CHAPTER II

LITERATURE REVIEW

Sericin

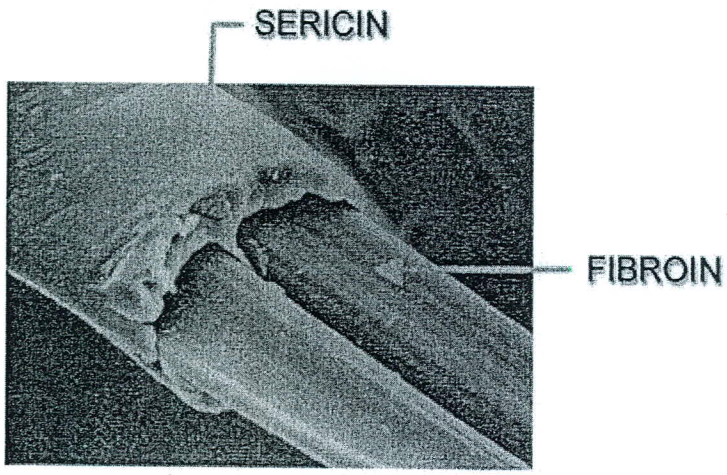


Figure 1 Fibrous core protein fibroin with surrounding sericin

Source: http://www.dermasilk.com.au/content.php?view=HEALTHCARE_PROFESSIONALS

General properties

Silk sericin is a natural water- soluble macromolecule protein from silkworm and composed of 18 kinds of amino acids. Sericin contributes about 20-30% of total cocoon weight and acts as a protein glue to fix fibroin fiber together in cocoon as show in Figure 1 [11]. In presence of sericin, the fibers are hard and tough. It becomes soft and lustrous after its removal [12]. Sericin can be divided into 2 classes: 1) α-sericin and 2) β-sericin. The α- sericin is presented in the outer layer of cocoon shell and β -sericin in the inner layer. The α-sericin contains low amount of C and H but higher amount of N and O comparing to the β-sericin. The solubility of α-sericin in the boiling water is more than that of β -sericin [13].

The specific characteristic of sericin is that the isoelectric point lies further on the acid side than that in the case of any other protein [14]. The isoelectric point of sericin is about pH 4.

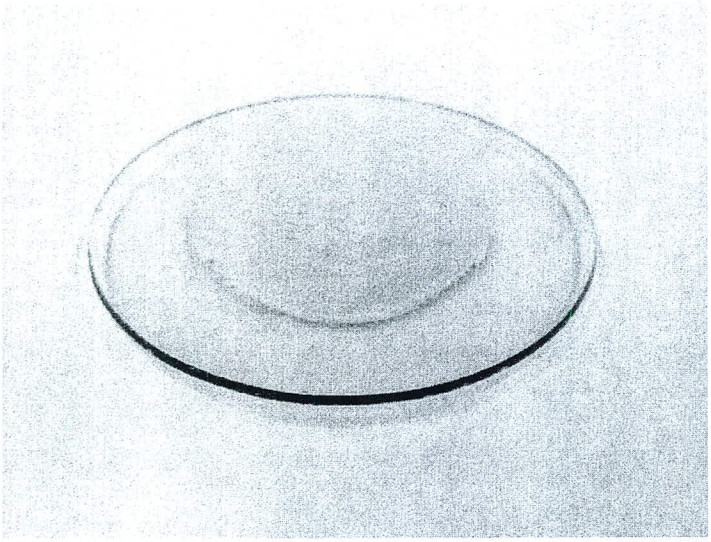


Figure 2 Sericin powder

Sericin used in this study was purchased from Thailand institute of nuclear technology. Specification of the sericin was showed in Table 1.

Table 1 Specification of the sericin

Item	Specification
Molecular weight (kDa)	10 – 250
Solubility at 25 °C	89.71%
Microorganisms	Sterilization with radiation
Stability and storage	1 year in cool place

Composition of sericin

The amino acid composition of sericin is presented in Table 2, It contains a high serine content of about 27.3% of the amino acids. Serine has strongly polar hydroxyl group, and is possibly related to the functional and physiochemical

properties of sericin. In addition, the hydrophilic amino acid amounts up to 70% of the18 kinds of amino acids, which provide good solubility and water absorbability of sericin [15].

Table 2 Amino acid composition of sericin

Amino acid	Percentage of total amino acid (%)
Serine	27.3
Aspertic acid	18.8
Glycine	10.7
Threonine	7.5
Glutamic acid	7.2
Arginine	4.9
Tyrosine	4.6
Alanine	4.3
Valine	3.8
Lysine	2.1
Histidine	1.7
Leucine	1.7
Phenylamine	1.6
Isoleucine	1.3
Proline	1.2
Methionine	0.5
Cysteine	0.3
Tryotophan	0.4
Hydrophilic	70%
Hydrophobic	30%
Aromatic	6.6%

Sericin structure

Seventy percentages of the total amino acid content in sericin is mainly of polar amino acids, especially serine and aspartic acid. Sericin consists of a group of

proteins ranging from 20 to 400 kDa [15]. The structure of sericin depends on preparation method. They are mostly found in random coil and β-sheet structure. Random coil structure was affected by water or organic solvent and changes to β-sheet structure to prevent the dissolution of sericin in water [16].

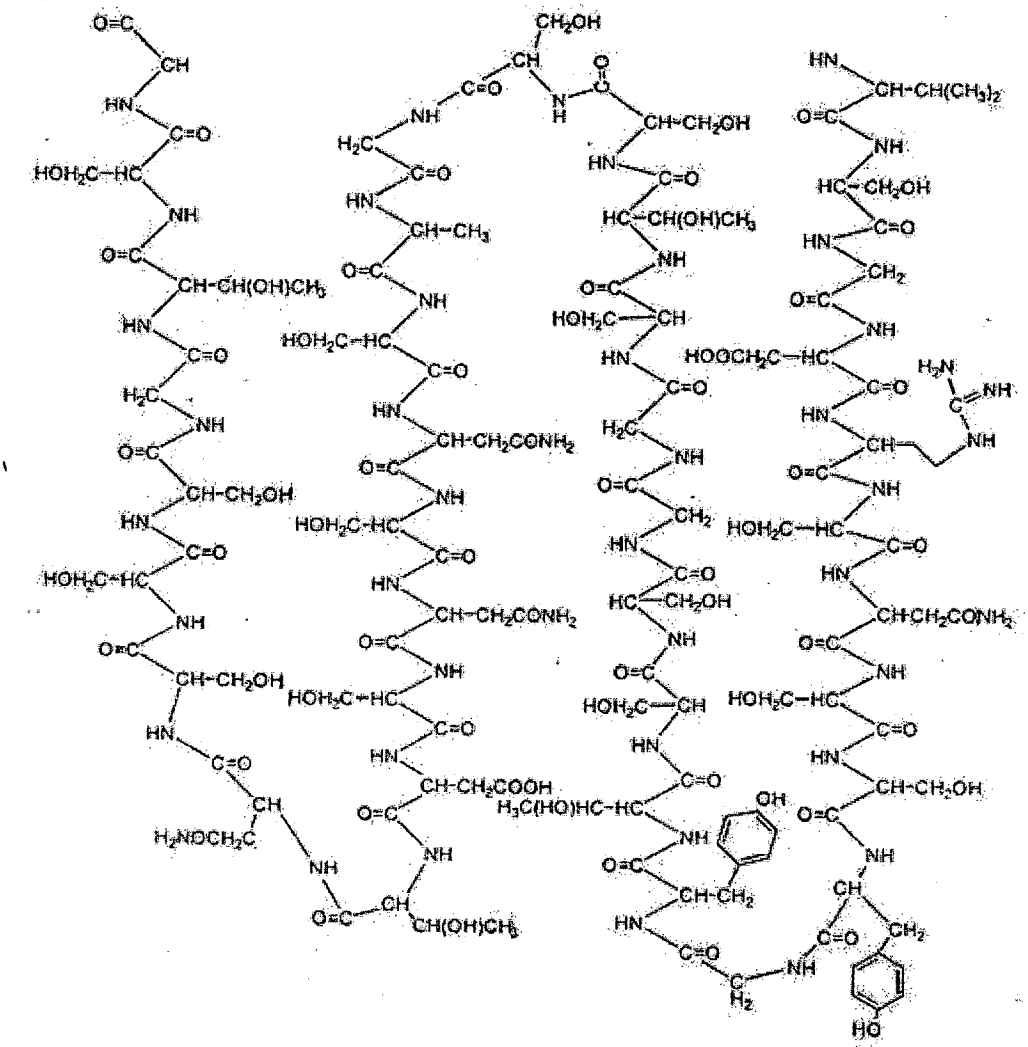


Figure 3 Sericin structure

Application of sericin

Serine-rich sequences in sericin responsible, together with aspartic acid, for its hydrophilicity are sensitive to chemical modifications. Sericin has been studied for various potential applications because of its unique biochemical and biophysical properties.

Cosmetic Application

There are 18 kinds of amino acids in sericin. Serine and aspartate present the highest contents. Besides that, it has other amino acids necessary to human body. About 70% amino acid has hydrophilic lateral group, only 1/3 is serine which water absorption is 50 times higher than that of glycerin. Sericin has excellent moisture absorption ability, therefore it is a good raw material. Sericin protein can form a film on the surface of skin and hair so that the water in skin can be preserved. Then the harm to skin cutin can be avoided. By applying sericin, the skin can be soft and smooth, and the hair can be soft and flexible. It also benefits for the shaping of hair.

Padamwar, et al. have shown the moisturizing property of sericin gel. It decrease skin impedance, increase hydroxyproline level, and hydration of epidermal cells, therefore the moisture are increased, whereas decreasing in the value of TEWL the later may be attributed to occlusive effect, which prevents water loss from the upper layer of the skin. Skin surface topography revealed the smoothness of the upper layer as a result of moisturization [17]. Dash, et al. [18] showed that the sericin obtained from tasar cocoons offers protection against oxidative stress and cell viability was restored to that of control on pre-incubation with the sericin. Fibroblasts pre-incubated with sericin had significantly lower levels of catalase; lactate dehydrogenase and malondialdehyde activity when compared to untreated ones. This report indicates that the silk protein sericin from the tropical silkworm serve as a valuable antioxidant [19]. Sericin also shows the antioxidant property by showing suppression *in vitro* lipid peroxidation [17] and protection against oxidative stress [18].

The soap containing 0.01 to 20% of sericin was reported to help nourish the skin. Therefore, the skin feel smooth and soften when used [20]. Sericin cationic nanoparticles applied to hair care were claimed that they can promote significant increase in hair gloss and volume reduction, along with improving softness and ease combing, abating roughness and repairing damage [21]. The cosmetic for nail was reported provided adding 0.02 to 20% by weight of sericin. The sericin in nail cosmetic was used for suppressing dryness of nails, keeping moisture, making nails glossy, protecting nails and preserving and improving healthiness of nails [22].

Biomedical and tissue engineering

Li, et al. [23] reported the antioxidant effect of sericin. The concentration of alcohol evidently decreased in serum and increased in urine in sericin treated mice as compared to alcohol-administered animals. The results indicated that sericin was able to hasten the alcohol elimination through urine directly and enhance the ethanol oxidation in liver. It showed that sericin may exert a protective effect against lipid peroxidation by scavenging reactive oxygen species and elevating the activity of antioxidant enzymes, in consequence prevented the peroxidative deterioration of structural lipids in membranous organelles, especially mitochondria and karyon [23]. Another report was conducted to assess protective effect of an antioxidant protein, sericin, on UVB-induced acute damage and tumor promotion in mouse skin. The intensity of red color and area of these lesions were inhibited by the topical application of sericin at the dose of 5 mg after UVB treatment. Immunohistochemical analyses showed that the application of sericin significantly suppressed UVB-induced elevations in 4-hydroxynonenal (4-HNE), expression of cyclooxygenase-2 (COX-2) protein, and proliferating cell nuclear antigen (PCNA)-labeling index in the UVB-exposed epidermis. The results suggested that sericin possesses photoprotective effect against UVB-induced acute damage and tumor promotion by reducing oxidative stress, COX-2 and cell proliferation in mouse [24].

Dash, et al. [18] described the potential use of sericin as a biocompatible natural biopolymer in its native form. The membranes were fabricated using sericin. The fabricated membranes were biophysically characterized and optimized for cell culture. The membranes did show robustness, good mechanical strength and high temperature stability. Cytocompatibility of the membranes was evaluated by MTT assay and cell cycle analysis using feline fibroblast cells. Morphology of growing cells was assessed by confocal microscopy that indicated normal spreading and proliferation on the sericin membranes. Nishida, et al. shows successful preparation of sericin gel, sponge and film. In each preparation, the release rate of the large molecular weight and negative charge model drugs were released for the longest period of time (1 week). The results suggested that sericin is usable as an aqueous sustained-release material for high molecular weight drugs and if drugs are charged, their release can likely be sustained for a longer period of time [25].

Silk proteins have been shown to be good candidates for biomedical materials. However, there have been some reports regarding immunological and allergic responses to silk sericin. [26, 27]

Delivery system

Drug delivery systems via sericin-based nanoparticles have been investigated. Several reports show the application of sericin in delivery system. Cho, et al. reported the successful preparation of sericin nanoparticles. The sericin–PEG conjugated was prepared by reacting act-PEG with sericin through hydrophobic interaction. The sizes of nanoparticles measured by dynamic light scattering was ranging about 200–400 nm in diameter. Shape of the particles observed by scanning and transmission electron microscopes was spherical (Figure 4) [6].

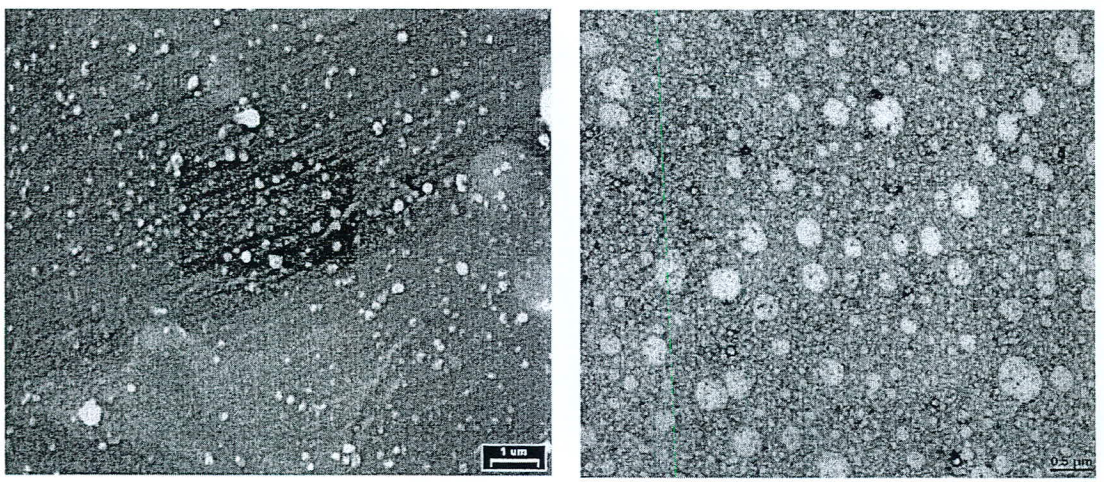


Figure 4 SEM (a) and TEM (b) photographs of sericin–PEG nanoparticles

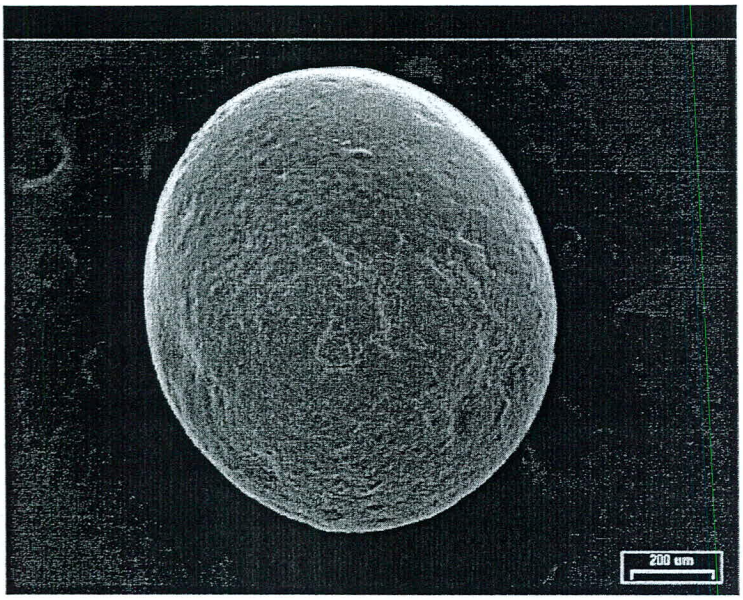


Figure 5 SEM image of sericin bead

Hanjin Oh, et al.[7] prepared sericin beads (Figure 5) using LiCl/DMSO as a solvent for oral administration. The result showed that pH and the presence of an enzyme greatly affected the dissolution rate of sericin beads. Whereas only 10 % of sericin beads were dissolved at pH 2.2 in the presence of pepsin. For more than 45 % of sericin beads were dissolved at pH 7.4 in the presence of trypsin. The release of drug was suppressed in a stomach-like environment but promoted in an intestine-like environment. The release behavior of sericin beads shows potential in oral drug delivery.

Sericin poloxamer nanoparticles [figure 6] were also found to be successful when use as universal drug carriers. The particles were achieved to capable of carrying both hydrophilic (FITC-inulin) and hydrophobic (anticancer drug paclitaxel) drugs (Figure 6). Nanoparticle sizes ranged between 100 and 110 nm in diameter as confirmed by dynamic light scattering. The fabricated sericin/poloxamer nanoparticles were not only stable in aqueous solution and smaller in size but also rapidly taken up by cells to achieve faster and prolonged delivery of drugs to the target site. Cytotoxicity assessment using MCF-7 cells showed both efficiency and efficacy of these drug-loaded nanoparticles when compared with the free drug [5].

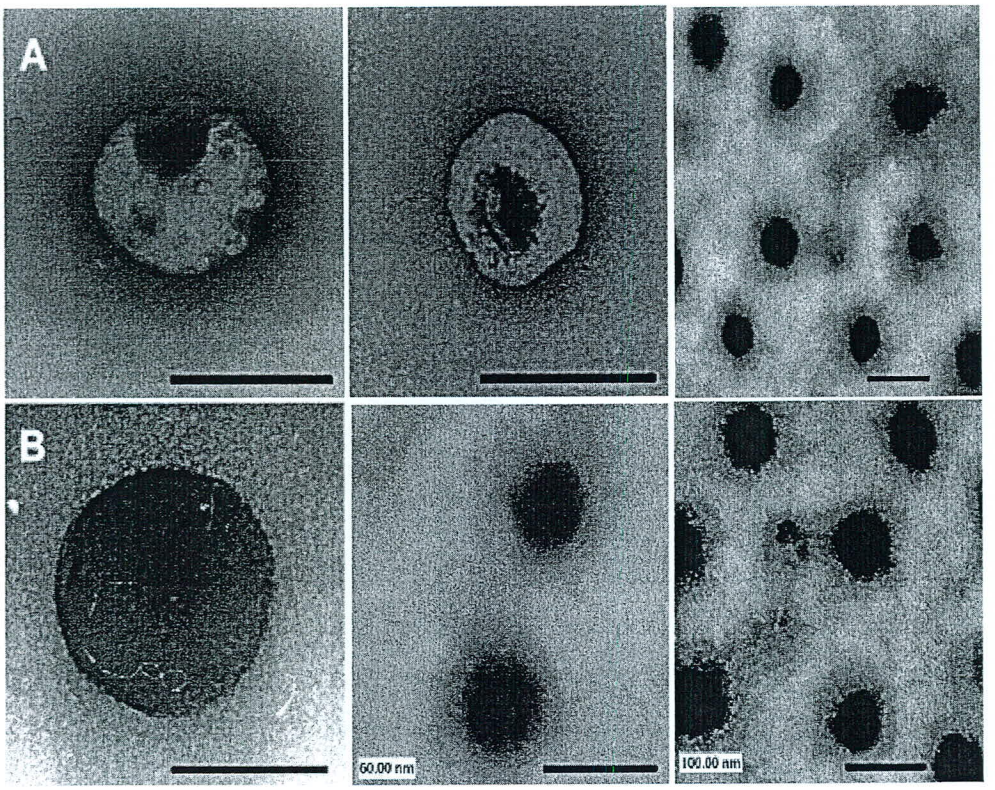
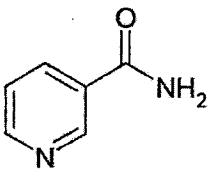


Figure 6 Nanoparticles (A) pluronic F-127 and (B) pluronic F-87

Niacinamide

Niacinamide is one of the water-soluble vitamins. Its chemical property is show in Table 3 [28].

Table 3 Chemical properties of niacinamide

IUPAC name	3-pyridinecarboxamide
Synonyms	nicotinamide, vitamin B3
Formula	$C_6H_6N_2O$
molecular structure	
molecular weight	122.11
p*Ka*	3.35–3.65
log P_{OW}	0.38
melting point	128–131 °C
boiling point	157 °C
flash point	182°C
density	1.4 g/cm3
bulk density	0.50–0.70 g/cm3
vapour pressure	31.4 hPa (at 25 °C)
solubility in water	690 g/L (at 20 °C)

Niacinamide is a moderately hydrophilic compound having an octanol/water partition coefficient of 0.38. At higher concentrations, it is much more hydrophilic due to aggregation in the water phase. The octanol and water solubilities at 30°C are 2.6 and 48.5 wt%, respectively. The low oil solubility and high water solubility of niacinamide make it an extremely sensitive probe of stratum corneum barrier function [28].

The niacinamide permeation

The niacinamide permeation profile shows in figure 7. From day 7 to day 14, the permeability dropped approximately 6-fold. At 2 weeks ex vivo, the permeability was 10-fold lower than on day 14 in vitro, and at 6 weeks ex vivo permeability was 40-fold lower [29].

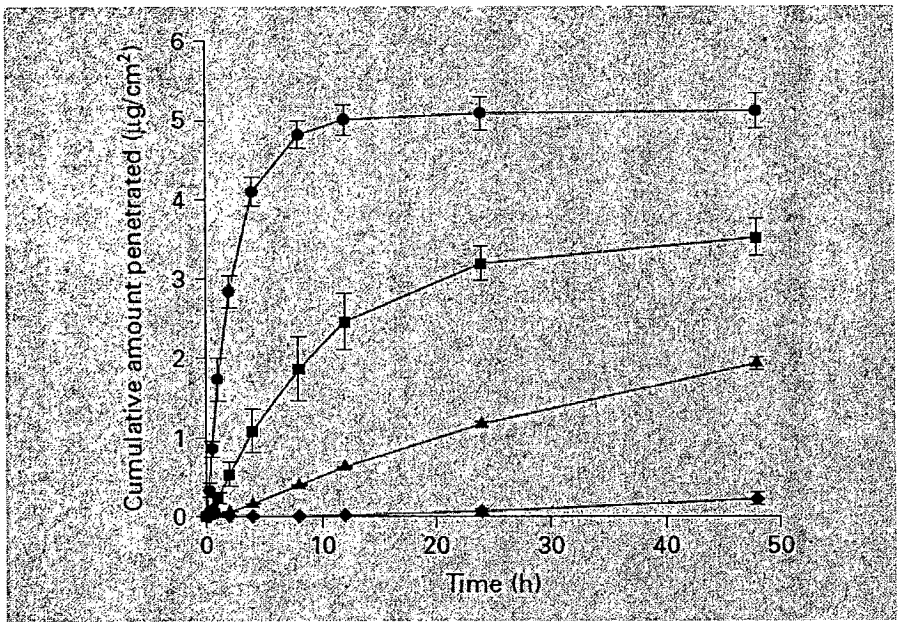


Figure 7 Representative plots of 14 C-niacinamide permeation in vitro and ex vivo. The time points of measurements are day 7 in vitro (●),/day 14 in vitro (■), week 2 ex vivo (▲) and week 6 ex vivo (◆)

Niacinamide is used widely in cosmetic product. The 4% niacinamide cream induced a decrease in pigmentation, inflammatory infiltrate, and solar elastosis [8]. Topical niacinamide lotion also showed skin improvement by reduction in fine lines and winkles, hyperpigmented spots, red blotchiness and skin sallowness [9].

Skin and transdermal drug delivery system

In past, the most commonly applied systems for dermatological disorders were topically applied creams and ointments. Drugs administered in the form of tablets, capsules, injection and ointment etc., usually produce wide ranging fluctuation in drug concentrations in the blood stream and tissues. Factors such as repetitive dosing and unpredictable absorption lead to the concept of controlled drug delivery system or therapeutic system. Transdermal systems can provide drug systemically at a predictable rate and maintain for extended periods of time thus eliminating numerous problems associated with oral products such as unpredictable or reduced bioavailability [30]

The skin

The skin is the largest organ in mammals and serves as a protective barrier at the interface between the human body and the surrounding environment. It guards the underlying organs and protects the body against pathogens and microorganisms [31]. There are three structural layers to the skin (Figure 8), the epidermis, the dermis and subcutis. Hair, nails, sebaceous, sweat and apocrine glands are regarded as derivatives of skin.

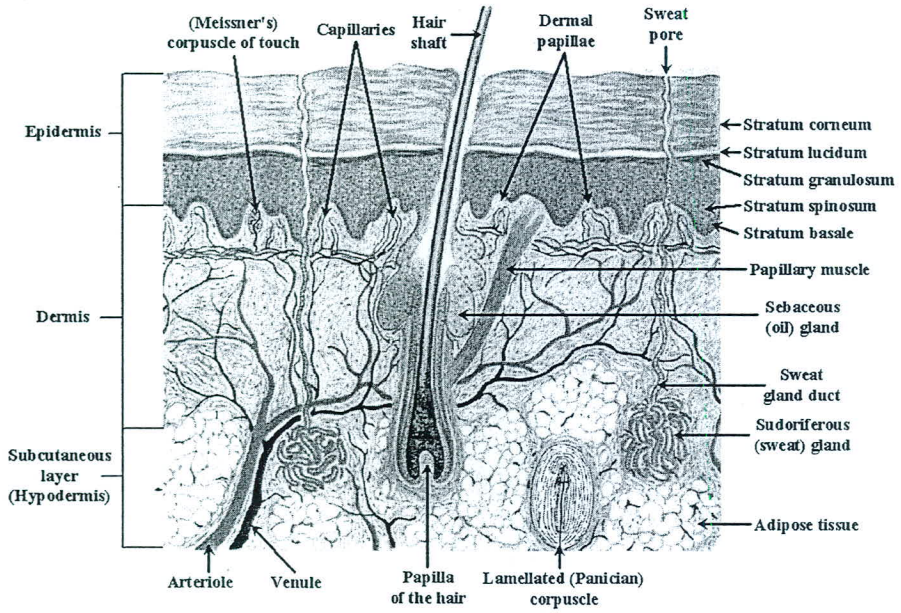


Figure 8 The anatomically skin structure

Source: http://healthfavo.com/labeled-skin-diagrams.html

Epidermis is a terminally differentiated stratified squamous epithelium, the major cell type of which is the keratinocyte. Keratinocytes synthesize keratin, a protein containing coiled polypeptide chains which combine to form supercoils of several polypeptides linked by disulphide bonds between adjacent cysteine amino acids. Keratinocytes also produce cytokines in response to injury [32].

The five layers of the epidermis are shown in Figure 9

1. Stratum corneum
2. Stratum lucidum

3. Stratum granulosum
4. Stratum spinosum
5. Stratum basale

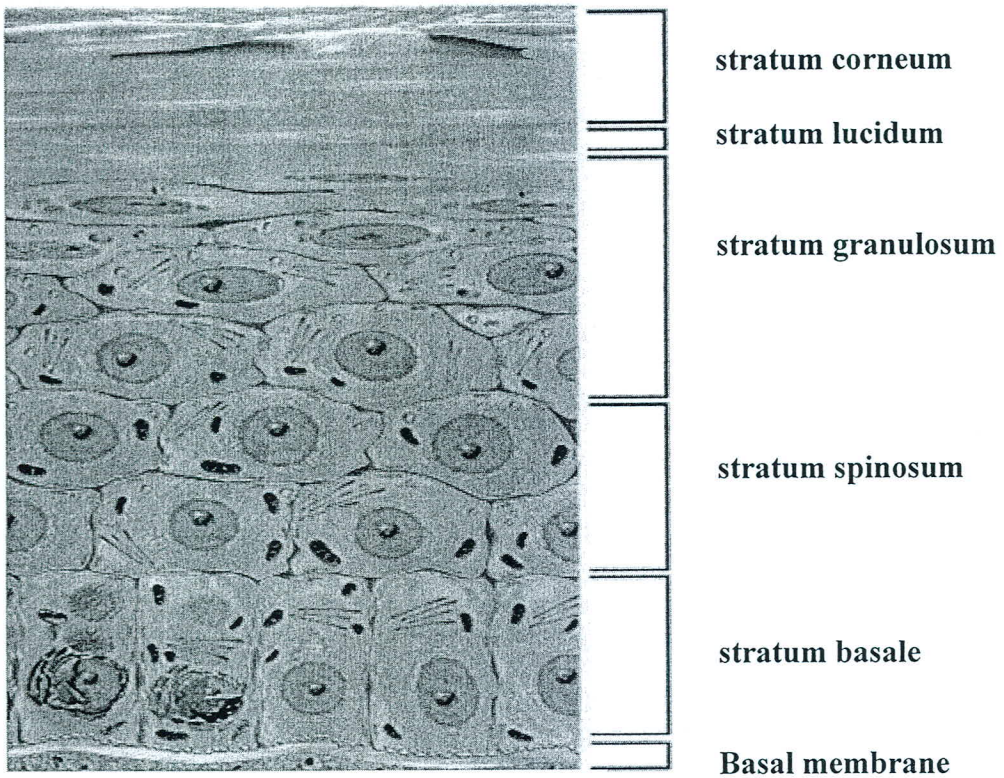


Figure 9 Five layers of epidermis

Source: http://www.eucerin.com/th/skin-expertise/about-the-skin/the-skin-and-its-cells/the-epidermis/

Dermis lies below the epidermis and supports it structurally and nutritionally. Its major component is fibroblasts and the collagen they produce. Collagen is a triple helix comprising three polypeptide chains. Fibres are packed in bundles and give enormous tensile strength. The dermis also contains elastin fibres that provide stretch. The fibres sit within a matrix of amorphous mucopolysaccharide ground substance. This binds water to facilitate passage of nutrients and other chemicals, acts as a lubricant to allow skin movement and provides bulk to aid shock absorption [33].

Barrier properties of the skin

Of the two component parts of the skin, it is the epidermis that provides protection from the environment. The dermis is very permeable once the epidermis is removed, a fact amply demonstrated in partial-thickness burns which readily soak overlying dressings with protein-rich exudates and serve as a focus for invasive infection [34]. The stratum corneum consists of a sheet of corneocytes embedded in an intercellular lipid matrix and is the primary barrier against pathogen entry. It is also largely responsible for the regulation of water loss from the body (transepidermal water loss). The epidermis comprises the physical, the chemical/biochemical (antimicrobial, innate immunity) and the adaptive immunological barriers. The physical barrier consists mainly of the stratum corneum, but the nucleated epidermis, in particular the cell–cell junctions and associated cytoskeletal proteins. The chemical/biochemical (antimicrobial, innate immunity) barrier consists of lipids, acids, hydrolytic enzymes, antimicrobial peptides and macrophages (Figure 10). The (adaptive) immunological barrier is composed of humoral and cellular constituents of the immune system [35].

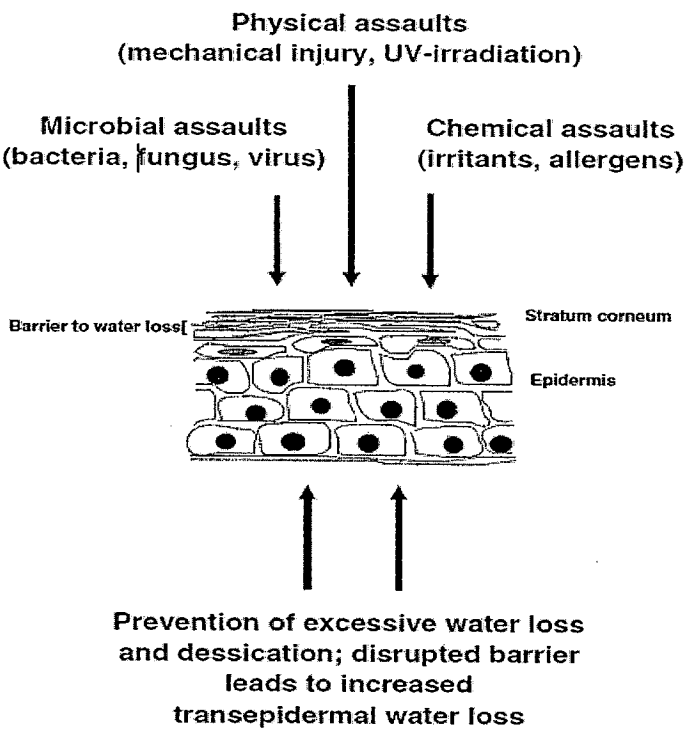


Figure 10 Functions of the epidermal 'inside-outside' and 'outside-inside' barrier

Transdermal delivery system

Delivery across skin offers many advantages compared to oral or intravenous routes of drug administration [36]. Transdermal delivery systems utilize the skin for the delivery of drug molecules from the surface of the skin, through its layers, to the circulatory system. Transdermal systems are noninvasive alternative to drug delivery through injection or by mouth. Transdermal drug delivery is the administration of a therapeutic agent through intact skin for systemic effect. Transdermal drug delivery offers the following advantages over the oral route for controlled drug delivery [37].

1. Avoidance of hepatic first pass metabolism.
2. Ability to discontinue administration by removal of the system.
3. The ability to control drug delivery for a longer time than the usual gastrointestinal transit of oral dosage form.
4. The ability to modify the properties.
5. Increased convenience to administer drugs which would otherwise require frequent dosing
6. Reduced side effects and improved therapy due to maintenance of plasma levels up to the end of the dosing interval

Route of penetration

Skin provides an alternative route for drug administration (Figure 11), allowing sustained drug delivery to the blood circulatory system and providing greater comfort for the patient, while avoiding several side effects of oral and parenteral administration.

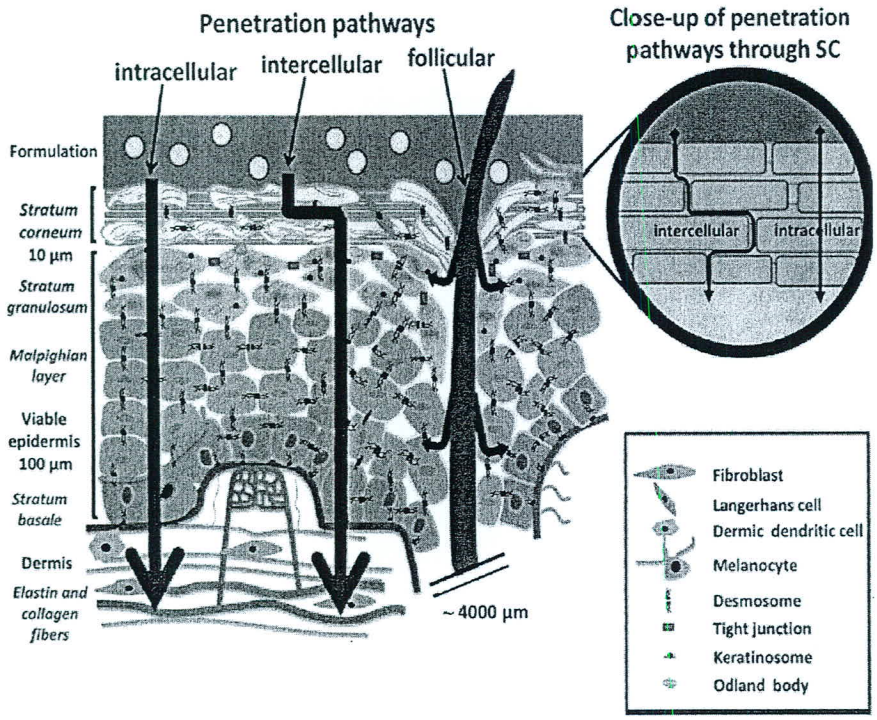


Figure 11 Sketch of the three penetration pathways: intracellular, intercellular and follicular. The upper right inset is a close-up of the *stratum corneum* showing the intracellular pathway and the tortuous intercellular pathway [38]

Intercellular pathway

Penetration between stratum corneum corneocytes is the pathway by which most compounds penetrate the skin. Since corneocytes are not stacked parallel to one another in the layers, when penetrating between them, a compound has a sinuous way to pass. This pathway is considered to enable free volume diffusion through lipid bilayers present between the cells. [39] This route is a significant obstacle for two reasons. Firstly, recalling the 'bricks and mortar model of the stratum corneum, the interdigitating nature of the corneocytes yields a tortuous pathway for intercellular drug permeation, which is in contrast to the relatively direct path of the transcellular route. It has been estimated that water has 50 times further to travel by the intercellular pathway, than the direct thickness of the horny layer. Secondly, the intercellular domain is a region of alternating structured bilayers. Consequently, a drug must

sequentially partition into, and diffuse through repeated aqueous and lipid domains. This route is generally accepted as the most common path for small uncharged molecules penetrating the skin [40].

Intracellular pathway

Drugs entering the skin *via* the transcellular route pass through corneocytes. Corneocytes, containing highly hydrated keratin, provide an aqueous environment for which hydrophilic drugs can pass. The cells are surrounded by a lipid envelope which connects the cells to the interstitial lipids. Separating keratinised skin cells are multiple lipid bilayers; there are estimated to be up to 20 such lamellae between each corneocyte. The physicochemical properties of the permeant will strongly influence whether the transcellular pathway is the predominate route taken. In particular, the relative ability to partition in and out of each skin phase is important. The transcellular pathway is, however, thought to be the predominant pathway for highly hydrophilic drugs during steady-state flux [41].

Follicular pathway

Transport of hydrophilic or charged molecules is especially difficult attributable to the lipid-rich nature of the stratum corneum and its low water content; this layer is composed of about 40% lipids, 40% protein, and only 20% water. Transport of lipophilic drug molecules is facilitated by their dissolution into intercellular lipids around the cells of the stratum corneum. Absorption of hydrophilic molecules into skin can occur through 'pores' or openings of the hair follicles and sebaceous glands, but the relative surface area of these openings is barely 0.1% of the total skin surface. However, these calculations did not take into the account that the hair follicles represent invaginations, which extend deep into the dermis with a significant increase in the actual surface area available for penetration. [41]

The hair follicles are surrounded by a dense network of blood capillaries, which is essential for drug delivery and ensures the systemic uptake. In addition, hair follicles are targets for regenerative medicine and immunomodulation (Figure 12), being the host of stem cells, surrounded by a high density of dendritic cells. Particles, at a size of some hundreds of nanometers, were shown to penetrate deeply into the hair follicles, where they are stored up to 10 days, which is 10 times longer than in the stratum corneum [42].

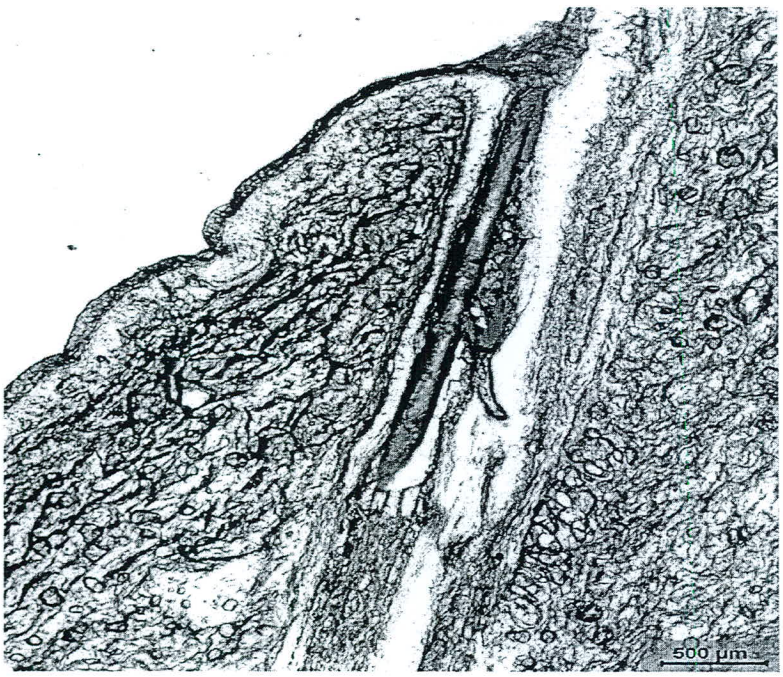


Figure 12 Follicular penetration; histological section demonstrating the penetration of fluorescence-labeled particles into a hair follicle