrook and Lowbury and their colleagues is noteworthy (Colebrook *et al.*, 1945; Colebrook, 1950; Cason and Lowbury, 1960; Lowbury; 1960).

Burns are sterile immediately after infliction, but are liable to be colonized very rapidly by bacteria. They may be infected from the hands or respiratory tracts of first-aiders, and there is a very high risk if patients are wrapped in unsterilized blankets. In hospital practice the majority of organisms originate from other patients in the vicinity. If a patient acquires an organism in his wound, within a short space of time it can be found in his dressings, his clothes and his bed-clothes. From these sites the organisms may be transferred directly through the air to other patients or transmitted indirectly by the hands, arms and clothes of nurses and doctors (Barclay, 1970, 1971). Hospital staff may carry streptococci in their throats, particularly after suffering from 'cold', and they may also harbor staphylococci in their noses. Less commonly the patient's burn may be infected by organisms which originate in his own nose and throat.

2.3 Treatment by Dressings

The purposes of dressing are:

(1) To provide a mechanical barrier to prevent bacteria-carrying particles from alighting on the wound.
(2) To absorb fluid exudates.
(3) To act as a vehicle for antibacterial substances.

Over the years there has been a shift of emphasis from one of these functions to another, and a further change has occurred again recently.

Prior to the introduction of antiseptics by Lister in 1967 the absorption of fluid exudates was considered the most important function of the dressing. The leading exponents of this view's Syme (1837) and Gamgee *et al.* (1876) who maintained that the wound surface produced by an absorptive dressing diminished the chances of the wound becoming septic. Although they were, at that time, ignorant of the bacterial nature of infection, subsequent work has confirmed the correctness of their observations; they must, however, have had failures, since their dressings were not aseptic. When Lister first introduced his antiseptic treatment with carbolic acid, he was much concerned with the maintenance of a high concentration of the antiseptic at the surface of the wound, and in order to achieve this he made repeated

applications of the carbolic acid to the inner dressing. Absorption of wound secretions was considered to be of secondary importance. Unfortunately carbolic acid, and indeed all the early antiseptics, were unselective in their action, and killed normal tissue cells as well as the bacteria. While this might be acceptable at the relatively small surface of a wound due to mechanical trauma, the tissue damage caused over the wide area of a burn was a serious drawback, and the results of antiseptic treatment of burns were disappointing. For this reason there was a swing back to the principles advocated by Gamgee, using a bland inner dressing such a paraffin-impregnated tulle gras covered by a massive absorptive dressing of gauze and cotton wool. Now, however, the dressings were presterilized, thus minimizing the chances of superadded infection. When sulphonamides and later penicillin and other antibiotics became available, these were added to the inner layer of the dressing which was then covered by an absorptive layer, thus apparently combining the advantages of the two methods – absorption and antisepsis. It was thought that the undisturbed dressing formed a good mechanical barrier against infection and that the risk of contamination was great only at the time of change of dressing. Furthermore, it was expected that the antibacterial substance in the inner layer would retain its potency for many days. For these reasons, the dressings were changed infrequently, for example only every 7 days or so. It now seems likely that these views were unduly optimistic. Infection often appeared in burns treated by dressings applied skillfully and under good conditions, and even when the outer layers of the dressings remained dry. During the first two or three days when the burn is in a state of rapid exudation, the antiseptic is so diluted or washed away that it is effective for only 24 or even 12 hours after it has been applied. On the other hand, there is now some evidence that if the antiseptic is changed frequently, even if this does increase the chances of contamination at dressing times, the rate of infection is reduced (Muir *et al.*, 1969). So the requirement, in burns treatment as so often elsewhere, is to choose from a number of different techniques, and to try to select the method which best fits the particular circumstances. In some of these circumstances the absorptive dressing with an inner antiseptic layer changed relatively infrequently will be appropriate. In other cases, however, and particularly with extensive burns being

treated by some of the newer potent antiseptics, a technique which involves frequent changes of the antiseptic, with minimal dressings, is more suitable.

2.3.1 Materials for Dressings

The properties of a satisfactory absorptive dressing must be such that it can absorb an adequate amount of fluid and distribute the fluid evenly throughout its substance so that pooling does not occur. The material which best fulfils these criteria is cotton gauze. Pads made entirely of gauze are the most satisfactory absorbent dressings at present available. These are not prepared commercially and must therefore be 'homemade'. The necessary thickness may be made up by a number of flat layers, but a more satisfactory method is to use a mass of fluffed gauze in a sandwich between two flat layers of gauze in as in the Brooke Army dressing (Davis *et al.*, 1953). Dressings of this type can be prepared beforehand in suitable sizes for individual patients. Because of the high cost of gauze the most commonly used absorptive dressing comprises an inner layer of gauze and an outer layer of cotton wool. Cotton wool is cheaper than gauze but it is not such an efficient absorptive and fluid tends to pool in the dressing rather than be distributed evenly throughout its fabric. Gamgee tissue, which is a sandwich of cotton wool between two sheets of gauze, is useful because of its ease of handling and because it is produced in suitably large size. Its absorptive capacity is that of the wool which composes most of its bulk.

2.3.1.1 Inner Layer

In the early stages the inner layer of dressing will contain an antiseptic. In addition, because of the tendency of dressings to stick to burn wounds, with subsequent pain and tissue trauma when the dressing is removed, it is an obvious advantage if the inner layer is non-adherent. Many commercial preparations are available, most which consist of tulle gras - a wide mesh gauze - impregnated with an antiseptic in a base either of paraffin or a water – miscible cream. These gauzes are acceptably non – adherent as long as they are not allowed to dry out. Alternatively, the antiseptic made up in a suitable cream can be spread by hand on sheets of cotton gauze which are then applied to the wound. Various other materials

such as perforated plastic sheeting or sponge – like foamed plastic have been tried but have not proved generally satisfactory.

2.3.1.2 Outer Layer – Pressure Bandages

When the absorptive dressing has been applied it is fixed in place with a bandage. Crepe bandages, because of the way they conform to the contour of the body, are undoubtedly most satisfactory, but are very expensive. Cotton and conforming bandages (Kling bandage) have some use but are not satisfactory if placed over joints. On the trunk many – tailed bandages are often useful. Some mention should be made here of the place of 'pressure bandaging' and 'pressure dressings'. The notion that the application of firm pressure on a wound is beneficial is of long standing and was stressed, among others, by Gamgee (1876) who stated: 'There must be no constriction – only equable adaption of surface to surface with the light pressure which always comforts. There must be no squeezing like that of an old college friend's hand when seen after long absence; such pressure as that, if continued, is intolerable constriction. The soothing surgical pressure is like that which you interchange with the hand of a lady, the pleasure of whose meeting is tempered by the respectable regard which she inspires.' The bandage should certainly hold the dressing in firm contact with the burn, so as to facilitate absorption of the discharge, and there is no reason to doubt that, in the limbs, sufficient pressure can be exerted to limit the amount of swelling which occurs after a burn and, particularly in the hand, this may be of value in expediting recovery of function. There is, however, no convincing evidence that pressure bandaging can influence the fate of an area of burned skin, nor that it can reduce the amount of oedema sufficiently to influence the development of shock. It should be remembered that over a large part of the body surface (face, neck and trunk), it is virtually impossible to apply even pressure for any length of time by means of bandages. We conclude, therefore, that bandages should indeed be applied so as to exert even pressure whenever possible, but that the merits of 'pressure dressings' have been exaggerated. It is important that the outer layers should be porous to allow evaporation of water, indeed the whole dressing has been described as an 'evaporating dressing. Attempts to prevent fluid coming through the dressing by incorporating an impervious layer resulted in the dressing becoming soggy and encourage bacterial growth. It will also

be apparent that, in circumferential burns, the side of the body or limb which is underneath will tend to become soggy and arrangements must be made to ventilate this as with a burn being treated by exposure. The dressing itself acts as padding and the dressed part can therefore lie on the netting of the frame. Any parts not padded by dressing can be protected by sections of polyurethane foam. Turning frames can also be used to give ventilation of alternate sides.

2.3.2 Hydrogel (Ottenbrite *et al.*, 1996)

Hydrogel is a three-dimensional network of hydrophlilic polymers in which a large amount of water is present. In general, the amount of water is at least 20% of the total weight. If water is composed of more than 95% of the total weight, then the hydrogel is called superabsorbant. The most characteristic property of hydrogel is that it swells in the presence of water and shrink in the absence of water. The extent of swelling is determined by the nature (mainly hydrophilicity) of polymer chains and the cross-linking density. If hydrogel is dried, the swollen network of the hydrogel is collapsed during drying due to the high surface tension of water. Thus, the dried hydrogel becomes much smaller in size than the hydrogel swollen in water. During swelling and shrinking process, hydrogels can preserve its overall shape.

To maintain the three-dimensional structures, polymer chains of hydrogels are usually cross-linked either chemically or physically. In chemical gels polymer chains are connected by covalent bonds, and thus it is difficult to change the shape of chemical gels. On the other hand, polymer chains of physical gels are connected through non-covalent bonds, such as Wan Der Waals interactions, ionic interactions, hydrogen bonding, or hydrophobic interactions. Since the bonding between polymer chains is reversible, physical gels possess sol-gel reversibility. Strong interest in biomedical applications of hydrogels was caused by many papers. Since then the research on hydrogel has been steadily increased. It is not until the end of 1970's, however, when the research on hydrogels began to take off. Hydrogels are commonly used as wound dressing materials, since they are flexible, durable non-antigenic, and permeable to water vapor and metabolites, while securely covering the wound to prevent bacterial infection. Hydrogel was prepared form

synthetic and natural polymeric materials. Natural polymeric materials were biopolymers and they were used for biomedical applications because their have many advantages such as good biocompatibility, biodegradability, minimal inflammatory reaction and non-toxic.

2.3.2.1 Biopolymers

2.3.2.1.1 Gelatin (Keenan, 1994)

Gelatin is a protein obtained by partial hydrolysis of collagen, the chief protein component in skin, bones, hides, and white connective tissues of the animal body. Type A gelatin is produced by acid processing of collagenous raw material; type B is produced by alkaline or lime processing. Because it is obtained from collagen by a controlled partial hydrolysis and does not exist in nature, gelatin is classified as a derived protein. Animal glue and gelatin hydrolysate, sometimes referred to as liquid protein, are products obtained by a more complete hydrolysis of collagen and thus can be considered as containing lower molecular-weight fractions of gelatin. Use of animal glues was first recorded ca 4000 BC in ancient Egypt. Throughout subsequent centuries, glue and crude gelatin extracts with poor organoleptic properties were prepared by boiling bone and hide pieces and allowing the solution to cool and gel. Late in the seventeenth century, the first commercial gelatin manufacturing began. At the beginning of the nineteenth century, commercial production methods were gradually improved to achieve the manufacture of high molecular weight collagen extracts with good quality that form characteristic gelatin gels. Uses of gelatin are based on its combination of properties; reversible gel-to-sol transition of aqueous solution; viscosity of warm aqueous solutions; ability to act as a protective colloid; water permeability; and insolubility to cold water, but complete solubility in hot water. It is also nutritious. These properties are utilized in the food, phramaceutical, and photographic industries. In addition, gelatin forms strong, uniform, clear, moderately flexible coatings which readily swell and absorb water and are ideal for the manufacture of photographic films and pharmaceutical capsules.

a) Chemical Composition and Structure

Gelatin is not a single chemical substance. The main constituents of gelatin are large and complex polypeptide molecules of the same

amino acid composition as parent collagen, covering a broad molecular weight distribution range. In the parent collagen, the 18 different amino acids are arranged in ordered, long chains, each having ~95,000 mol wt. These chains are arranged in a rod-like, triple-helix structure consisting of two identical chains, called α_1, and one slightly different chain called α_2. These chains are partially seperated and broken, i.e., hydrolyzed, in the gelatin manufacturing process. Different grades of gelatin have average molecular weight ranging from ~20,000 to 250,000. Molecular weight distribution studies have been carried out by fraction precipitation with ethanol or 2-propanol and by complexing with anionic detergent molecules. The coacervates are isolated and recovered as gelatin fractions.

Analysis shows the presence of amino acids from 0.2 % tyrosine to 30.5 % glycine. The five most common amino acids are glycine, 26.4-30.5%; proline, 14.8-18%; hydroxyproline, 13.3-14.5 %, glutamic acid, 11.1-11.7 %; and alanine, 8.6-11.3 %. The remaining amino acids in decreasing order are arginine, aspartic acid, lysine, serine, leucine, valine, phenylalanine, threonine, isoleucine, hydroxylysine, histidine, methionine, and tyrosine. Table 2.1 shows name and chemical structure of 18 different kinds of amino acids in gelatin. Warm gelatin solutions are more levorotatory than expected on the basis of the amino acid composition, indicating additional order in the molecule, which probably results from Gly-Pro-Pro and Gly-Pro-Hypro sequences as show in Figure 2.2. The α-chain form of gelatin behaves in solution like a random-coil polymer, whereas the gel form may contain as much as 70 % helical conformation. The remaining molecules in nonhelical conformation link helical regions together to form the gel matrix. Helical regions are thought to contain both inter- and intramolecular associations of chain segments.

Table 2.1 Name and chemical structure of amino acids in gelatin

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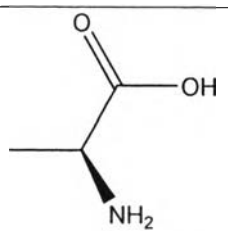
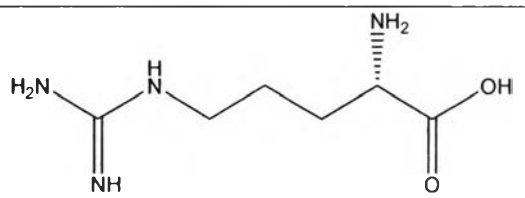
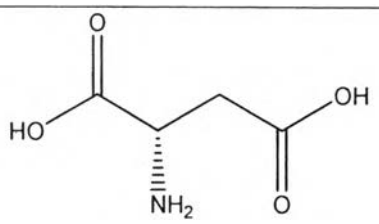
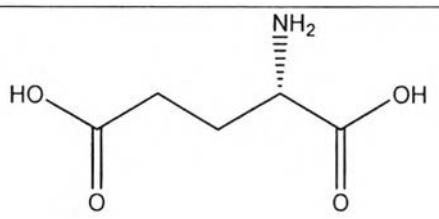
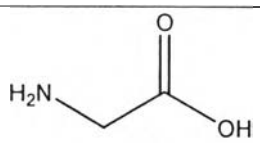
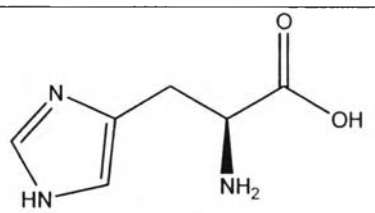
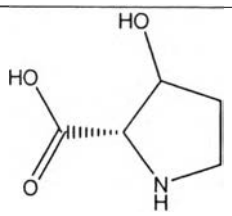
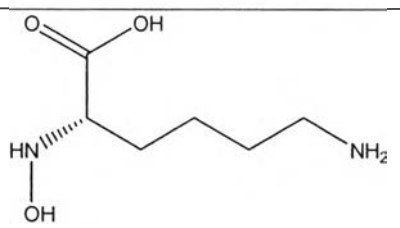
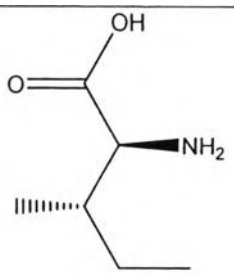
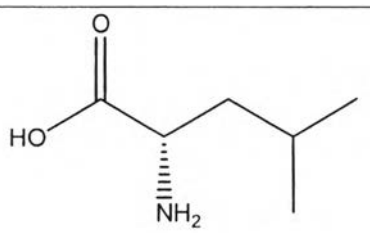
 alanine	 arginine
 aspartic acid	 glutamic acid
 glycine	 histidine
 hydroxyproline	 hydroxylysine
 isoleucine	 leucine

Table 2.1 Name and chemical structure of amino acids in gelatin

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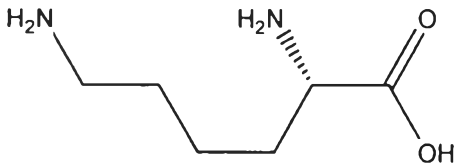
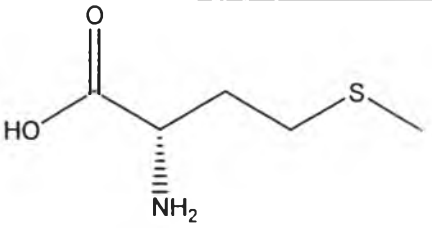
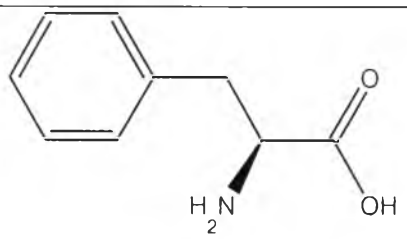
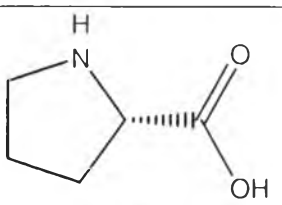
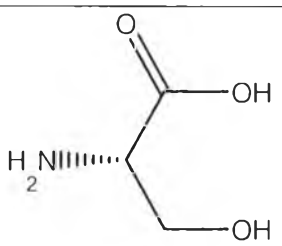
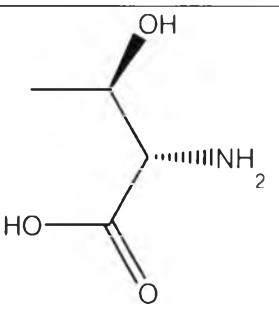
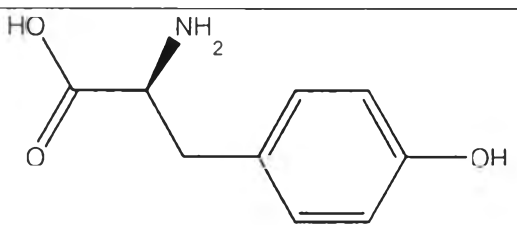
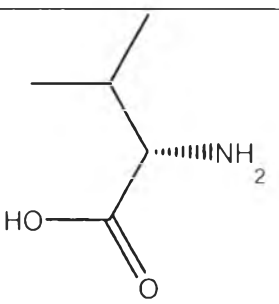
 lysine	 methionine
 phenylalanine	 proline
 serine	 threonine
 tyrosine	 valine

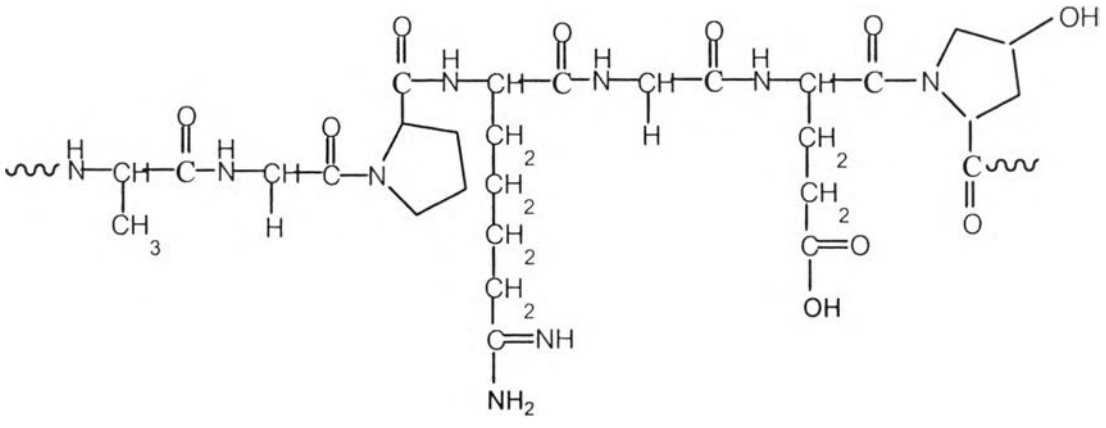


Figure 2.2 The general structure of gelatin is-Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-.

b) Physical and Chemical Properties

Commercial gelatin is produced in mesh sizes ranging from coarse granules to fine powder. In Europe, gelatin is also produced in thin sheets for use in cooking. It is a vitreous, brittle solid, faintly yellow in color. Dry commercial gelatin contains about 9-13 % moisture and is essentially tasteless and odorless with specific gravity between 1.3 and 1.4. Most physical and chemical properties of gelatin are measured on aqueous solution and are functions of the source of collagen, method of manufacture, conditions during extraction and concentration, thermal history, pH, and chemical nature of impurities or additives.

Gelatin perhaps the most useful property of gelatin solution is its capability to form heat reversible gel-sols. When an aqueous solution of gelatin with a concentration greater than about 0.5 % is cooled to about 35 to 40 °C, it first increases in viscosity, then forms a gel. The gelation process is thought to proceed through three stages:

- rearrangement of individual molecular chains into ordered, helical arrangement, or collagen fold;
- association of two or three ordered segments to create crystallites; and
- stabilization of the structure by lateral interchain hydrogen bonding within the helical regions.

The rigidity or jelly strength of the gel depends on the concentration, the intrinsic strength of the gelatin sample, pH, temperature, and additives.

Because the economic value of gelatin is commonly determined by jelly strength, the test procedure for its determination is of great importance. Commercially, gelatin jelly strength is determined by standard tests which measure the force required to depress the surface of a carefully prepared gel by a distance of 4 mm using a flat-bottomed plunger 12.7 mm in diameter. The force applied may be measured in the form of the quantity of fine lead shot required to depress the plunger and is recorded in grams. The measurement is termed the bloom strength after the inventor of the lead shot device. In the early 1990s, sophisticated testing equipment utilizing sensitive load cells for the measurement are commonly used. The conversion temperature for gelatin is determined as setting point, i.e., sol to gel, or melting point, i.e., gel to sol. Commercial gelatins melt between 23 and 30 °C, with the setting point being lower by 2-5 °C. Melting point determination utilizes test tubes filled with gelatin solution that are gently chilled to form a gel. The tubes are tilted and colored carbon tetrachloride solution is placed on the gelatin surface. The tube is gradually warmed and the end point is determined when the descent of the colored solution is observed. Several methods have been used to determine the setting point of gelatin.

2.3.2.1.2 Silk

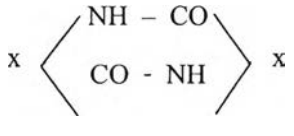
a) Chemical Constitution

The glands of silkworm appear to secrete two transparent liquids. The one : <u>fibroin,</u> constitution from one – half to two – thirds of the entire secretion, forms the interior and larger portion of the silk fiber; the other : <u>sericin</u> ; also called silk – glue, forms the outer coating of the fiber. The latter substances is yellowish in color, and is readily soluble in boiling water, hot soap, and alkaline solutions. The amount of sericin present in raw silk is about 23 % , and this causes the fiber to feel hard and to be stiff and coarse. Before being manufactured into textiles, the raw silk is subjected to several processes with a view to making it soft and glossy. The

first treatment is called degumming, and has for its purpose the removal of the silk – glue. It is really a scouring operation, the silk being worked in a soap solution at a temperature of 205 °F. In this process thrown silk loses from 20 to 30% in weight, but becomes soft and glossy. Alkaline carbonates are not to be recommended for silk scouring, as they are liable to injure the fiber, especially at elevated temperatures. Soft water should also be employed, as lime makes the fibers brittle.

2.3.2.1.3 Fibroin

The fibroin of the silk filament is a protein similar in composition to the sericin gum surrounding it. The silk fibroin is formed by the condensation of α - amino acids into polypeptide chains. It differs from the hair proteins in that it does not contain sulfur : the long chain molecules are not linked together by the disulfide bridge as they are in wool. Some observers suggest the following structural formula for fibroin, allowing x to represent a hydrocarbon residue :



And the decomposition of fibroin by sponification with potash would then be :

Fibroin is composed of about 15 amino acids. There is considerable different of opinion among authorities about the arrangement of the acids ; however, most agree that the simple amino acids such as glycine, alanine, serine, and tyrosine make up the largest part of the fiber. When silk fibroin is hydrolysed by treatment with strong acids, it yields a mixture of amino acids, including :

	percent (grams of amino acid per 100 grams of protein)
glycine	41.2
alanine	33.0
serine	16.0
tyrosine	11.4

a) *Molecular structure of fibroin*

The molecular weight of fibroin is not known with any accuracy. It has been estimated as between 84,000 and 220,000. X – ray analysis of silk fibroin has shown that there is a high degree of crytallinity in the silk filament. The polypeptide chains are able to pack together in such a way that they from regions of regularity typical of a crystal. The atoms of the molecules in this crystalline regions are exerting natural forces of attraction which hold the molecular chains tight against one another. The closely packed and aligned molecule of silk fibroin are associated into regions of high order or crystallinity whilst regions of disorder also occur where the fibroin is amorphous and the molecules are not orientated. Individual fibroin is amorphous and the molecules are not orientated. Individual fibroin molecules may form part of several crystalline regions, and of the amorphous in between. The fully extended nature of the silk molecule and the high degree of orientation accounts for the low elasticity of silk. The molecules cannot unfold like wool molecules when a filament is subjected to a pull. There is only a small amount of distortion before slippage of the molecular chains takes place. The close packing of the silk molecules and the high degree of crystallinity confer great strength on the silk filament. The natural forces of attraction between the molecules can operate with maximum effect.

Motta *et al.* (2004) prepared silk fibroin hydrogels for biomedical applications and studied the *in vitro* cell culture with human osteoblast-like cells. They found that silk fibroin hydrogels were non-toxic and provided the cells to proliferate.

2.4 Antibacterial Substances for Inclusion in the Dressing

It will be remembered that the early wound dressings used by Lister (1907) were antiseptic dressings, and the idea of using inert sterile dressings was a later development.

Immediately after being burned, the surface of the skin is sterile and the only organisms present are those deep in skin glands, which are of low pathogenicity. Some organisms will usually alight on the burn between its occurrence and the application of the first dressing, but it should theoretically be possible to kill these by a suitable antiseptic, and then prevent further infection by a simple sterile dressing. Practical experience, however, shows that under these conditions infection frequently occurs. The presence of discharged serum in the dressing and on the skin surface, the warmth, and lack of light under the dressing all provide an ideal environment for organisms. It needs only a few organisms to gain entrance along the skin surface or through the dressing to result in a substantial infection. If the dressing becomes soaked through to the outside the entrance of organisms is greatly facilitated, and can take place in a very short space of time.

The earlier antiseptics used, such as carbolic acid, mercurial compounds and aniline dyes, had the disadvantage that their action was unselective, causing damage to surviving tissue cells, ad well as to bacteria, and therefore often did as much harm as good.

Sulphonamide was the first substance to have a strong action against bacteria without a toxic effect on tissue cells, and was shown by Colebrook and his colleagues (1945) to be very successful in reducing infection by haemolytic streptococci. When penicillin became available, this was also shown to have a powerful action against streptococci and in addition against staphylococci. Unfortunately, it was not long before many strains of staphylococci appeared which were resistant to the action of penicillin and, as new antibiotics were developed and used, this same problem of resistant strains of staphylococci recurred. The commonly used antibiotics – the penicillins, the tetracyclines and others – had the added disadvantage that they possessed little or no inhibiting action on Gram – negative organisms. A number of antibiotics and antiseptics were produced which

were not suitable for systemic use but were suitable for local application. Most of these – gramicidin, neomycin, bacitracin, polymyxin, framycetin, nitrofuazone, chlorhexidine – had the advantage that organisms did not develop resistance to them. These substances have all been used singly and in various combinations. They have proved useful in the past and many of them are still useful under certain circumstances. However, they all failed to prevent serious infection in extensive burns, and none gave satisfactory protection against *Pseudomonas* which for a long period had been the main source of anxiety in burns units. The search therefore continued for more suitable antiseptics which would be active against Gram – negative organisms as well as against Gram – positive organisms. In 1965, Moyer *et al.* showed that excellent prophylaxis against *Pseudomonas* and other organisms could be achieved b using dressings which were kept continuously wet with a 0.5 per cent solution of silver nitrate. This treatment, however, had certain disadvantages. It was prophylactic only and did not control established infection; there was a loss of electrolytes which required to be made good by electrolyte supplements; some patients complained of discomfort from lying in a continuously wet environment; the silver nitrate caused black staining of the patient, the bedclothes and the nurses.

Sulfamylon (Mafenide), introduced in 1964 (Lindberg *et al.*, 1965), superseded silver nitrate in most centres. Sulfamylon is a sulphonamide derivative which has been known since the early 1940s, but was not then extensively investigated because it was found to be relatively inactive against Gram – positive organisms, particularly streptococci, which at that time were the main cause of trouble. It does, however, have a strong action against *Pseudomonas*, and not only is it effective in prophylaxis, but it also diffuses readily into tissues without loss of potency and is thus effective in the treatment of established infections. Disadvantages are that it sometimes causes severe pain and that it is relatively ineffective against Gram – positive organisms, although experience suggests that this is more obvious *in vitro* than *in vivo*, and in fact staphylococcal infection has not been a serious problem in patients treated with sulfamylon.

Silver sulphadiazine cream was introduced as an antiseptic for application to burns by Fox in 1968, and after nearly two decades of very widespread use no serious side – effects have been reported.

Topical application of gentamicin, a powerful antibiotic effective against both Gram – positive and Gram – negative organisms, has found favour in many centres, but in the authors' view this drug should be reserved exclusively for systemic use.

2.4.1 Silver

Silver has a long history as an antimicrobial agent (Klasen, 2000; Klasen, 2001), especially in the treatment of burns. Silver (as well as copper) is used as a disinfectant in hospital and hotel water sanitization systems. It is used in the food industry in chicken farming and oyster cleaning to inhibit bacterial and fungal growth and in the space program to sterilize recycled water aboard the MIR space station and the NASA space shuttle (Searle, 1919). Although not employed in allopathic mainstream medicine, colloidal silver taken orally has been used as a complementary health aid, the overuse of which in some cases has lead to argyria (also referred to as "blue skin disease," where the reduced silver is deposited in dermal cells. This explains how the term "blue blood" originated). Silver also is used as a preservative in cosmetics and toiletries and has been incorporated into plastics of various forms to protect against microbial contamination.

Different silver delivery systems exist, including those that deliver silver from ionic compounds, such as silver calcium phosphate and silver chloride, and those that deliver silver from metallic compounds, such as nanocrystalline silver (Warriner and Burrell, 2005; Kirsner *et al.*, 2001). However, the difficulties with many current topical silver antimicrobials lie in their low silver release levels, the limited number of silver species released, the lack of penetration, the rapid consumption of silver ions, and the presence of nitrate or cream bases that arempro-inflammatory negatively affecting wound healing. Other issues include staining, electrolyte imbalance, and patient discomfort. Over the past few years, there has been a rapid increase in the number of silver dressings made available to physicians to address these issues (Warriner and Burrell, 2005; Kirsner *et al.*, 2001, Wright *et al.*, 1999).

2.4.1.1 Types of Silver Products

Various available silver products may be summarized as follows (Atiyeh *et al.*, 2007):

2.4.1.1.1 Colloidal Silver Solutions—Electrically Charged

This is the most common delivery system prior to 1960. Charged pure silver particles (3–5 ppm) are held in suspension by small electric currents. Positive ions repel each other thus remain in solution even when applied topically to a wound.

2.4.1.1.2 Silver Proteins

Consist of silver complexed to small proteins in order to improve stability in solution. These however proved to possess much less antibacterial action than pure ionic silver and were rapidly replaced by silver salts in the 1960s.

2.4.1.1.3 Silver Salts

Delivery system becomes more stable when positively charged silver ion is complexed to negatively charged ions (AgCl, $AgNO_3$, and $AgSO_4$). 0.5% Silver nitrate is the standard and most popular silver salt solution used for topical burn wound therapy. Concentrations exceeding 1% silver nitrate are toxic to the tissues. Ionic silver solutions are highly bactericidal, with no reported resistance and have a beneficial effect in decreasing wound surface inflammation. The solutions, however, are unstable and when exposed to light produce typical black stains therefore extremely unpractical. On the other hand, nitrate is toxic to wounds and to cells and appears to decrease healing offsetting to some degree the beneficial antibacterial effect of silver. Moreover, the reduction of nitrate to nitrite causes oxidant induced cell damage. This effect is most likely the reason for the impaired re-epithelialization reported with its use in partial thickness burns or donor sites. Bacterial resistance to $AgNO_3$ has been described.

2.4.1.1.4 Silver Compounds—Silver Sulfadiazine

Silver sulfadiazine (Flammazine®, Silvadene®) was introduced by Fox (1967) in 1970s as an antibacterial agent for topical treatment of substituting a silver atom for a hydrogen atom in the sulphadiazinemolecule and combining the inhibitory action of the silver with the antibacterial effect of sulphadiazine (Stanford *et al.*, 1969; Klasen, 2000,). This silver complex acts on the

bacterial wall in contradistinction to the silver ions which act on the bacterial energy system. All kinds of combinations of sulpha drugs with silver were tested in vitro, but silver sulphadiazine appeared to be the most effective (Stanford *et al.*, 1969). A possible explanation of this effectiveness could be the relatively strong bonding of silver sulphadiazine to DNA (Klasen, 2000) which differs from that of silver nitrate or other silver salts (Fox and Stanford, 1971; Klasen, 2000).

2.4.1.1.5 Sustained Silver Releasing Systems-Nanocrystalline Silver

Various silver-based dressings have been introduced in the past few years and have become the latest and greatest ''innovation'' in wound care products. The ''innovation'' involved in these new wound care products is the simple fact that silver itself is incorporated within the dressing rather than being applied as a separate salt, compound, or solution. The basic issues in choosing a silver-containing dressing can be broadly conceptualized in terms of: (1) the characteristics of the ''carrier'' dressing and (2) the delivery of silver by the dressing to the wound. Keeping these basic issues in mind can help make sense of some of the media marketing blitz accompanying these products (Mooney, 2006). The following list of available silver dressings is not intended to be exhaustive, as the list is growing rapidly. Rather, it should be seen as illustrating various carrier dressing materials used in conjunction with various silver delivery ''reservoirs'' (Mooney, 2006).

- Acticoat-7 (Smith & Nephew, Hull, United Kingdom) dressing consists of three layers of polyethylene mesh coated with nanocrystalline (<20 nm diameter) silver and two layers of rayon polyester. The nanocrystalline silver provides an initial large bolus of silver to the wound followed by a sustained release.
- Actisorb Silver 220 (Johnson & Johnson, New Brunswick, N.J.) is an activated charcoal dressing to which silver is bound. Actisorb works by adsorbing bacteria onto the charcoal component, where they are killed by silver. The ''odoreating'' nature of the charcoal is used as a marketing focus.

- Aquacel-Ag hydrofiber (Convatec, Skillman, N.J.; 70:30 sodium: silver carboxymethylcellulose hydrofiber) is an absorptive dressing. Silver ion is displaced from the carboxymethylcellulose carrier as it is hydrated, thereby achieving a gradual, sustained slow release.
- Arglaes (Medline, Mundelein, Ill.) is silver-impregnated polymer film. The silver reservoir is Ag/CaPo4, formed as glasses co-extruded in a polymer matrix.
- Contreet-H (Coloplast, Marietta, Ga.) is a dense hydrocolloid dressing that has silver bound to the hydrocolloid.
- SilvaSorb (Medline) is a polyacrylate matrix with a silver halide reservoir.
- Silverlon (Argentum LLC, Willowbrook, Ill.) is a polymeric fabric coated with metallic silver by autocatalytic electroless chemical plating. A marketing focus is the three-dimensional fabric, which has a large surface area and is flexible.burns and wounds. Silver is complexed to propyleneglycol, stearyl alcohol, and isopropyl myrislate and mixed with the antibiotic Sulfadiazine producing a combined formulation made from silver nitrate and sodium sulphadiazine by

2.4.2 Silver Nanoparticles

Metal nanoparticles and nanostructured materials are novel classes of materials, which have attracted great attention in catalysis (Lewis, 1993; Daniel, 2004), optics (Hayward, 2000; Ispasoiu *et al.*, 2000), electronics (Poizot *et al.*, 2000; Kiesow *et al.*, 2003) and biomedicine (Daniel, 2004; Geckeler and Rosenberg, 2006) as well as quantum-size domain applications (Wang *et al.*, 2001) due to their unusual physicochemical properties that are quite different from those of the bulk solids. The synthesis of metal nanoparticles is a major research area in nanoscience and technology. Chemical reduction (Lisiecki and Pileni, 1993), co precipitation (Chen *et al.*, 2002), carbon nanotubes (Kim *et al.*, 2006) and polymer protection (Yanagihara *et al.*, 2001; Gao *et al.*, 2004) has been extensively used as the best way to obtain metal nanoparticles with a narrow size distribution.

Nanotechnology has provided a way of producing pure silver nanoparticles. This system also markedly increases the rate of silver ion release (Fan and Bard, 1999). Silver nanoparticle is one of the most effective antimicrobial agents because of the high specific surface or volume fraction so that a large proportion of metal atoms are directly contact with the environment. Silver nanoparticle is an effective antimicrobial agent, is non-toxic to human tissue and can kill a wide range of bacteria. Moreover, it can help in wound healing process.

2.4.3 Release and Mechanism of Silver Ions Against Bacteria

Pal (2002) showed that, in an aqueous medium containing a nucleophile (e.g., $NaBH_4$, SCH^-, and I^-), the dissolution of silver is possible due to the significant decrease in the reduction potential and the redox reaction for silver dissolution can be written as

$$4Ag + O_2 + 2H_2O \leftrightarrow 4Ag^+ + 4OH^-$$

Here, it is postulated that the as-formed elemental Ag^0 dissolved readily upon the contact with the releasing medium and both the remnant and the dissolved Ag^+ ions were released into the medium during the release studies (Figure 2.3).

The increasing number of commercially available silver-based dressings, there is a distinct lack of comparative data on their clinical effectiveness. What is known is that silver can be effective against a wide range of microorganisms, including aerobic, anaerobic, Gram-negative and Gram-positive bacteria, yeast, fungi, and viruses. Elemental silver (Ag^o) appears to have no antibacterial, whereas its cation (Ag^+) is highly reactive (Lansdown, 2002; Brett, 2006; Maillard and Denyer, 2006), particularly at a concentration between 5 and 40 mg/l (Burrell, 2003), and its low concentration component means it retains efficacy even when dilute.

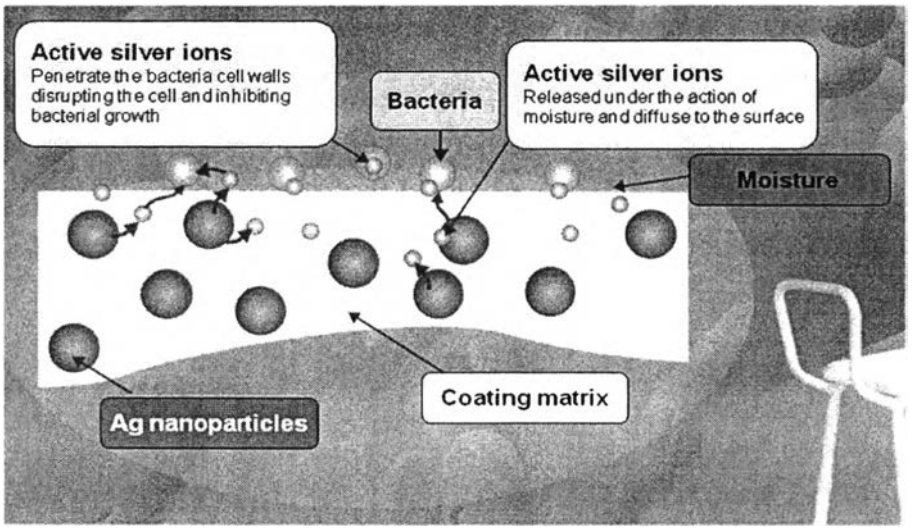


Figure 2.3 Release of active silver ions.

The antimicrobial effect of silver can be explained by various mechanisms:

1. The inhibitory action of silver is due to its strong interaction with thiol groups present in the respiratory enzymes in the bacterial cell (Lansdown (silver 1), 2002; Lansdown (silver 2), 2002). (Unlike antibiotics, silver is toxic to multiple components of bacterial cell metabolism. These include damage to the bacterial cell wall, and membrane permeability leads to gross cellular structural changes, blockage of transport and enzyme systems such as the respiratory cytochromes, alteration of proteins and binding of microbial deoxyribonucleic acid and ribonucleic acid to prevent transcription and division.)

2. Silver has also been shown to interact with structural proteins and preferentially bind with DNA nucleic acid bases to inhibit replication (Lansdown (silver 1), 2002; Lansdown (silver 2), 2002). (Like other antiseptics, silver is soon inactivated by protein binding, but this inactivation can also be caused by tissues and anions such as chloride, phosphate and sulphide.)

For this reason, silver has recently been shown to be highly toxic to keratinocytes and fibroblasts and may delay burn wound healing if applied indiscriminately to debrided healing tissue areas (Cooper, 1990; Lansdown (silver 1), 2002).

Dressings that can sustain release of silver do not need to be changed so often, thereby representing a nursing management time benefit. A reduced number of dressing changes could affect positively a patient's quality of life, particularly in burn management.

Organisms do vary in their susceptibility to silver, but there is good evidence that silver has activity against the common pathogens, *S. aureus* and *Pseudomonas spp.*, which are commonly encountered in chronic wound care. The newer dressings present silver ions differently from silver nitrate and SSD. These include forming unique Ag^{+}/Ag^{o} complexes by the use of nanocrystalline technology, or a high silver availability (Ag^{+}) through other means, to give a large and effective sustained bolus delivery (Wright *et al.*, 1998). Clinical evidence of bacterial resistance to silver ions, involving organisms cultured from chronic wounds, is awaited, but it would be inappropriate to discount that the possibility could occur. Local staining by silver dressings does not appear to be a major complication and is usually temporary. This probably relates to sustained release and high bioavailability, which is furnished by many of the new dressings. Although the level of staining relates to the silver concentration presented by dressings at the wound–skin interface, penetration into the tissues is small. This is more likely with the use of silver nitrate (Walker *et al.*, 2005). Systemic toxicity, argyria, is unlikely as absorption from dressings is so small and probably depends on wound size (Lansdown, 2002). This systemic risk is probably overstated, just as the risk of thyroid disorder is after the use of povidone–iodine in chronic wounds. Nevertheless, argyria may theoretically result when there is a very large open wound and dressings that release large amounts of silver ions are used. There have been no consistent reports of silver allergy, unlike the use of topical antibiotics, such as neomycin, and some other antiseptics.