The dermis, collagen synthesis, and adnexa other than the hair follicle

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THE DERMIS

The dermis (aka: corium) is of mesodermal origin and represents most of the body's connective tissue. It constitutes the majority of the skin and is responsible for skin depth/ density. The dermis contains insoluble fibers (collagen and elastin) that form a fibrous matrix which contributes to the tensile strength of skin; as well as soluble polymers (e.g.- proteoglycans, hyaluronan) which make up the diffuse and filamentous matrix and contribute to the compressive resistance of skin. These components together make up the extracellular matrix (ECM). The dermis also houses various cell types including resident cells (fibroblasts, macrophages, mast cells, melanocytes, dermal dendrocytes) and transient, circulating cells of the immune system (neutrophils, eosinophils, lymphocytes, histiocytes, plasma cells). Scrotal dermis is unique in that it has numerous smooth muscle bundles.

The dermis provides structure to the skin and is responsible for the skin's pliability, elasticity, and tensile strength. Its functions include thermal regulation; water binding; cell growth, differentiation, and migration; wound repair and remodeling; and protection against mechanical injury.

There are two main regions to the dermis in most species: the superficial dermis (termed the papillary dermis in humans) and the deep dermis (reticular dermis in humans). The deep dermis contains tightly woven, thick bundles of collagen and elastin. The superficial dermis is composed primarily of finer, more loosely arranged elastin and collagen. In the horse there is a third dermal region, the "horse mirror" or Ross-Spiegel layer, which is composed of collagen fibers in a tree-like arrangement that appears shiny on gross examination. The horse mirror is present in the skin over the rump, back and upper half of the chest.

Fibroblasts

Fibroblasts are the major cell type in the dermis and are of mesenchymal origin. They have a branched cytoplasm with extensive rough endoplasmic reticulum (rER) related to their function as the major producer of extracellular matrix proteins (glycosaminoglycans, glycoproteins, procollagen, elastin). These cells are crucial for wound healing and tissue damage induces fibroblast mitosis. Other cellular functions include the degradation of matrix proteins and the synthesis of cell mediators needed for cell growth, differentiation, and migration, as well as mitigation of inflammation (e.g.- TGF-β, MMP-1).

Collagen

Along with elastic fibers, collagen forms the fibrous portion of the ECM. Collagen makes up 75-80% of the dry weight of the dermis. There are 28 (I- XXVIII) collagens that have been identified. Types I, III, V and XI are fibril-forming collagens. The most abundant collagen types in the adult dermis are types I (80- 87% of the fibers); III (10%); and V (3-5%), all of which are

rod-like and relatively large. Fetal skin contains primarily type III collagen. Type III collagen is also the first collagen produced during wound healing. Types III and V are concentrated around blood vessels. Type VI forms dermal microfibrils that stabilize and organize the larger fibrils of the ECM. Types XII and XIV (among others) are fibril-associated collagens with interrupted triple helices (FACIT) and are necessary for the organization and stability of the fibril-forming collagens (I, III, V). Type IV, VII, and XVII are integral parts of the basement membrane zone (BMZ) and dermal-epidermal junction.

Type I, III, and V collagens are all composed of 3 polypeptide α chains that coil around each other to form a triple helix. Each α chain is composed of amino acids (aa's). Type I collagen fibers have repeating triplets of aa's where the smallest aa glycine (Gly) constitutes 1/3 of the total aa's in the fiber and is evenly distributed throughout the fiber in a Gly-X-Y repeating pattern. "X" and "Y" may be occupied by a number of aa's, however, the "X" position is usually occupied by proline and "Y" is usually hydroxyproline (combined, proline and hydroxyproline make up ~22% of human type I collagen). Type III collagen has an increased concentration of hydroxyproline, glycine and cysteine residues as compared to type I. Proline and hydroxyproline are required for cross-linking between α chains. Hydroxyproline is essential to stabilize the triple helical structure. The repetition of glycine is essential to the triple helical structure. Lysine and hydroxylysine are essential for cross linking between collagen fibrils.

<u>Collagen synthesis</u>

Collagen synthesis begins in the intracellular space and is completed extracellularly. It is stimulated by vitamin C, TGF-β, IL-1, IL-4, IGF-1, IGF-2, superoxide generating system, bleomycin, and PDGF. It is inhibited by glucocorticoids, retinoids (although quiescent cells can be stimulated by retinoic acid to activate collagen gene expression), vitamin D3, parathormone, prostaglandin E2, IFN-γ, D-penicillamine and minoxidil.

1. Gene transcription occurs in the fibroblast, mRNA leaves the nucleus and enters the rER
2. mRNA is translated on ribosomes of the rER into pre-proα chains
    a. pre-proα chains contain an amino-terminal
3. Amino-terminal of the pre-proα chain is enzymatically removed by signal peptidase
    a. proα chain released into lumen of rER
4. Hydroxylation of prolyl and lysyl residues to form hydroxyproline and hydroxylysine
    a. Catalyzed by prolyl and lysyl hydroxylase enzymes
        i. Require molecular oxygen, ferrous iron, α-ketoglutarate, and a reducing agent (i.e.- ascorbate/ vitamin C)
            - Scurvy results in poor wound healing, decreased tensile strength of connective tissue
            - Hyperbaric oxygen may aid wound healing by increasing the activity of prolyl hydroxylase
        ii. Corticosteroids inhibit prolyl hydroxylase
    b. Hydroxylation of prolyl residues is the rate limiting step for collagen synthesis
    c. Hydroxyproline is required for proper folding of α chains into triple helices
5. Glycosylation of some hydroxylysyl groups

    a. Manganese co-factor
6. Formation of procollagen which is then released into the cytoplasm
    a. Interchain disulfide bonds
    b. Triple helices form from 3 proα chains
    c. Contain globular amino and carboxy terminals
7. Secretion of procollagen into the extracellular space
8. Amino and carboxy terminals on procollagen are cleaved by proteases
    a. Deficiency in one of these proteases can cause dermatosparaxis
9. Spontaneous assembly into insoluble collagen fibrils
10. Cross-linking of collagen fibrils via covalent bonds between molecules (usually lysine or hydroxylysine)
    a. Deamination of hydroxylysine and lysine
    b. Lysyl oxidase is an important enzyme
        i. Requires copper
        ii. Inhibited by β-aminopropionitrile found in some legumes and is the cause of lathyrism

Collagen is degraded by collagenases which are matrix metalloproteinases (MMP) and require zinc and calcium for proper functioning. Denatured collagen is gelatin- this is then further degraded by other MMP's like gelatinases. Collagen turnover is directly correlated to the amount of hydroxylysine and hydroxyproline in the urine as these amino acids are relatively unique to collagen (elastin fibers contain small amounts of hydroxyproline). On histopathology, collagen fibers are highlighted by staining with Masson-Trichrome.

<u>Elastic fibers</u>

Elastic fibers are the second component of the fibrous matrix of the dermis. Elastin composes ~1-2% of the dry weight of the dermis. Elastic fibers are in highest concentration in the aorta, arteries, and lungs. They form a type of "mesh" between collagen fibers. There are three types of elastic fibers in the dermis: elastin; elaunin; and oxytalan. Mature elastic fibers have a core of elastin surrounded by microfibrils such as fibrillins, fibulins and type VI collagen. In the deep dermis, insoluble, cross-linked elastin fibers are arranged horizontally with vertically oriented oxytalan fibers attached. The oxytalan fibers are composed of microfibrils and they anchor the deep dermis to the superficial dermis. Oxytalan fibers are also found in the superficial dermis and help to anchor it to the BMZ. Elaunin fibers are associated with elastin and consist of small amounts of amorphous cross-linked elastin with bundles of microfibrils. They are found in the superficial dermis and form an arcade-like arrangement.

Elastin production starts with the formation of tropoelastin, the basic molecular unit of elastin. Once genes are transcribed in fibroblasts and smooth muscle cells, they are translated on the rER into tropoelastin which is then secreted into the extracellular space. Similar to collagen, ~1/3 of the aa's that compose elastin are glycine, however, it is not evenly distributed as in collagen. The other two most common amino acids in tropoelastin are valine and proline. Once in the extracellular space, tropoelastin spontaneously associates with microfibrils and cross links are formed between the molecules of tropoelastin to finalize the formation of elastic fibers.

The insolubility of elastin is due cross links formed by two amino acids: desmosine and isodesmosine which are unique to elastin. As with collagen cross links, the copper (and oxygen) requiring enzyme lysyl oxidase is essential in the formation of desmosine/ isodesmosine cross link formation.

Elastin production is stimulated by TGF-β and IGF-1; production is inhibited by TNF-α, vitamin D3 and colchicine. Elastases are responsible for degradation of elastin. Most elastases are serine proteases, however, some are metalloenzymes and require calcium for proper functioning.

On histopathology, elastin fibers can be highlighted by staining with Verhoeff-van Gieson and acid orcein-Giemsa.

Diffuse and filamentous matrix

The diffuse and filamentous matrix is made of glycoproteins, proteoglycans (PG's) and glycosaminoglycans (GAG's). The ground substance is composed of PG's and GAG's and fills the space between the fibrous matrix elements to allow nutrients, electrolytes, and cells to pass through the dermis. It functions in water storage and homeostasis, wound healing, and resists compressive forces. PG's are composed of a core of protein with covalently linked GAG's and are soluble molecules. PG's are found intracellularly (e.g.- serglycin in eosinophils and mast cells), on the cell surface (e.g.- syndecans and glypicans), and in the extracellular space. PG's in the ECM include large aggregates like aggrecan and versican which bind to hyaluronic acid in the cartilage and dermis respectively; and small aggregates like decorin which "decorates" type I collagen.

GAG's are polysaccharides that are usually found bound to PG's and include heparan sulfate, chondroitin sulfate, keratan sulfate, and dermatan sulfate. Hyaluronic acid (aka hyaluronan) is unique in that it is the only GAG that does not have a core protein. Hyaluronic acid aids in wound healing and cellular movement and expands the ECM- it is in highest concentrations in fetal skin and allows healing without scar formation; concentration reduces with age leading to reduced skin turgor.

Fibrillins, fibulins, fibronectins, vitronectin, tenascin, and mucin are glycoproteins (proteins with carbohydrate groups attached) and form the filamentous portion of the ECM. Fibrillins are the largest and align head to tail to form microfibrils. Fibulins are found within elastic fibers and fibronectins cover collagen and elastic fibers in the dermis. Fibronectins are concentrated around the vascular and nervous tissue and are also found in the BMZ. Fibronectins modify cell to cell interactions and cellular adhesion to substrates. In the vascular tissue they mediate vascular permeability and microvascular integrity. Vitronectin is found on all elastic fibers except oxytalan. Tenascin is found around collagen fibrils, epidermal appendages and is involved in the development of collagen as well as wound healing. It binds to the GAG chain of decorin. Mucin is concentrated around appendages and the main component is hyaluronic acid. Shar-Peis have increased mucin content in their dermis which is attributed to increased hyaluronan synthase. Mucin is blue staining with hematoxylin- eosin and can be highlighted with Alcian blue stain.

SELECT DISEASES OF THE DERMIS

Cutaneous asthenia

Cutaneous asthenia, Ehlers-Danlos, and dermatosparaxis are inherited diseases of the connective tissue that are characterized by a similar phenotype which presents as excessive skin fragility and hyperextensibility. In animals, the term cutaneous asthenia is most commonly used when describing this phenotype, however, in people this term is reserved for patients in whom both collagen and elastin are affected. Dermatosparaxis means “torn skin” and describes a condition with extraordinarily fragile skin- it is a specific type of Ehlers-Danlos (VIIC) in humans where the amino-terminal of procollagen is not removed because of defective protease activity. Dermatosparaxis due to a similar mechanism has been described in cattle and sheep as well as singular reports in a Himalayan cat and a Doberman pinscher dog.

Animals with cutaneous asthenia present with hyperextensible skin that has a decreased tensile strength and tears easily. Tears are often wide and gaping and although they heal readily they leave irregular, thin, visible white scars. The skin may hang in folds around the legs and throat and may seem excessively thin on palpation. Histopathology findings are variable and may appear relatively normal. Masson trichrome staining helps to highlight changes to the collagen fibers which are often disorganized and bundled together in larger, irregular bundles or may be fragmented and shortened. The fibers may by blurred and hypereosinophilic and are often surrounded by excess mucin. Collagen abnormalities may also be present in and affect the eyes, ligaments, tendons, and blood vessels in affected animals.

Hereditary equine regional dermal asthenia is a specific type of cutaneous asthenia found in quarter horses and their crosses. It is due to an autosomal recessive mutation in the cyclophilin B/ peptidylprolyl isomerase B (PPIB) gene and genetic testing is available through the UCDavis. The genetic mutation causes impaired collagen synthesis, folding, and post-translational modifications of lysine. The average age of presentation is 1.5 years when riding exercises begin. The disease is not truly regional as all of the skin is affected, however, symptoms of ulcers, scarring, seromas, hematomas and loose or hyperextensible skin are often found primarily along the dorsum, lateral thorax, withers and croup. These areas may be painful on palpation. Diagnosis should not be based on histopathology alone due to variability of findings, however, biopsies of the back, croup and especially the neck are more likely to facilitate the diagnosis by having fewer false negative results. The dorsum from the withers to the croup has a larger magnitude of difference in skin thickness in affected individuals as compared to normal horses.

Treatment of cutaneous asthenia involves reducing trauma to the skin through husbandry. Vitamin C supplementation may be helpful in some dogs.

Warmblood fragile foal syndrome

This is a lethal, autosomal recessive genetic disorder that results in non-functional lysyl hydroxylase. Affected foals are non-viable or still born with fragile skin, skin defects, abnormally flexible joins, and incomplete closure of the abdominal wall. Genetic testing is available through the UCDavis.

Solar elastosis

Solar elastosis is due to the degeneration of collagen and elastic fibers of the superficial dermis in response to chronic exposure to solar radiation. UV light upregulates collagenase production in fibroblasts which likely contributes to the changes seen in solar damaged skin. Microfibrils are also found to be altered in solar damaged skin. On histopathology, solar damage presents as pale, homogenous collagen superficially which may progress to fibrosis. Collagen and elastin appear as thickened, wavy, basophilic fibers and are highlighted by van Gieson stain.

Scleroderma

Scleroderma represents a thickened deep dermal collagen layer and has been described in humans and horses. The localized form is called morphea. Generalized scleroderma results in progressive fibrosis that affects the skin, lungs, GI tract, kidneys, and heart.

Cutaneous mucinosis

Excessive accumulation of mucin can occur in hypothyroidism, acromegaly, alopecia mucinosa, dermatomyositis, and discoid lupus erythematosus. There is also an indiopathic form which is most common in the Shar-Pei. Shar-Peis naturally have more mucin than other breeds but some animals are affected by mucinous vesiculation and exaggerated folds of the face, ventrum and distal extremities. Affected Shar-Peis have large accumulations of hyaluronic acid in excess of unaffected Shar-Peis. Treatment for the idiopathic form usually involves corticosteroids.

SEBACEOUS GLANDS

Each hair follicle has a sebaceous gland and together they form the pilosebaceous unit. In general, during ontogeny, the follicular appendages develop on the cranial side of primary hair follicles and on the caudal side of secondary hair follicles. Sebaceous glands are present in all mammals except whales and porpoises and are not present on the paw pad or nasal planum. Sebaceous glands may be simple or branched and are composed of 2-8 lobules. They are most numerous and largest near mucocutaneous junctions, in the interdigital spaces, near the coronet in horses, on the dorsal neck and rump/ along the mane in horses, on the chin (the submental organ) and on the dorsal tail (the tail gland/ supracaudal gland/ preen gland) in carnivores. The tail gland is an oval region on cats and dogs around the 5^{th}-7^{th} coccygeal vertebrae that has stiff, coarse hairs from simple follicles and where the skin is slightly yellow and waxy due to excess sebum from the large sebaceous glands. An excess of these glands and their secretions is the cause of stud tail.

Sebaceous glands are holocrine glands whose ducts are lined with squamous epithelium and open into the infundibulum of the hair follicle. The basement membrane of the gland contains the reserve cells that become lipidized as they mature and eventually disintegrate to form sebum. Lipid is accumulated from the circulation via two receptors: the LDL receptor and the FATP4 receptor found on sebocytes. Sebum secretion is under hormonal control: androgens induce hypertrophy and hyperplasia; estrogens and glucocorticoids induce involution; retinoids reduce sebum production.

Sebum is composed of triglycerides, wax esters, squalene, cholesterol and cholesterol esters and composition varies among species. Equidae are the only species with lactones present in their

sebum and epithelial surface lipid. Sebum mixes with resident lipase producing bacteria in the infundibulum (primarily *Propionibacterium* and *Staph* spp. in dogs) to produce free fatty acids which have antimicrobial properties. Sebum also functions to increase the softness and pliability of the skin, it retains moisture and provides hydrophobic protection. Sebum carries pheromones and in combination with sweat provides a physical and chemical barrier against pathogens. The musky odor and greasy feel associated with ferrets is due to increased sebaceous gland activity in this species.

Sebaceous adenitis

Sebaceous adenitis is an inflammatory condition resulting in T-cell-mediated, immunologic destruction of the sebaceous glands. The pathogenesis is not fully understood but does appear to have a genetic basis. Sebaceous adenitis is inherited as an autosomal recessive trait in Akitas and poodles. Other breeds (e.g.- Havanese, Lhasa apsos, chow chows and springer spaniels) may also be predisposed. Sebaceous adenitis has been reported in other species including humans, rabbits, cats, and horses. It may be associated with other diseases including hypothyroidism and Leishmaniasis.

Affected animals present with varying degrees of alopecia, hyperkeratosis, seborrhea, follicular casting, pruritus, and secondary infections. Long coated patients may initially present with a change in hair color and/ or a change in hair texture from curly/ wavy to straight. Symptoms often start on the head or cervical region and pinnae.

The diagnosis is made on histopathology. Inflammation is variable and dependent on breed and stage of disease. If present, inflammation is focused on the sebaceous gland or in the region of the sebaceous gland. In chronic cases the glands may be completely destroyed with minimal inflammation and perifollicular fibrosis.

No treatment is 100% effective but systemic cyclosporin has been shown to reverse histopathological changes in dogs. Other systemic treatments include fatty acid supplementation, systemic retinoids, vitamin A, as well as tetracycline and niacinamide. Topical treatments including keratolytic shampoos, propylene glycol, oil soaks, and topical spot-ons and sprays are often helpful and the combination of topical and systemic treatments usually results in the most significant improvement for patients.

EPITRICHIAL SWEAT GLANDS

Historically, epitrichial sweat glands were called apocrine glands and this is still the terminology used in some texts as well as much of the human literature. Strictly speaking, epitrichial sweat glands are NOT apocrine in their mechanism of excretion and are instead merocrine, so epitrichial is a more correct terminology in this context.

Epitrichial sweat glands are found adjacent to primary hair follicles and are increased in size where there is a decreased follicular density. Although they are located deep to sebaceous glands, their ducts empty into the infundibulum above the level of the sebaceous gland. Similar to sebaceous glands they are largest and most numerous near mucocutaneous junctions, in the interdigital spaces and over the dorsal neck and rump. In horses they are concentrated around

mucocutaneous junctions, in the submandibular region, near the mane and coronet. The volume of epitrichial sweat glands increases in thoroughbreds in the summer. There are no epitrichial sweat glands in rodents or ferrets and they are only present around the lips in rabbits.

These glands are coiled and saccular to tubular. The secretory cells are a singular row of flat to columnar epithelial cells with microvilli and secretory vacuoles that fill the cytoplasm. In horses, a water channel called aquaporin-5 has been identified in the secretory cells which facilitates rapid movement of fluid during thermoregulatory sweating. The duct is composed of a double layer of cuboidal cells. The secretory cells are surrounded by a single layer of fusiform myoepithelial cells in most species. The horse is unique in that the myoepithelial cells form a loose basket-weave around the gland and the gland has a rich blood supply. Epitrichial sweat glands are not directly innervated.

Although the glands are not directly innervated, neural control is the primary regulator of epitrichial sweating which is stimulated by adrenergic agonists. In the horse, autonomic adrenergic nervous control is the main mechanism of sweat control, but humoral control by adrenergic agonists secreted from the adrenal medulla during exercise is also important. There also appears to be some autocrine control of the secretory process. Equine epitrichial glands can become refractory to adrenergic agents if continuously exposed. Triggers for epitrichial sweating in dogs and cats are unclear. In horses, epitrichial sweating is induced by fever, exercise, heat, pain, hypoglycemia, hyperadrenocorticsm, and catecholamine release from excitement, stress or a pheochromocytoma.

Epitrichial sweat contains antimicrobial salts, pheromones, and IgA. In horses, epitrichial sweat contains a high concentration of protein, particularly glycoproteins called latherins, compared to other species.

Anhidrosis

Anhidrosis is the inability to sweat. It may have a sudden onset or may be more gradual. It is a serious condition that can result in life threatening hyperthermia in horses. It occurs most commonly in hot, humid climates (e.g.- Gulf coast regions of the USA) and is more common in thoroughbred and warmblood breeds. It is a conditioned response to continuously high levels of epinephrine that leads to desensitization, downregulation of cell receptors and aquaporin-5 and eventual degeneration of secretory cells. Macrolides, especially erythromycin, can also cause anhidrosis in foals being treated for *R. equi* infections. Initial symptoms include patchy to inadequate sweat response, reduced ability to cool down after exercise with persistent hyperthermia and tachypnea. Chronically, horses can have a dry coat with excess scale and partial alopecia of the face and neck. Acutely, animals can collapse and die. Diagnosis is based on history and response to intradermal testing with epinephrine, terbutaline, or other β-2 agonists at increasing doses (1:1,000,000 to 1:1,000). Normally, sweating would occur over the test site within minutes at all dilutions, in anhidrotic animals, sweating only occurs at the highest concentrations of epinephrine and only after 5+ hours. Treatment involves moving the patient to a drier, colder environment where recovery can be seen within 4-6 weeks.

ATRICHIAL SWEAT GLANDS

These are also called eccrine glands in the literature. They are the most important sweat glands for cooling in humans. They are only present in the paw pads of carnivores, rats, mice and hamsters. Horses do not have atrichial sweat glands.

Atrichial glands are small and tightly coiled and reside in the deep dermis and subcutaneous tissues. The secretory cells are cuboidal to columnar and are found in a single layer, lined by a singly layer of fusiform myoepithelial cells. The excretory ducts connect directly to the paw pad surface and consist of a double layer of cuboidal cells. They have a rich nerve supply and sweating increases due to agitation and excitement.

CERUMINOUS GLANDS

These are modified apocrine (epitrichial) glands. Cocker spaniels, English spaniels and Labrador retrievers have a higher concentration of ceruminous glands compared to Greyhounds and mongrel dogs. These glands may become hyperplastic with chronic otic disease and may initially appear as prominent white specks along the canal. Cocker spaniels are particularly prone to ceruminous gland hyperplasia and ectasia in end stage ear disease whereas other species have an increased predisposition toward fibrosis with end stage otitis. In one study (Kaimio et al.), 87% of American cocker spaniels with otitis externa as well as some clinically normal cocker spaniels with no history of otitis or skin disease displayed evidence of ceruminous gland hyperplasia and ectasia.

ANAL SACS

Anal sacs are paired invaginations of the skin located between the muscular layers of the anal sphincter. They have a single duct that opens at the mucocutaneous junction in dogs and lateral to the anus in the cat. The anal sacs and ducts are lined by keratinizing squamous epithelium. The walls of the sacs have fibrous connective tissue. Sebaceous and epitrichial sweat glands line the sacs. The contents of the anal sacs are a mixture of cellular debris as well as fatty and serous secretions from the sweat and sebaceous glands. They are normally expressed during defecation and may be used for defense (e.g.- skunk) and territory marking (e.g.- wolves).

PERIANAL GLANDS

Also called circumanal glands or hepatoid glands (the mature cells in the gland lobules resemble hepatocytes), the perianal glands are modified sebaceous glands. They are ductless glands located in the perianal region. Hepatoid glands are also found on the dorsal and ventral aspects of the tail, around the prepuce, around the mammary glands, on the caudal aspect of the hind limbs and on the midline of the dorsum and thorax. The glands are androgen responsive.

ARRECTOR PILI MUSCLE

The arrector pili muscle is a band of smooth muscle that attaches to the cranial bulge region/ at the lowest portion of the follicular isthmus. It inserts onto the follicular epithelium via elastic fibers. It is anchored in the superficial dermis where the smooth muscle fibers splay between collagen bundles and anchor to the ECM via integrins. These muscles are largest on the dorsal

neck and rump and contraction (typically in response to epinephrine and norepinephrine) results in piloerection.

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