

## TARGETING HAIR FOLLICLES - AN EXQUISITE REVIEW

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### ABSTRACT

The pilosebaceous unit is a complex structure that undergoes a specific growth cycle and comprises a few important drug targeting sites. For example, drugs can be targeted to the bulge region with stem cells or to the sebaceous glands. Interest in pilosebaceous units is directed towards their utilization as reservoirs for localized therapy and also as a transport pathway for systemic drug delivery. Improved investigative methods, such as differential stripping, are being developed in order to determine follicular penetration. This article reviews relevant aspects of effective follicle-targeting formulations and delivery systems as well as the activity status of hair follicles, and variations in follicle size and distribution throughout various body regions. Each of these factors strongly affects follicular permeation. We provide examples of improved penetration of particle-based formulations and of a size-dependent manner of follicular penetration. Contradictions are also discussed, indicating the need for detailed future investigations.

**Keywords:** Hair, Follicle, Pilosebaceous, Penetration, Transdermal, Targeting.

### INTRODUCTION

The transdermal pathway of drug application outweighs other methods of administration, e.g., oral or parenteral, because the active substance avoids the hepatic first-pass effect and variances in the gastrointestinal tract that may influence absorption. In addition, percutaneous application minimizes the risk of irritation of gastrointestinal tract by a drug, and eliminates pain and other complications of parenteral administration. The skin barrier, more precisely the highly lipophilic stratum corneum of the epidermis, significantly limits the skin permeation of exogenous substances including drug substances. Thus, various approaches, such as methods related to the modification of drug molecules, the properties of the stratum corneum, and electrically supported methods, as well as bypassing or removing the stratum corneum, are undertaken to weaken or avoid the skin barrier and enable percutaneous absorption of drug substances<sup>1-5</sup>. Recently, the microneedle method for bypassing the stratum corneum has been extensively reviewed by Arora et al.<sup>6</sup>. When considering the skin structure, one can perceive that hair follicles are connected with a network of blood

capillaries and that below the entrance of the sebaceous gland duct to the hair canal there is no mature stratum corneum. Thus, the molecules that penetrate the hair follicles can get to the tissue surrounding the follicle and reach the blood circulation through the dense network of blood capillaries, thereby avoiding the stratum corneum barrier. For example, it was shown that when caffeine was applied in a shampoo formulation, the caffeine was detected in the blood 5 min after application<sup>7</sup>. It seems that hair follicles, as well as sweat glands and microlesions in the interfollicular horny layer, are an ideal target for drug delivery and may represent an alternative to the intercellular route of skin permeation.

Determination of the penetration into and the permeation through the hair follicles is possible, whereas permeation through the sweat glands and microlesions is still difficult to investigate and very rarely studied. The hair follicles can serve not only as a major entry point but also as a reservoir for dermally applied substances<sup>7-9</sup>. This article reviews relevant aspects of effective follicle-targeting formulations and delivery systems as well as the activity status of hair follicles, and variations in follicle size and

distribution throughout various body regions. The review is addressed to the dermatologists and other physicians interested not only in skin diseases, but also in investigating the mechanisms of skin barrier permeation. In addition, the pharmaceutical technologists studying dermal/transdermal drug forms may find this review useful.

### Types of hair follicles

The androgen-independent hair (eyebrows, lashes) and hair on hormone-dependent body regions (scalp, beard, chest, axilla, pubic region) consist of terminal hair shafts, which are long (>2 cm), thick (>60  $\mu\text{m}$  in diameter), pigmented and medullated<sup>10,11</sup>. The medulla is located in the large terminal hair fibers, but most scalp hair is not medullated<sup>12</sup>. Terminal hair usually extends more than 3 mm into the hypodermis. The rest of the body in adults is covered with vellus hair – short (<2 cm), thin (<30  $\mu\text{m}$  in diameter), often unpigmented and extending just 1 mm into the dermis. Some hair follicles can exist in a transitional phase between terminal and vellus forms<sup>10,13–15</sup>. The only skin regions devoid of hair follicles are the palms, soles of the feet, lips, and portions of the external genitalia<sup>12,16</sup>. In the scalp, the hair follicles are typically arranged in the follicular unit composed of 1–4 terminal hairs and 1–2 vellus hairs, encircled by the same arrector pili muscle<sup>13,14</sup>.

### Size and distribution of the hair follicles

Historically, follicular penetration was disregarded, mainly because it was assumed that hair follicles occupy less than 0.1% of the total skin area. Recent studies indicate that this is true for the inner side of the forearm, which is commonly used as an investigational area for skin permeation. However, there are significant variations across body regions in hair follicle density, size of follicular orifices, hair shaft diameter, as well as volume and surface of the infundibula. Knowledge of hair follicle density and size is necessary to calculate follicular penetration.

Hair shaft diameters show relatively little variation (16–42  $\mu\text{m}$ ); the highest shaft diameter is observed in the sural (42  $\mu\text{m}$ ) and thigh (29  $\mu\text{m}$ ) regions, with the lowest on the forehead (16  $\mu\text{m}$ )<sup>17</sup>. The highest average hair follicle density is found on the forehead (292 follicles/cm<sup>2</sup>). The highest follicular infundibula volume, which is interpreted as a potential follicular reservoir for dermally applied substances, is on the forehead (0.19 mm<sup>3</sup>/cm<sup>2</sup>) as well as in the sural region (0.18 mm<sup>3</sup>/cm<sup>2</sup>). The follicular reservoir on the forehead is comparable to the reservoir of the

stratum corneum, assuming that all follicles are open for the penetration process<sup>17</sup>. For the scalp and face, the combined areas of follicular openings can be as much as 10% of the total skin area<sup>10,18</sup>.

### Hair follicle structure and drug targeting sites

The term pilosebaceous unit describes the integrated structure of the hair follicle, hair shaft, adjoining arrector pili muscle and associated sebaceous gland(s). The hair shaft is composed of the medulla, the cortex with melanosomes, and the cuticle, represented by flat cornified cells arranged similarly to roof tiles<sup>13,14</sup>. Figs. 1 and 2 demonstrate the structure of the human hair follicle. It can be divided in the following manner

- The infundibulum (the part between the skin surface and the point of the sebaceous gland duct opening to the hair canal);
- The isthmus (between the sebaceous gland duct opening and the bulge region);
- The suprabulbar zone, where various layers of anagen follicles begin to differentiate and can be easily identified at this level;
- The hair bulb with the dermal papilla connected to the blood vessels.

The outer root sheath that surrounds the hair follicle is a stratified epithelium that is continuous with the epidermis<sup>14</sup>. The superficial part of the hair follicle infundibulum (acroinfundibulum) is lined by epidermis including a well-developed stratum corneum and a stratum granulosum layer. The lower part of the infundibulum, called the infrainfundibulum, may experience a continuous loss of epidermal differentiation occurring towards the isthmus and creating the major entry point for applied substances<sup>7,9,19</sup>.

The sebaceous glands also represent an important therapeutic target site as they are involved in the etiology of acne and also androgenetic alopecia, the latter by expressing 5 $\alpha$ -reductase (especially in face and scalp regions), which converts testosterone to more potent 5 $\alpha$ -dihydrotestosterone<sup>12,13</sup>. The sebaceous glands produce sebum – a fungistatic and bacteriostatic mixture of short chain fatty acids – a lubricant for the hair and skin that repels water and microorganisms. In addition, an extensive capillary network associated with the upper dermal vasculature supplies the upper follicle and sebaceous glands with blood, creating the possibility of systemic drug delivery. Another attractive targeting zone is the bulge region, responsible for follicle reconstitution. It contains stem cells with a high proliferative capacity and multipotency. These

cells are the target for gene delivery to facilitate long-term gene correction of congenital hair disorders or genetic skin disorders. The hair bulb region, including hair matrix cells, controls hair growth and pigmentation<sup>10,13,16,20</sup>.

### Hair cycle

Hair follicles undergo a growth cycle comprising three major phases: anagen (growth phase – cells proliferate rapidly and continuously to form the inner root sheath, and migrate upward to form the hair shaft), catagen (involution – the end of mitosis, reabsorption and cell death of the lower follicle segment) and telogen (resting phase prior to the hair being shed)<sup>13</sup>. Recently, the two other stages of the hair cycle have been described: exogen (release of telogen fibers from hair follicles) and kenogen (lag time between exogen and new anagen fiber development)<sup>21</sup>.

Usually, 85–90% of scalp follicles are within the anagen phase (lasting 2–6 years), 1–2% of scalp follicles represent the catagen phase (2 weeks) and about 10% are in the telogen phase (2–4 months)<sup>15,21</sup>. The rate of scalp hair shaft elongation is between 0.3 and 0.4 mm per day, but some authors quote 0.5 mm per day<sup>15,22</sup>. The rate depends on the proliferation and subsequent differentiation of the matrix keratinocytes in the hair bulb. The thickness of the hair shaft relates to the size of the hair bulb<sup>15,21</sup>.

The majority of hair growth disorders result from changes in the hair cycle. Androgenetic alopecia is caused by a shortening of the anagen stage, with the clinical consequence of increased hair loss, accompanied by a transformation of terminal to vellus hair follicles (miniaturization). On the other hand, a prolonged anagen phase with conversion of vellus hair follicles into terminal can be observed during hypertrichosis and hirsutism<sup>15,23</sup>.

### Techniques for investigating follicular penetration

In the search for satisfactory experimental models to estimate the importance of the transfollicular route, different animal models were used. In guinea pigs, hairy skin regions were compared to non-hairy regions. Healthy rodents' skin was compared to scarred skin without appendages<sup>8,9</sup>. Barry<sup>24</sup> introduced the skin sandwich model consisting of human stratum corneum and epidermis (with its own stratum corneum). In this model, the membranes come from the same human skin sample and the top stratum corneum blocks all the shunts in the bottom epidermis.

In order to distinguish between transepidermal and transfollicular permeation, a method to block the follicles selectively with an avarnish–wax mixture was developed. It was used to determine the follicular penetration of chemical and physical UV filters and curcumin<sup>25</sup>, as well as the in vivo follicular penetration of caffeine applied in a shampoo formulation<sup>7</sup>. Differential stripping allows us to selectively determine the amount of topically applied substance that penetrates into the hair follicles. The method combines the tape-stripping technique (removing the stratum corneum layer by layer), followed by cyanoacrylate skin surface biopsies (removing the content of the follicular infundibulum, the “follicular cast” consisting of a mixture of keratinized material, cell detritus, lipids and bacteria)<sup>17,26–28</sup>.

Confocal laser scanning microscopy (CLSM) is a non-invasive method that was used to visualize skin samples at multiple depths parallel to the sample surface<sup>13</sup>. On-line CLSM is a recently developed tool to visualize the diffusion of a model dye in a cross-sectional view of fresh unfixed skin with subcutaneous fat. It enables the investigator to observe the diffusion of a dye into the upper part of hair follicles in real time and depth<sup>29</sup>.

### Follicular drug delivery

Not all hair follicles are accessible for penetration. It was found that substances penetrated only into the “active” (open) hair follicles that are characterized by hair growth and/or sebum production. This can be explained by the fact that plugs from shed corneocytes and dry sebum, which close some of the hair follicles, are pushed out by growing hair or flowing sebum. “Inactive” (closed) hair follicles exhibit neither growth nor sebum production<sup>8,30</sup>. Studies revealed the significant role of mechanical peeling of the skin, which is able to open closed follicles for the penetration process<sup>8</sup>. Furthermore, pretreatment of the skin with cyanoacrylate skin surface stripping (CSSS) removes the superficial part of the stratum corneum with cellular debris and sebum from the follicular openings, facilitating penetration. One CSSS removes approximately 30% of the stratum corneum; the remaining stratum corneum and the viable interfollicular epidermis are left intact<sup>31</sup>. To perform CSSS, a drop of cyanoacrylate adhesive is placed on the surface of the treated skin and covered with a glass slide under slight pressure. After polymerization (approximately 2 min), the cyanoacrylate is strongly linked with the upper layers of the

stratum corneum, the hair shafts, and the casts of follicular infundibula<sup>27</sup>.

Examples of active substances examined for their follicular penetration include: minoxidil<sup>32-34</sup>, plasmid DNA encoding IL-1ra<sup>34</sup>; finasteride, cimetidine, melanin<sup>35,36</sup>; anti-androgen RU58841-myristate<sup>37</sup>, hydrocortisone, testosterone<sup>36,38</sup>; titanium dioxide<sup>8,25</sup>; chemical UV filter (Eusolex 6300)<sup>25</sup>; curcumin<sup>25,39</sup>; inulin<sup>40</sup>; caffeine, niflumonic acid, paminobenzoic acid<sup>7,9</sup>; brilliant green<sup>26</sup>, podophyllotoxin<sup>30</sup>, estradiol, corticosterone, aldosterone, deoxyadenosine, adenosine<sup>36</sup>, a-interferon, cyclosporin-A<sup>41</sup>.

Many studies suggest that the follicular pathway, in contrast to the conventional transdermal pathway, is especially favorable for highly hydrophilic and high-molecular weight substances<sup>42</sup>, as well as particle-based drug delivery systems<sup>43</sup>. On the other hand, the upward movement of sebum may moderate drug transport, especially for hydrophilic drugs<sup>13</sup>. Sebum excretion rates in the forehead may vary from 0.74 to 1.56 mg/cm<sup>2</sup>/min<sup>44</sup>; however, sebum flow out of the follicles is a slow process in comparison to the penetration of drugs in nanoparticle form. Studies concerning nanoparticles that can reach deeper parts of the follicles after a short time prove that the penetration process overcomes sebum flow<sup>27</sup>.

It is possible to achieve systemic delivery via the follicular route, which was demonstrated in vivo with caffeine applied in shampoo. The relevant amount of caffeine in the blood was detectable 5 min after topical application (compared to 20 min after permeation through the stratum corneum of the interfollicular epidermis)<sup>7</sup>.

Lademann et al.<sup>18</sup> studied penetration of titanium dioxide microparticles into the stratum corneum and the hair follicles. They observed that in the lower half of the horny layer the titanium dioxide particles were localized exclusively in the pilosebaceous orifices, but penetration into the viable tissue was not observed.

Follicular drug delivery is highly dependent upon the vehicle used in the formulation. Lipophilic rather than hydrophilic vehicles are able to improve follicular penetration<sup>13</sup>. Teichmann et al.<sup>39</sup> demonstrated improved follicular penetration of lipophilic dye – curcumin – applied in an oil-in-water microemulsion as compared to amphiphilic cream. Another study demonstrated that water-in-oil nanoemulsions could facilitate follicular transport of water-soluble compounds encapsulated within water nanoemulsion droplets<sup>40</sup>. A recent study showed higher

minoxidil follicular accumulation after application in formulations containing ethanol in comparison to formulation without ethanol. It reflects the partitioning of ethanol into lipid-rich skin compartments (including follicles), since minoxidil is six times more soluble in ethanol than in water<sup>33</sup>.

The follicular penetration of nano- and microparticles has been widely investigated recently. Apparently, the penetration depth depends on the size of the particles. Particle-based delivery systems can therefore be used to target specific regions of the follicular duct. Table 1 presents different carriers for follicular delivery and their penetration depths. The results are not very similar because there are a variety of techniques used to study follicular penetration.

Shim et al.<sup>32</sup> studied the permeation of minoxidil-loaded polymer nanoparticles through rodents' skin, and concluded that the follicular pathway is a primary penetration route for the particles. Furthermore, decreased nanoparticle size promotes permeation. Vogt et al.<sup>45</sup> found that particles in the size range of 750 nm remain in the superficial parts of the infundibulum, while particles sized 40 nm penetrate deeper into the follicular duct and also through the follicle epithelium.

It is important to emphasize the role of massage (following application) on follicular penetration as many studies use it to improve follicular penetration<sup>8,10,27,31,46</sup>. Some authors claim that the massage can mimic in vitro the hair movement that occurs in vivo<sup>8,14</sup>. However, a standardized massage technique and device are needed, as some authors state that the formulation "was massaged into the tissue for 3 min with a massage appliance"<sup>27</sup>, while the others discussed the topical application "for 2 min using a commercially available massage instrument with a frequency of 3000 oscillations per min"<sup>31</sup>.

Lademann et al.<sup>46</sup> demonstrated in vitro, using porcine ear skin, the superiority of particle-based versus non-particle formulations for follicular penetration and storage. They also demonstrated that application with massage significantly increases the penetration depth for both particulate and non-particulate systems. The authors proposed a "geared pump" hypothesis that explains particle penetration into the hair follicles as a process in which particles are entrapped underneath the cuticular cells and guided further down as the hair moves back and forth<sup>8,46</sup>.

Toll et al.<sup>31</sup> investigated in vitro follicular penetration of polystyrene microspheres using human skin. The results emphasize the

importance of the CSSS technique. CSSS removes the content of the follicular infundibulum consisting of a mixture of keratinized material, cell debris, lipids, and bacteria and occasionally parts of vellus hairs. In skin samples without prior CSSS, the microspheres tend to aggregate on the skin surface and the maximum follicular penetration depth was 1500 nm. In contrast, skin samples treated with CSSS exhibit a penetration depth of 2300 nm. Microsphere penetration depth also depends on size: 0.75 µm microspheres show the deepest penetration and 3 µm microspheres show the lowest.

Using CLSM for the full-thickness porcine ear skin, Alvarez-Roman et al.<sup>47</sup> visualized the cutaneous localization of the fluorescent polystyrene nanoparticles with mean diameters of 20 and 200 nm. They concluded that the nanoparticles preferentially accumulate in the follicular openings in a time-dependent manner. Green fluorescence was seen in the hair follicles after only 0.5 h, while after 2 h the preferential distribution in the follicles was clear. It was observed for both 20- and 200- nm particles. The smaller nanoparticles demonstrated higher accumulation in the follicular regions, proving that the particle distribution depends on the particle size, which is in accordance with other studies<sup>10,31,45</sup>.

The role of particle size can also be observed in the penetration of crystalline material. After application of material of <1 µm in size, it can be found in both the upper layers of the stratum corneum and in the follicular orifices. Crystalline particles with a size range of 3–10 µm penetrate only into the follicles (not into the stratum corneum), whereas a size >10 µm forces them to remain on the skin surface and particles <3 µm are distributed into the follicles and stratum corneum<sup>10</sup>. Follicular penetration of solid lipid nanoparticles (SLN) was also studied, showing that lipophilicity of the carrier improves drug uptake by the hair follicles<sup>37,48</sup>. It was possible to detect SLN in the hair follicles 24 h after application, which presents SLN as a slow-release system<sup>37</sup>.

Topical *in vivo* application of liposomes with calcein and melanin in mice showed much higher follicular penetration in comparison to free calcein or melanin molecules, which were trapped in the stratum corneum. Liposome-entrapped molecules were only delivered into the hair follicles without entering the epidermis, dermis or blood circulation<sup>49</sup>.

Jung et al.<sup>50</sup> studied penetration of different liposomes into porcine hair follicles and concluded that amphoteric and cationic liposomes reached a penetration depth that was 69% of follicle length. However, they do not

mention the hair cycle phase of investigated hair follicles, which would be vital information. They also observed no correlation between the diameters of the liposome types and their maximum penetration depths. Tabbakhian et al.<sup>35</sup> indicated the superiority of niosomes (nonionic surfactant based vesicles) and liposomes to conventional formulations (e.g., aqueous-alcoholic solutions) in targeting drugs to the pilosebaceous unit. It was noted that liposomes in the liquid-state facilitated the deposition of finasteride in the sebaceous gland region. Earlier studies indicated that liposomal formulations facilitate follicular drug deposition independently of the drug hydrophobicity/hydrophilicity<sup>41</sup>.

Human and mouse hair follicles were successfully targeted by liposomes loaded with DNA, resulting in efficient *in vivo* transfection of hair follicle cells<sup>51</sup>. In another *in vivo* study, the chimeric oligonucleotide in liposomes was topically delivered to hair follicles<sup>52</sup>. Such *in vivo* topical delivery of genes into follicular keratinocytes is a simple method, but usually allows only transient expression<sup>20</sup>. Li and Hoffman<sup>53</sup> showed the selective targeting of the lacZ reporter gene to the hair follicles in mice after topical application of the gene entrapped in liposomes. These results demonstrate that highly selective and safe gene therapy for the hair process is feasible. Zhao et al.<sup>54</sup> showed that histocultured albino-mouse skin, when infected with the pLme/SN retrovirus, produced melanin pigments in hair bulbs and hair shafts, suggesting that tyrosinase-gene vector was transferred and expressed in hair matrix melanocytes.

In comparison to stratum corneum, the hair follicles are efficient long-term reservoirs (up to 10 days), creating the possibility of retarded delivery and lower application frequency<sup>27</sup>.

### Contradictions

Model studies suggest that the follicular penetration pathway favors highly hydrophilic substances<sup>42</sup>, while, on the other hand, the presence of lipophilic sebum may favor follicular uptake of lipophilic molecules<sup>13</sup>. *In vitro* studies by Frum et al.<sup>36</sup> suggested that lipophilicity could be an important modulator of drug absorption into follicular orifices only above a critical logarithm of the octanol–water partition coefficient value. Below this value, lipophilicity does not apparently influence the follicular contribution. Previous research showed that the lipophilicity of the penetrant has a great influence on follicle targeting in the presence of surfactant and propylene glycol<sup>55</sup>. For surfactant/propylene glycol containing



formulations, an increase in the lipophilicity of model dyes resulted in a gradual increase in dye accumulation in the follicles.

The follicular penetration of particles is best explained by the "geared pump" hypothesis, with a significant dependence on the dimensions of the penetrating particles<sup>8,46</sup>. Particles with a size similar to hair cuticles are pushed into the follicles by the movement of the hair<sup>56</sup>. However, Lekki et al.<sup>43</sup> claim that it is the formulation with nanoparticles, which is pushed into the follicles, and particle diameters are less important. While particle penetration was proven to be size-dependent, some observe no relationship between the diameters of liposomes and their follicular penetration depths<sup>50</sup>.

The "active" hair follicles, that is those exhibiting hair growth (anagen) and/or sebum production, are open for the penetration process<sup>10,30</sup>. That is why anagen follicles should be accessible for penetrating substances. Lademann et al.<sup>30</sup> demonstrate that the active hair growth determines the follicular penetration in the same way as active sebum production.

While most authors distinguishing "active" and "inactive" hair follicles claim that the state influences follicular penetration, Tollet et al.<sup>31</sup> found no correlation between hair follicle growth and microsphere penetration in vitro, studying the follicular unit (Meijie's res trio group). Lekki et al.<sup>43</sup> offer quite a simple explanation, supposing that the spreading of the formulations can be inhomogeneous and therefore some follicles are not exposed to penetration.

It has also been shown that the hair follicles create a long-term reservoir for topically applied substances, but they can represent the same reservoir for microorganisms residing on the skin and after disinfection, may be the source of reinvasion<sup>26</sup>.

The safety of microparticles and nanoparticles applied into hair follicles is still an inscrutable problem. It can be considered in two dimensions: the safety of the carriers only and the safety of active substances delivered via these carriers. The particles as drug carriers seem to be safe because they are mostly made of biodegradable materials and as long as their dimension is above around 40 nm they are not able to enter epidermal cells<sup>45</sup>. Some studies claim that particles larger than 100 nm can be removed out after some time (and after releasing the active substance) because of sebum production and excretion<sup>46</sup>. However, still there are scarcely any studies focused on the long-term fate of particles in the hair follicles, and this aspect definitely needs investigation.

Alvarez-Roman et al.<sup>47</sup> observed the skin samples only after 2-h contact with fluorescent polystyrene nanoparticles (20 and 200 nm in diameter) and found no evidence for their uptake away from the follicles. The second issue of the safety is the active substance applied transfollicularly. Depending on its physicochemical properties and the carrier type, it can reach the blood circulation. This can be a safety problem if such penetration is not intended and/or uncontrolled. Well-known fatal anaphylactic reactions to hair dye may be the example here.

## CONCLUSIONS

The delivery of active substances to specific target sites within the pilosebaceous unit (specific compartments, cell populations) offers opportunities not only in hair therapy and in the treatment of hair follicle-associated diseases, but also in gene therapy, wound healing, immunotherapy and systemic delivery<sup>13,14</sup>. For instance, 40-nm nanoparticles, which were shown to cross the skin barrier via hair follicles, may be an example of how particle-based systems can be used to transdermally deliver active vaccine compounds<sup>45</sup>. There is also a variety of formulation aspects (vehicles, carriers, penetration enhancers) that have to be taken into consideration while creating an effective hair follicle targeting system<sup>57</sup>. Each body region possesses its own hair follicle characteristics, which, in the future, should lead to a differential evaluation of skin permeation processes and to choosing a specific region appropriate for follicular penetration<sup>17</sup>.

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