CHAPTER 12

Dandruff and Seborrheic Dermatitis: A Head Scratcher

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Introduction

The visual perception of individually distinguishable flakes on the scalp, in the hair, or on the clothing is considered an abnormal condition frequently referred to as dandruff, seborrheic dermatitis, or multiple other names.[1-2] This condition is, however, most often referred to as dandruff, especially in the public domain of non-technical literature and advertising. In the medical literature, the same disorder, though often in a more severe form, is most commonly referred to as seborrheic dermatitis. Historically, there have been multiple other descriptive names reflecting the fungal cause of this condition, such as pityriasis simplex and pityriasis capitis (referring to *Pityrosporum*) and furfuracea (referring to *Malassezia furfur*). As all of these names remain in use, we simply need to remember *they represent a continuum of the same symptoms based on the same causes and with similar treatment.* [1,3] More than 50% of adults may be affected by these conditions, which suggests a high socioeconomic impact. For dandruff and seborrheic dermatitis alone, the health care direct, indirect, and intangible costs exceeded $1.4 billion in the United States in 2004.[4] Study of dandruff and seborrheic dermatitis is more important than is often perceived, as its presence is now documented to lead to significant psychological trauma, manifesting in the loss of self-esteem and the

generation of negative social image.[5-7] Further, the field has been recently re-invigorated by the discovery of the fungal cause and the sequencing of the *Malassezia* genome.

What is Dandruff?

The relationship between dandruff and seborrheic dermatitis has at times been controversial. While most investigators regard seborrheic dermatitis of the scalp as severe dandruff, others believe that dandruff should be used to describe any flaking of the scalp.[8-11] A normal scalp has few flakes and healthy looking, smooth skin (**Figure 1**).

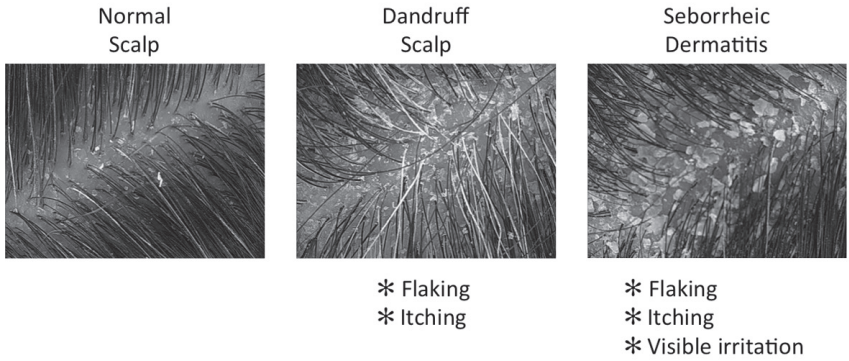

Figure 1. Topical presentation of dandruff and seborrheic dermatitis.

Dandruff is characterized by patches of loosely adherent flakes, usually accompanied by itching. Dandruff has the clinical feature of small white or gray flakes that accumulate diffusely on the scalp in localized patches. It does not exhibit apparent inflammation and is confined to the scalp. In seborrheic dermatitis, the flakes have progressed to being greasy with a yellow color. Seborrheic dermatitis flakes are frequent enough to appear as adherent mounds accompanied by inflammatory changes (seen as surface erythema). Seborrheic dermatitis varies in appearance, presenting as patches of red, flaking, greasy skin and differs from dandruff in that it can appear beyond the scalp, particularly the nasolabial folds, ears, eyebrows and chest. The key differentials in diagnosis of dandruff versus seborrheic dermatitis are visible redness or erythema and the

presence of flakes and irritation beyond the scalp.[2]

It is also clear that dandruff and seborrheic dermatitis are more than just superficial disorders of the stratum corneum. Instead, the epidermis is substantially altered, with hyperproliferation, excess intercellular and intracellular lipids, interdigitation of the corneal envelope, and parakeratosis (**Figure 2**).[12-13] Interestingly, these abnormalities are seen throughout the scalp of affected individuals, not just in areas of flaking.

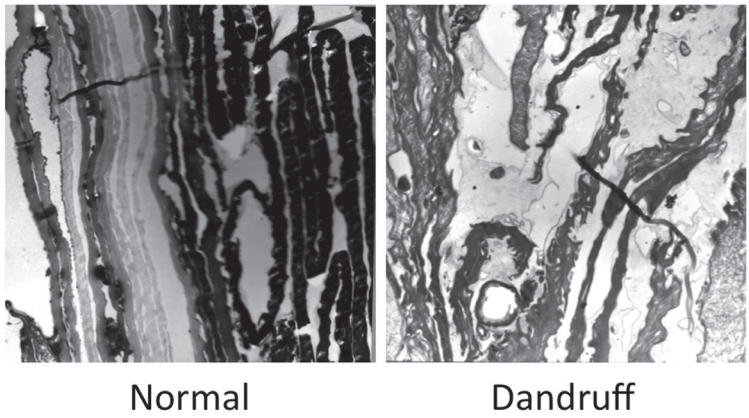

Figure 2. The physiology of seborrheic dermatitis.

The vast majority of current literature views dandruff and seborrheic dermatitis as a continuum of symptom severity with the same etiology. Furthermore, the concept of dandruff and seborrheic dermatitis as a continuum of symptoms from the same etiology is supported by the presence of inflammatory markers in dandruff even though the inflammation is not visibly apparent.[14] Because these two entities share a similar mechanism and treatment for both is similar, we shall refer to these jointly (D/SD) for the remainder of this chapter simply as "dandruff."

Etiology

Based upon the most recent evidence, the etiology of dandruff and seborrheic dermatitis appears to be dependent upon three factors: sebaceous gland secretions, microfloral metabolism, and individual susceptibility (**Figure 3**).[15-16] This chapter will describe

recent advances in the understanding of these factors, especially the role of the yeast *Malassezia*.

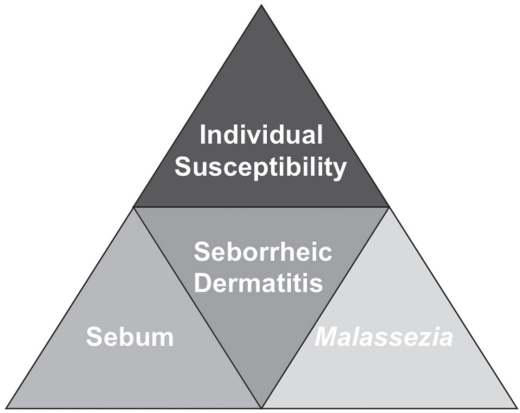

Figure 3. A three-factor causal model for dandruff and seborrheic dermatitis

Sebum

The role of sebum in dandruff is implied by the strong temporal correlation with sebaceous gland activity. This correlation includes increased incidence during infancy (cradle cap), low incidence from infancy to puberty, an increase in adolescence and young adulthood, and a decrease later in life.[17-19] In addition, dandruff occurs exclusively on skin in areas with high levels of sebum.

The function of human sebum has been and remains controversial, but recent advances in analytical technology have made some progress possible. Sebum is involved in epidermal development and barrier maintenance,[20] transporting antioxidants,[21] protection, body odor, and generation of pheromones.[22] Sebum is directly involved in hormonal signaling, epidermal differentiation, and protection from ultraviolet (UV) radiation.[23-24]

Human sebum is a complex mixture of triglycerides, fatty acids, wax esters, sterol esters, cholesterol, cholesterol esters, and squalene (**Figure 4**).[16,25] When secreted, sebum consists of triglycerides and esters which are broken down by microbes into diglycerides, monoglycerides, and free fatty acids (**Figure 5**). The free fatty acids play a key role in initiation of the irritant response, which is involved in scalp hyperproliferation. The role of sebaceous secretion also

underlies the impact of stress and hormones on dandruff, as it is well known that these are affecters of sebum secretion and impact dandruff incidence and severity.[26-28]

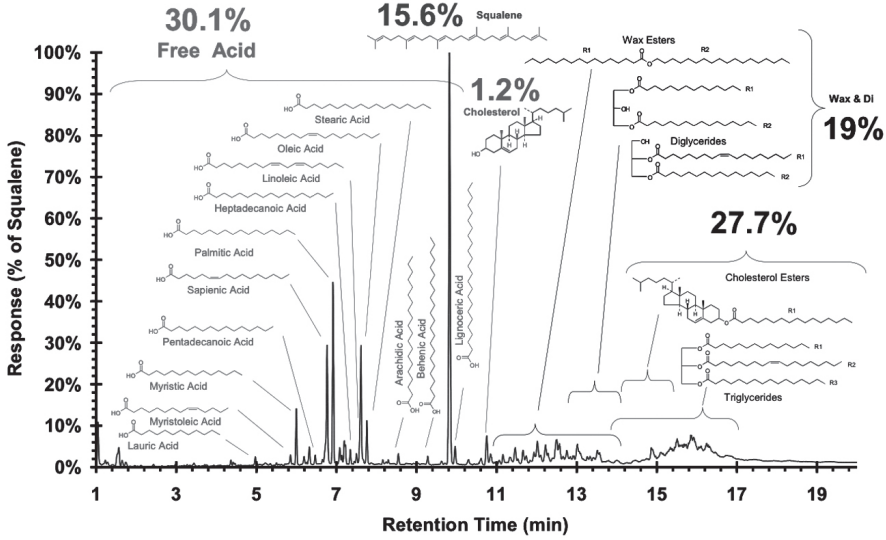

Figure 4. Components of human sebum

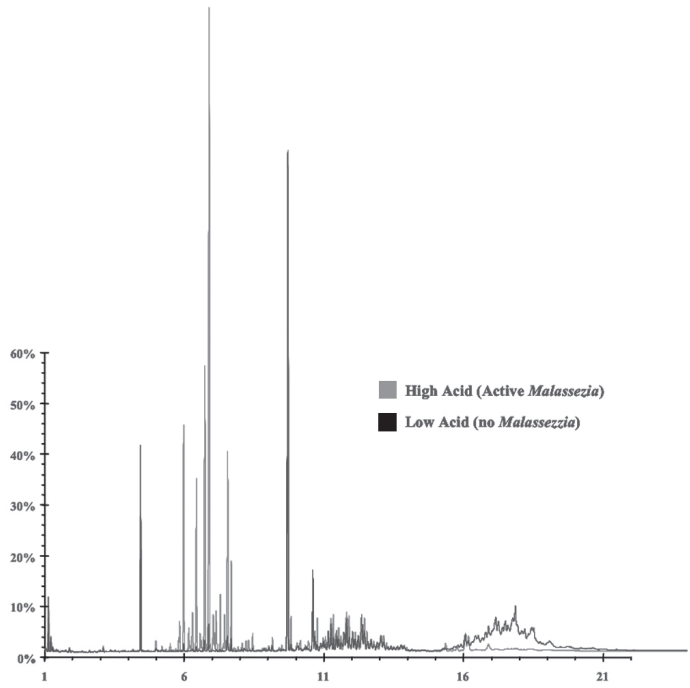

Figure 5. Human sebum in the presence and absence of M*alassezia*. Note the reduced triglycerides and increased fatty acids when M*alassezia* are present.

Malassezia

The microbial origin of dandruff centers on the causal role of yeasts of the genus *Malassezia*.[29-30] The vast majority of recent data supports a direct causal link between *Malassezia* fungi and dandruff. First, effective treatment of the condition can occur with a wide range of material types, from zinc and selenium salts to highly specific azoles, with the only known functional link between these materials being antifungal activity.[30] The second supporting factor is that improvement in dandruff correlates considerably with reduction in scalp *Malassezia* level.[31-32] While the absolute level of *Malassezia* correlates less well with dandruff, its reduction amongst those individuals that express the symptoms strongly supports its role.

Originally named *Malassezia* by Malassez in 1898,[33-34] this genus was renamed and referred to as *Pityrosporum* during the second half of the 20th century.[35-36] At one time, members of *Malassezia* were classified into two species: a lipid-dependent species, *M. furfur*, and a non-lipid-dependent species, *M. pachydermatis*. In the mid 1990s studies of the morphological, ultrastructural, physiologic and genomic differences in *Malassezia* led to the identification of multiple lipid-dependent species (including *M. globosa, M. restricta, M. furfur, M. obtusa, M. slooffiae, M. sympodialis, M. japonica, M. nana, M. dermatis,* and *M. yamatoensis*), in addition to the non-lipid-dependent, primarily zoophilic, species, *M. pachydermatis*. Use of molecular markers is generally required to correctly differentiate between the various lipid-dependent species.[37-41]

Although members of the normal cutaneous microflora, yeasts of the genus *Malassezia* have been known for many years to play a role in human skin diseases including dandruff, seborrheic dermatitis, pityriasis versicolor, and *Malassezia* folliculitis, and they may likewise play a role in the exacerbation of atopic dermatitis and psoriasis.[42-43] The study of this genus has been complicated by their fastidious culture requirements and a complex series of changes in nomenclature.[42] The one exception to antifungal hypotheses

is steroidal anti-inflammatory agents. The effectiveness of these materials is not in conflict with the fungal hypothesis of dandruff genesis; it is only intervention downstream of the original insult. Treatment options including both antifungal and anti-inflammatory agents will be discussed in the treatment section of this chapter.

Using a molecular technique (terminal fragment length polymorphism) to eliminate any potential culture bias, we previously identified *M. globosa* and *M. restricta* as the predominant species present on the scalp of dandruff sufferers.[44] The *Malassezia* yeasts are most common on sebum-rich areas of the body and degrade sebum. Specifically, the organisms contain lipases that hydrolize triglycerides, freeing specific saturated fatty acids that the yeast requires to proliferate (**Figure 5**). To demonstrate that *Malassezia* generated free fatty acids can induce dandruff like flaking in humans, we applied a marker fatty acid, in the form of oleic acid, to human scalp tissue. Even when *Malassezia* have been removed from the scalp, oleic acid was able to elicit a flaking response in dandruff susceptible individuals (**Figure 6** and below).[16]

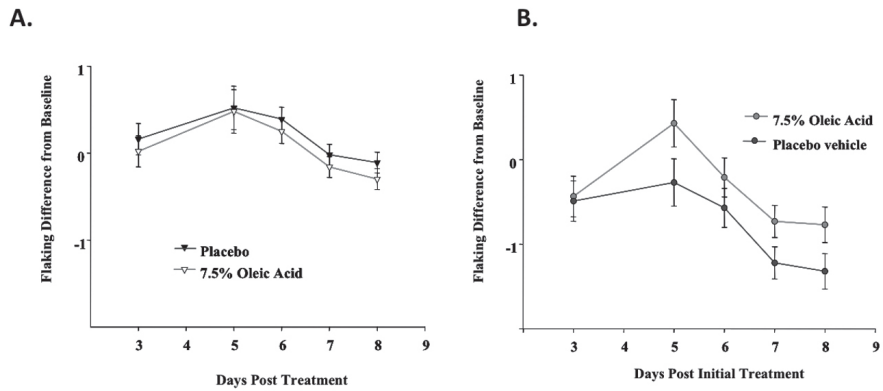

Figure 6. a) Oleic acid induced dandruff-like flaking in non-dandruff human subjects; b) Oleic acid induced dandruff-like flaking in dandruff sufferers.

A detailed model for the metabolic pathways involved in dandruff genesis has been formally proposed (**Figure 7**). *Malassezia globosa* reside on the surface of the scalp and in the follicular infundibulum. These cells secrete hydrolytic enzymes, including

lipase, into the extracellular milieu. The lipase enzymes cleave sebaceous triglycerides into free fatty acids and glycerol. The *Malassezia* consume the saturated fatty acids necessary for their proliferation and leave behind an increased amount of irritating unsaturated free fatty acids. These unsaturated fatty acids penetrate into the epidermis, and in susceptible individuals (discussed below) induce a breach of the skin's barrier function, inducing either directly or indirectly irritation and a subsequent hyperproliferation and flaking.

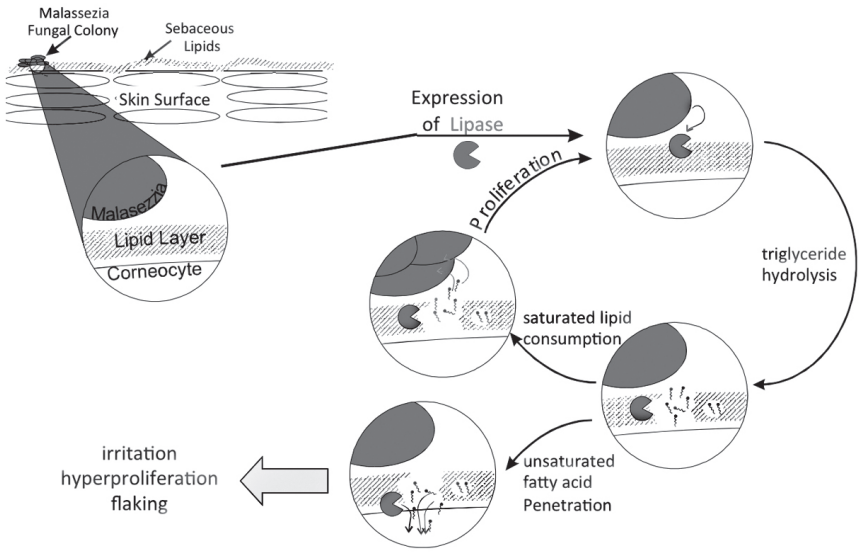

Figure 7. The role of Malassezia lipid metabolism in dandruff genesis.

Lipases have been shown to play a key role in the lifestyle of *Malassezia* species on skin.[45] In order to better understand this role, we isolated a lipase from *M. globosa*. One highly expressed lipase was sequenced and the corresponding lipase gene (*LIP1)* cloned and sequenced. This work was a first step toward a molecular description of lipid metabolism on the scalp and a more complete understanding of the role of microbial metabolism in the etiology of dandruff. Based on the limited activity of LIP1, it was postulated that additional lipases were present in *Malassezia*, and sequencing of the *Malassezia* genomes has revealed that to be the case.

The M*alassezia globosa* Genome

In order to further our understanding of *Malassezia* and human scalp biology and their unique lipid dependence, we sequenced the complete genomes of *M. globosa* and *M. restricta*.[46]

The *M. globosa* genome is 9 Mb, among the smallest of free-living fungi.[47-48] To assist in identification of protein open coding frames, we sequenced a cDNA library, resulting in the prediction of 4,289 protein coding genes. Even with this small gene compliment, the genome contains all of the necessary components for glycolysis, the TCA cycle, synthesis of all twenty amino acids and the five nucleic acid bases, among others. The key deficiencies linked to lipid dependence are the absence of a fatty acid synthase and a d-9 desaturase. *Malassezia* seem to have complimented their need for fatty acid assimilation by duplicating a high number of secreted lipases (13) and phospholipases (9). Reverse transcription Polymerase Chain Reaction (RT-PCR) and proteomics experiments from cultured cells and isolated from human scalp confirm the expression of multiple lipase and phospholipase genes. Also, multiple genes for generation of peroxides were identified, making it likely that *Malassezia* are involved in damage to the hair shaft as well as the scalp. This hypothesis is also supported by recent work indicating that hair sampled from dandruff sufferers was less healthy than that isolated from non-dandruff subjects.[49]

Of course, these hydrolytic enzymes require extracellular secretion to interact with host skin. We therefore performed proteomics experiments (on cultured cells) to identify over 50 secreted proteins. The most abundant of the identifiable secreted proteins were, as hypothesized, lipases. In addition, many other secreted proteins were identified, including aspartyl proteases, members of the phospholipase C family, glucose-methanol-choline (GMC) oxidoreductases, known *Malassezia* allergens,[50] cell wall modifying enzymes, and unknown proteins. Because these proteins are secreted, they would be the most likely to interact with skin and would therefore mediate *Malassezia* pathogenicity and be relevant therapeutic targets.

Role of Individual Susceptibility

It is well known and often cited as a confounding fact that while *Malassezia globosa* is present on almost all humans only one-half to three-quarters of people suffer from dandruff. One hypothetical explanation of this phenomenon is the possibility that there exists a fundamental difference between dandruff suffers and non-dandruff individuals.

To test this hypothesis we applied a fatty acid *Malassezia* metabolite, oleic acid, to the scalp of human volunteers who were clinically assessed as dandruff or non-dandruff. In this experiment, oleic acid dosed ata physiologically relevant concentration was able to induce a flaking response which was indistinguishable from dandruff by visual observation or electron microscopy in dandruff susceptible patients but not in non-susceptible patients.[16] This finding provides evidence for a direct role of these fatty acid metabolites in dandruff and suggests an underlying difference amongst individuals that predisposes some to the development of dandruff or seborrheic dermatitis. The difference between dandruff susceptible and non-susceptible individuals remains unclear. Multiple possibilities exist, including innate differences in stratum corneum barrier function, skin permeability, and immune response to free fatty acids or proteins and polysaccharides from *Malassezia*. Further work will be necessary to fully understand the susceptibility response.

It will be necessary to conduct significantly more research into *Malassezia* biology and its interaction with human skin to understand the fundamentals of the interactions. The sequencing of these genomes, in conjunction with the already sequenced human genome, will allow a detailed investigation of the metabolic interactions between human skin and *Malassezia*. As new pathways are elucidated, new intervention targets will arise. This new, groundbreaking research will enable development of new technologies to interrupt dandruff, which may not be dependent on and complimentary to existing antifungal treatments.

Work on *Malassezia* physiology will provide insight into the mechanisms by which basidiomycete fungi have adapted to the mammalian skin environment. This research will also provide new opportunities to dissect specific interactions between commensal fungi and skin. A deeper understanding of these interactions may lead to new treatment paradigms and ways to intervene in the effects of *Malassezia* on human and animal health. Hopefully, new, more fundamental understanding of the interactions between *Malassezia* and human skin will enable development of new tools to manage both the number and the activity of these unique fungi.

Prevalence

Dandruff is the most common scalp disorder in adolescence (post-pubescence) and adulthood, but is rare and mild in children. [51-53] Historically, it was thought that about 50% of humans were affected to some degree, with onset at puberty and peak incidence and severity at about 20 years of age and becoming less frequent after the age of 50.[51-52] A more recent study of 1,408 Caucasians, African Americans and Chinese from the states of Minnesota and Georgia in the United States, Beijing, Shanghai, and Guangzhou, China, suggests that severity and prevalence of noticeable dandruff and seborrheic dermatitis is much higher in adults than first thought, at 81-95% in African Americans, 66-82% in Caucasians, and 30-42% in Chinese (see **Table 1**) [6]. Additionally, the prevalence of dandruff was as high in US teens as their adult counterparts with prevalence at 75-95% in Caucasian and African American teens. [5,54] Based on this survey, dandruff occurs in 60-90% and seborrheic dermatitis in 3-5% of immunocompetent adults. In AIDS patients, the prevalence of seborrheic dermatitis increases to 30-33%. [55]

Dandruff does not seem to vary with climate, as incidence and severity are similar from regions north to regions south in both the United States and China. [5,54] As may be predicted, more frequent shampooing results in lower severity in all populations,[5] but the use of specific antidandruff products must be considered. Despite

higher shampoo frequencies and the availability of effective over-the-counter and prescription antidandruff shampoos in the United States, the most recent prevalence study shows dandruff is occurring at a much higher rate and severity in the United States than in China.[5] The higher prevalence of dandruff in the United States is most likely associated with a lower use of antidandruff products (10-20%) than in China (40-52%).

Table 1. Adherent scalp flaking severity scores in adults and teens in the United States and China

Category by Creed/Gender	Adults	Teens
African American Females	29.3	27.1
African American Males	23.4	26.0
Caucasian Females	22.7	22.8
Caucasian Males	21.3	23.7
Chinese Females	12.1	12.4
Chinese Males	13.6	11.2

Pathology

The visible symptoms of dandruff and seborrheic dermatitis, superficial flaking and redness, are manifestations of abnormal epidermal structure and function.[56] Flakes are generally believed to occur in "patches" on the scalp and that these lesions randomly "move" about the scalp over time. However, the underlying stratum corneum irregularities occur throughout the scalp of affected individuals,[56] suggesting the actual flakes are the end result of a cycle of skin distress that may or may not be visible to the unaided eye.

The stratum corneum of dandruff-affected individuals shows striking features consistent with a hyperproliferative state, which is supported by functional studies that measure accelerated epidermal maturation times.[51] The physical features accompanying hyperproliferation are dramatic.[6,53] An electron microscopic study of stratum corneum[56] revealed that dandruff-affected stratum corneum exhibits parakeratotic nuclei, lipid droplets within corneocytes, a

decreased number of desmosomes, irregular corneocyte envelope structure, intercellular *Malassezia* yeasts, and massive quantities of unstructured intercellular lipids (refer to **Figure 7**). All of these features are consistent with a state in which the feedback between epidermal synthesis and maturation rate is lost and uncontrolled growth leads to corneocytes that are immature and not ready to be shed as individual cells reaching the surface.

The intercellular lipid abnormalities are striking in amount as well as lack of order. As expected, there is a lack of true intercellular lipids (ceramides), with most of the lipids being sebaceous in origin.[57] This is indicative of a lack of a temporally ordered series of events, resulting in low epidermal lipid secretion and a lack of proper organization into a functional stratum corneum. Simply topically applying such lipids is unlikely to be meaningful, as they cannot displace the sebaceous lipids, nor will they be able, in abstentia from the normal physiology, to initiate the formation of missing features such as a tight lamellar structure and the other characteristics required for proper function or orderly desquamation.

The pathophysiological features observed at the symptom/sign and structure/function levels are also observed at the molecular level. Using new noninvasive biomarker sampling techniques, biomarkers associated with each pathophysiological step (i.e. inflammation, hyper-proliferation and barrier disruption) have been observed as significantly altered in D/SD populations vs. non-D/SD control groups. Inflammatory bio-markers such as IL-1a[14] and histamine[58] are dramatically elevated in the D/SD condition, supporting the macro observations. Likewise, biomarkers of hyper-proliferation/differentiation (involucrin, specific keratins) and barrier disruption (human serum albumin and ceramides) are shown to be perturbed compared to a normal population.

Treatment of dandruff and seborrheic dermatitis will be discussed below (for which discussion, please refer to **Figure 8**, as well), but it is appropriate to mention here that as certain treatments of the initiating cause, fungal interaction with the scalp, not only is the outward symptom of flakes improved, but the underlying

skin condition is also being restored.[56] There is a direct correlation between clinical flaking and the severity of the stratum corneum abnormalities, suggestive of the cause and effect relationship between the sub-surface (morphology) and superficial symptoms (flaking).

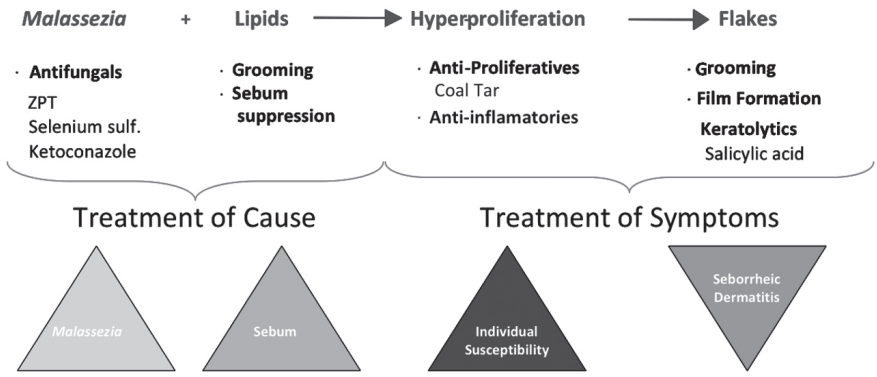

Figure 8. Treatment strategies for D/SD.

General Therapy Concerns

Multiple topical agents are effective therapies for the treatment of dandruff. These agents include pyrithione zinc,[2,59-62] selenium sulfide,[2,8,63-64] salicylic acid,[60] sulfur,[60] coal tar,[60,65] hydrocortisone,[60] and ketoconazole.[2,8,59,62] in the United States. In addition, piroctone olamine, ciclopirox olamine and climbazole are commonly used to treat D/SD in other countries. A common mechanism of most effective actives is their antifungal activity against *Malassezia*. In vitro fungistatic and fungicidal tests of ketoconazole,[8,56,66-69] pyrithione zinc,[8,56,66-67,70] and selenium disulfide[8,56,66-67,70] have demonstrated low inhibitory concentrations of growth (MICs) against *Malassezia furfur.*[56] Coal tar[71] was also demonstrated to possess activity against fifty-four *Malassezia* strains isolated from patients with dandruff, seborrheic dermatitis and pityriasis versicolor, but with a much lower potency. Other anti-mycotic agents, such as itraconazole, terbinafine, bifonazole, climbazole, fluconazole, clotrimazole, dithranol, and liquor carbonis, also have

the ability to inhibit *P. ovale* (presumed to be *M. furfur,* due to culture conditions).[67-68]

Salicylic acid, sulfur and liquor carbonis possess exfoliative qualities expected to improve the appearance of scaling, while the antimitotic effect of topical corticosteroids and coal tars might also be involved in reducing the hyperproliferation associated with dandruff scaling.

Traditionally, non-scalp seborrheic dermatitis has been treated with either topical or oral steroids.[31] However, renewed interest in the role of *Malassezia* yeasts and the known side effects of topical steroids have made antifungal medications an increasingly popular choice. Tacrolimus has been shown to have potent antifungal activity against *Malassezia furfur* in vitro.[72] Tacrolimus and pimecrolimus may be effective as they possess both anti-inflammatory and antifungal activity.

The role of commensal fungi in dandruff causes it to be a refractory condition. As *Malassezia* are commensal, cessation of antifungal therapy results in a relapse of the condition. When considering any topical therapy for long-term prophylaxis, particularly when impacting cosmetic attributes of hair, it must be cosmetically acceptable enough to maintain compliance. This highlights the assertion that for dandruff treatment the use of cosmetic antidandruff shampoos should be the first choice, with less cosmetically acceptable shampoos, lotions, and foams reserved for use in severe or refractory cases.[73-74]

Optimal treatment of D/SD requires controlling scalp flaking and itching at the lowest possible cost and inconvenience.[52,65,73-74] Since the 1960s, shampoos, conditioners and treatments have been marketed as over-the-counter or prescription products for the treatment of dandruff. Many of these products not only treat the scalp, but also provide the hair grooming needs of cleansing and conditioning.[56] The importance of antidandruff hair care products with no trade-offs in aesthetics is extremely important for effective therapy because they can be incorporated into a routine hair care regimen and lead to high consumer compliance.[56,65]

Therapeutic Actives and Their Mechanisms of Action

As there are three factors involved in dandruff etiology (*Malassezia*, sebum, and individual susceptibility), there are several potential avenues for treatment. One may treat the causes or one may treat the symptoms. Treating the causes would mean removal of the fungi with antifungal treatments or suppressing the secretion of sebum. Treatment of the symptoms would involve calming the inflammation with anti-inflammatory steroidal agents, minimizing cell proliferation with anti-proliferatives, or by simply grooming away the resultant flakes. Using non-medicated shampoos to simply wash away the flakes is minimally effective; they are simply generated too quickly. Anti-proliferative therapies have also been poorly effective due to the minimal efficacy of available treatment materials. Sebum suppression has also proved to be very difficult, with few if any effective topical treatments available at this time. Treatment with anti-inflammatory steroidal agents can be effective in the short term but cannot be used for long-term prophylaxis due to limiting adverse effects. This leaves the most effective treatment with the most flexible options being antifungal treatment.

Zinc pyrithione (ZPT) is a biocide whose rational development in the 1950s was based on aspergillic acid, the natural antibiotic from *Aspergillums*.[75] ZPT was included in the evaluation of over 1,000 candidates for controlling the yeast of the genus *Malassezia* relevant in dandruff etiology. [76] ZPT has many properties which make it especially useful to deliver in the complex vehicle of a shampoo; it is:

- only sparingly water-soluble, allowing efficient scalp retention after rinsing;
- affordable for regular usage;
- and it allows galenic formulations due to lack of color and odor impact on product cosmetics.

These attributes have led to ZPT becoming the most common material used for dandruff treatment globally. Antidandruff efficacy

and safety were demonstrated in the early 1960s, which served as the basis for acceptance by the US Food and Drug Administration; since then, ZPT shampoo and conditioning rinse-off products have been widely marketed. This category of antidandruff products has been approved for over-the-counter use in the United States for dandruff treatment at 0.3-2% in shampoo and rinse-off products,[60,64,77-78] and 0.1-0.25% in leave-on products.[77-78] The efficacy of these products has been demonstrated in many clinical trials.[8,35,60,64,66-67,77-79] While ZPT possesses high intrinsic antifungal activity against *Malassezia*,[35,60,64,66-67,77-78] its practical efficacy is dependent on multiple vectors, including but not limited to particle size, particle shape, deposition amount, coverage, and availability of the deposited material. These parameters can all be varied to deliver optimal efficacy. For example, platelet ZPT at a particle size of 2.5 microns is optimal for deposition on the scalp through shampooing and for providing scalp surface coverage (**Table 2**).[56]

Table 2. Optimization of active particle size increases dandruff efficacy of marketed 1% pyrithione zinc shampoos.

1% pyrithione zinc shampoo	Avg. particle size (μm)	Median Effect Size	Range of Effect Sizes
Shampoo A	13	0.85	1 study only
Shampoo B	5	1.41	[1.36, 2.93]
Shampoo C	2.5	2.08	[0.55, 2.14]
Shampoo D	0.5	1.74	1 study only

Individual effect sizes are standardized mean differences between active and placebo shampoos of the reduction in scalp flaking after six weeks of use. The results were taken from 14 separate studies. Since some pyrithione zinc shampoos appeared together in the same study, the effect sizes were computed accounting for the correlation and for unequal variances.

In products containing particulate actives like ZPT, the efficacy is effected by the size and shape of those particles, as these factors affect the amount deposited, the persistence of the deposit during rinsing, and the degree of scalp surface coverage. Clinical efficacy testing has demonstrated that particle size is a significant variable in ZPT-based

product efficacy and that not all ZPT-based shampoos can be assumed to work equivalently. In practice, products containing 2.5 micron platelet ZPT appear to be the most effective.[56, 80-81]

Despite widespread human use, until recently there has been little known of the antifungal mechanism of action. Ermolayeva and Sanders[82] and Chandler and Segel[83] showed that ZPT can depolarize membranes and prevent membrane transport, although the ZPT concentrations used (> 100 μM) are much higher than required to inhibit fungal growth. More recently, Yasokawa et al.[84] used microarray analysis to show that ZPT induces iron starvation, suggesting the antifungal mechanism is due to iron starvation. Recently, Reeder et al.[85] demonstrated a new hypothesis on the mechanism of action of ZPT, namely that ZPT inhibits *S.cerevisiae* growth through copper influx. The data supporting this conclusion are 1) an increase in cellular copper content, 2) gene expression responses indicative of excess intercellular copper, 3) a requirement for environmental copper for ZPT activity, and 4) the observation that mutant cells more sensitive to copper are likewise more sensitive to ZPT. The molecular mechanism of ZPT-mediated inhibition of *S. cerevisiae* is copper-mediated loss of function of iron-sulfur proteins. Where possible, parallel studies were performed with the scalp fungus *M. globosa* where ZPT was also acting through intracellular copper.

Selenium sulfide has been approved for over-the-counter use at levels of 0.6% (micronized form) and 1%.[60,64,77-78] Shampoos containing selenium sulfide have proven efficacy. [10,86-87] Since selenium sulfide is a particulate, efficacy is dependent on the particle size to optimize coverage. Differences in efficacy may be related to the particle size of the selenium sulfide in the shampoo. Further, selenium sulfide is a complex mixture of multiple isoforms and the relative constitution of formulations affects efficacy. The mechanism of antidandruff activity is presumed to be based on its antifungal activity, but the molecular mechanism of its antifungal action remains unknown.

Ketoconazole is an imidazole antimycotic agent and has been used orally for the treatment of multiple mycoses. Several large antidandruff efficacy studies have demonstrated efficacy against pityriasis capitis and seborrheic dermatitis.[10,30,64,88] Ketoconazole has been approved for topical over-the-counter use at 1% in shampoos and for prescription use at 2%. Twice-weekly treatments are currently recommended for ketoconazole-containing shampoos. To achieve efficacy, these products are recommended to be left on the scalp for 5 to 10 minutes before rinsing, thereby requiring a change in shampooing habits and practices. As an antifungal, ketoconazole is a member of the imidazole family and blocks fungal synthesis of ergosterol. Ergosterol is an essential constituent of fungal cell membranes. Ketoconazole binds and inhibits cytochrome P450 14-alpha-demethylase. This enzyme is required in fungal thesterol biosynthesis for the formation of ergosterol from lanosterol.

Climbazole is another azole antifungal with similar activity and efficacy to ketoconazole. Climbazole is used in antidandruff shampoos in Europe, with high in vitro and in vivo efficacy against *Malassezia* evaluated for efficacy and safety.[89] This shampoo is not marketed in the United States.

Coal tar, approved for over-the-counter treatment of dandruff, seborrheic dermatitis and psoriasis at levels of 0.5-5% (tar equivalent),[60,64,77-78] reduces the number and size of epidermal cells, decreases epidermal proliferation and dermal infiltrates. Coal tar may also have slight antifungal activity, which could explain its minimal antidandruff efficacy.[60,64-65,77-78] Coal tar-containing shampoo and treatment products have been marketed for decades, mainly for psoriasis.

Salicylic acid, approved for over-the-counter treatment of dandruff, seborrheic dermatitis and psoriasis at concentrations of 1.8-3%,[60,64,77-78] is an exfoliant that loosens weakly adherent flakes, enabling them to be washed away. Sulfur is approved for over-the-counter treatment of dandruff at levels of 2-5%. Combinations of salicylic acid and sulfur have not been approved for over-the counter use in the United States.

Methods of Measurement

The primary efficacy measure of antidandruff activity in clinical trials is adherent scalp flaking severity. This assessment is based on a subjective 11-point flaking scale ranging from 0 (no scaling)[60,70,79] to 10 (very heavy scaling). [60,63,90] The scalp is divided into sixor eight octants and the flaking density is scored after parting the hair at each site.[60-62] The score from each site is summed across all sites (total of 60 or 80). An alternative method, the Colorimetric method (Chroma C*), called squamometry, assesses the flakes obtained on D-squame tapes collected from the most severely affected area at pre-treatment and the same site after treatment. In addition to the adherent scalp flaking scores, assessment of loose dandruff, global involvement in the disease process, and subjective assessment of itch and dandruff severity serve as secondary efficacy measures. Other secondary efficacy endpoints include the assessment of *Malassezia* density.[91-93] These have been complimented by more accurate molecular genetic techniques[32,40,94-97] in species identification and quantification. The most recent advance in assessing the therapeutic resolution of D/SD utilizes noninvasive sampling methods for molecular biomarkers. Such methods are compatible with the high capacity needed in a clinical setting and are objective endpoints. Using these tools, therapeutic resolution for ZPT-based shampoos has been demonstrated for inflammatory biomarkers (IL-1a, IL-8, histamine), hyper-proliferation (involucrin, keratins) and biomarkers of barrier integrity (human serum albumin, ceramides).[14,58]

Summary

Dandruff is characterized by adherent or loose white flakes that accumulate on the scalp, in the hair, and on the clothing. It is accompanied by pruritis, and carries a significant social stigma in most developed countries. Seborrheic dermatitis is a more severe form of dandruff, with all the same symptoms plus visible inflammation, and often extends to the nasolabial fold, mustache and beard area of the face, and possibly the eyebrows.

Dandruff is more than just superficial flaking, as demonstrated by significant structural changes in the stratum corneum and changes in inflammatory biomarkers. Dandruff and seborrheic dermatitis share a similar etiology based on metabolic activity of the cutaneous commensal yeast, *Malassezia globosa*, the presence of sebaceous lipids, and individual susceptibility.

Today, dandruff can be successfully treated by multiple antifungal or topical steroidal anti-inflammatory agents. Due to the cost, poor cosmeticity, and adverse effects of steroidal agents, they should be confined to severe or refractory cases. When considering treatment options, one must consider that long-term, chronic therapy will be required, as *Malassezia* are commensal microbes and will return upon cessation of treatment. This necessitates that patients be highly compliant to any therapy. As hair is a significant driver of cosmetic appeal, for any subject to remain compliant to long-term therapy the treatment must be cosmetically appealing as well as effective.

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