Chapter 2

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“Never be ashamed of the scars that life is left with. A scar means the hurt is over, the wound is closed, pain endured”

- Anonymous

2.1. Skin as an organ

Skin, being the largest organ, covers the external surface of the human body. It serves as a major site of interaction between internal and external environment. It plays a key role as a protective barrier through which it supports the internal tissues from any trauma or injury, radiations such as UV, extreme temperatures, toxins and also from pathogens like bacteria. Skin prevents the entry of dangerous chemicals and invading organisms like bacteria, virus etc., because of its physical structure. It also offers resistance against shocks, for the more sensitive tissues lying underneath it.

Morphologically, thin and thick types of skin can be recognized. Variations in the thickness of skin can be seen based on anatomical site, gender and age of the person. Thickest skin (1.5 mm thick) is present on the palms and soles of the feet while the thinnest (0.05 mm thick) skin is found on the eyelids and in the post auricular region.

Skin also presents sexual dimorphism as skin of male individual is naturally thicker than female in all anatomic sites. Relatively thin skin is observed in children, which thickens progressively until 40-50 years of life when it begins to become thin (Burns et al., 2004).
At birth itself, typical multilayered skin is formed. Embryologically, a single cell ectoderm and underlying mesoderm begin to multiply and differentiate during fourth week of development (Carlson, 1994). Epidermis is a derivative of surface ectoderm, but it is populated by specialized cells from different embryonic origin. These cell populations include melanocytes, Langerhans cells and Merkel cells. Melanocytes are pigmented cells and of neural crest in origin. Langerhans cells are antigen presenting cells, are of bone marrow in origin. Merkel cells are pressure sensing cells derived from neural crest cells (Carlson, 1994).

Structurally, skin presents two mutually dependent layers, the epidermis and dermis, which rest on a fatty subcutaneous layer. Microscopically, dermis of the skin presents two distinct layers. The more superficial layer is the papillary layer, predominantly comprising of capillary vessels, few elastic fibers, reticular fibers and some collagen fibers. The deeper layer, reticular dermis, consists of abundant dense connective tissue with larger blood vessels, coarse bundles of collagen fibers arranged parallel to the surface and intimately interlaced elastic fibers. The cell populations of reticular layer are fibroblasts, mast cells supplemented with nerve endings, lymphatics and epidermal appendages. These components of the dermis are surrounded by gel-like ground substance made up of hyaluronic acid based mucopolysaccharides, glycoproteins and chondroitin sulfates. Therefore, the difference in the dermal thickness results in varying thickness of the skin due to its thinning of the skin is characterized by the deficiency of elastic fibers, loss of epithelial appendages, and ground substance of the dermis (Burns et al., 2004). This
is in contrast to changes in the thickness of the epidermis which is relatively constant, irrespective of the anatomical location and it is also constant throughout life.

The dermal-epidermal junction present flattening as their histopathological feature. This manifests the reduction in the ability of the nutrients to cross the layers which could be visualized as the photo-aging consequence. The presence of disorganized collagen fibrils showing a pronounced reduction in the contents of collagen levels and abnormal elastin storage together is termed solar elastosis. (Rabe et al., 2006; Bauman 2007).

Collagen and elastic fibers are the two major dermal connective tissue contents. Both these fibers play a vital role in supporting and to maintain its and elasticity which can avoid wrinkles, age spots, and prevent the appearance of other signs of aging. These proteins are abundant in youth but deplete as the age advances. Dermal collagen and elastic fibers are responsible for providing tensile strength to the skin to resist injury from trauma. They also allow the skin to return to its resting state after being stretched or compressed. Therefore, these characteristic features are known to exhibit their functional significance during wound healing process.

2.2. Dermal collagen fibers

Collagen is the most abundant and ubiquitous protein in nature and also a major constituent of the human body. Though they are found throughout the body, their types and organization are dictated by the functional role played in a particular region or structure. In the skin, being the major content of the dermis, it displays
morphologically different texture both in reticular and papillary layers. Normal adult human skin contains type I and type III collagen in an approximate 6:1 ratio. With age, the proportion of type III collagen increases in the ratio, probably due to impaired synthesis of type I collagen in aged skin (Lovell et al., 1987). Of the total collagen, type I collagen accounts for about 85% of it and the remaining 15% is by Type III (Carlson, 1994).

Biochemical investigations have confirmed the presence of type III collagen in papillary dermis and type I collagen in reticular dermis (David & Rosalie, 2008). However, Immuno-fluorescence tests done to localize the various types of collagens in normal adult skin confirmed the predominant occurrence of type I collagen in entire dermal layer and type III collagen in the papillary dermis (Wilhelm et al., 1977). The skin of the human fetus contains large percentage of type III collagen, in contrast to the skin of an adult (David et al., 2008).

The arrangement of dermal collagen is mostly in random form. It appears less parallel in deep dermis when compared to superficial dermis of the normal skin (van Zuijlen et al., 2003) and its bundles show a basket weave like pattern with random organization (Linares, 1996).

Similar to its diverse morphology, its pattern of distribution also differs within the dermis. Thin collagen fiber filaments in a loose meshwork arrangement are seen in papillary dermis and thick coarse collagen bundles are the main feature of the reticular dermis. Structural display of dermal collagen is therefore designed for its
major role in strength and function of the human integument and its importance is widely recognized.

Significant correlation between concentration of collagen and age as implicated by presence of thinner filaments of collagen in reticular dermis of persons aged between 20 to 40 years than older persons has been observed by the histomorphometric analysis (Branchet et al., 1991).

**Biosynthesis of collagen**

The polypeptide precursors of the collagen molecule are formed in fibroblasts and are secreted into the extracellular matrix. After enzymatic modification, the mature collagen monomers aggregate and become cross-linked to form collagen fibers. A complex step involved in the biosynthesis of collagen is summarized in the following flow chart (Pamela et al., 2005).
Flow chart: Summary of collagen bio-synthesis (Pamela et al., 2005)

### 2.3 Dermal elastic fibers

Elastic fibers appear in the dermis much later than the collagen fibers. Unlike collagen fibers, that are tough and have high tensile strength, elastic fiber with the elastin protein in it exhibit rubber like nature. They can be stretched to several times their normal length, but are capable of recoiling to their original shape when the stretching force is relaxed (Kielty et al., 2002). Elastin is secreted as monomer but rapidly matures to form polymer by the cross-linking process between tropoelastin units as well as with other elements such as fibrillins, fibulins etc (Taddese et al., 2008).
Structurally the dermal elastic fibers present two components: the micro fibrils and the elastin matrix. The micro-fibrils are electron dense and are aggregated at the periphery of an elastic fiber. Elastin is electron lucid, amorphous substance that incorporates the microfibrils within it. Microfibrils accounts for 15% of elastic fiber, whereas elastin makes up 85% of elastic fibers. Histologically, it is the elastin that stains with the biological stains. Elastic fibers undergo extensive changes during life. These changes represent either aging of skin or elastotic degeneration of elastic fibers. The elastotic degeneration is often due to chronic sun exposure. In aged persons the fragmentation and disintegration of elastic fibers may also be observed (David & Rosalie, 2008).

Elastic fibers play a significant functional role by resisting deformational forces and returning the skin to its resting shape (Carlson, 1994). Dermal elastic fiber network is a strong determinant of skin pliability, texture and quality. But, they are not adequately regenerated following a burn injury. In addition to its structural and mechanical roles, elastin has natural cell signaling properties that uphold a varied range of cellular responses including chemotaxis, cell attachment, proliferation and differentiation (David & Rosalie, 2008).

The elastic fiber content in the dermis also differs and its distribution is found to be depending on the topographic area of the dermis as it is different among the subjects (Pasqualil & Baccarani, 1997).
2.4. Staining properties of collagen and elastic fibers

Both, collagen and elastic fibers are formed elements that are found in intercellular substances of connective tissues. In majority of staining methods these fibers are demonstrated by the contrasting colors. This principle highlights these structures and enables its easy identification and differentiation from other structures.

van Gieson’s stain is a very useful and special stain for the collagen fibers. The picro-fuchsin solution of van Gieson is also used as the counter stain in demonstration of other connective tissue fibers as in Verhoeff’s staining method of elastic fibers to exhibit collagen fibers. This combined usage of special stains as a single technique of stain has been utilized in the current research work by performing Verhoeff - van Gieson method for the collagen and elastic fibers.

Mechanism of action of Verhoeff - van Gieson staining

Verhoeff’s stain is used to demonstrate the elastic fibers which appear black in color and the van Gieson stain is for collagen fibers which appears pink and its shades of color

2.4.1. Principle of collagen stain by van Gieson method

Collagen is stained selectively by acid aniline dyes (acid fuchsin) from fairly strong acid solution (picric acid). The small molecules of picric acid stains all the unstained tissue components rapidly including collagen, but are firmly retained in close textured tissue parts. The larger molecules of acid fuchsin displace picric acid from collagen. Collagen is a structure which can be penetrated easily by acid fuchsin by displacing picric acid. Since the Picric acid used in the stain is saturated, acid
fuchsin dye cannot displace it from cytoplasm, smooth muscle etc. Selective staining of collagen involves three factors (Lynch, 1969; Brown GG, 1978; Bancroft and Gamble, 2002).

a. **pH**

Acid fuchsin and picric acid are both anionic substances which stain cationic tissue components. Picric acid is more acidic than acid fuchsin.

b. **Molecular weight of the dyes**

Dyes with high molecular weight have a tendency to stain more easily by penetrating tissues like collagen. Dye with low molecular weight stain less penetrable structures such as cytoplasm and muscles.

c. **Tissue permeability**

Acid fuchsin can stain more easily penetrable component like collagen by displacing picric acid. However, acid fuchsin cannot displace saturated solution of picric acid from cytoplasm or smooth muscles.

**2.4.2. Principle of elastic fibers staining by Verhoeff’s method**

Elastin, the major protein content of elastic fibers, is cross linked by dis-sulphide bridges. It is oxidized and converted to sulfonic acid derivatives by iodine (oxidizing agent). These derivatives being anionic are strongly basophilic and are capable of taking up a basic dye, haematoxylin.

Verhoeff’s haematoxylin, is an iron haematoxylin incorporated with the ferric chloride mordant. Ferric chloride also acts as an oxidizing agent to convert haematoxylin to haematein. In addition to this, differentiation is also carried out by iron salts because of strong oxidizing ability of the solution containing ferric salts.
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The elastic tissue is readily penetrated by the stain in the form of a metallic dye-lake complex. The iodine in the solution accelerates the conversion of haematoxylin to hematein, which in turn acts as a trapping agent for the cationic dye-lake. This mechanism eventually retards its extraction during differentiation with ferric chloride (Lynch, 1969; Brown GG, 1978; Bancroft and Gamble, 2002).

The major disadvantage of the Verhoeff's haematoxylin is its over oxidation property. To prevent it, the mordant – oxidant solution and haematoxyllin solution is prepared separately and are combined together with Verhoeff's iodine solution just before use.

Resulting colors of tissue structures stained by Verhoeff-vanGieson special stain

<table>
<thead>
<tr>
<th>Elastic fibers</th>
<th>Black in color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen fibers</td>
<td>Red (or pink) in color</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Grey to black in color</td>
</tr>
<tr>
<td>Muscles</td>
<td>Yellow in color</td>
</tr>
</tbody>
</table>

2.5. Skin injury and wound healing

Wound healing is a complex process that begins at the moment of injury. Various events that take place during the period of wound healing can be briefly summarized as the following.

The normal healing cascade begins with the process of haemostasis and fibrin deposition. This in turn activates the inflammatory cell cascade. The inflammatory phase is followed by attraction and proliferation of fibroblasts and collagen
deposition. In the final stage, remodeling by collagen cross-linking and scar maturation takes place. This orderly sequence of events may be altered due to certain pathological conditions such as fibrosis or chronic ulcers. Opposition of wound edges marks the primary healing process and hence it is the most crucial stage in the process of wound healing (Norman et al., 2008). Tissue loss results in separation of wound edges, that in turn lead to failure of opposition and hence rapid closure is not possible. In such cases, the angiogenesis and fibroblast formation forms the granulation tissue. This event contracts the wound to reduce wound area and facilitates epithelialization, which finally results in wound closure. The shrinkage of wound area by the contracture results in the appearance of disfiguring scar (Norman et al., 2008).

Three major types of wound healing are healing by primary, secondary and tertiary intentions.

The process of healing by primary intention (primary union) is manifested when there is no tissue loss. The incised wound is held together by a blood clot or sutures or surgical clamps. The epithelial cells at wound margin seal the wound to cover it. This event is concluded by the formation of thin scar as a result of maturation of granulation tissue.

Healing by secondary intention (secondary union) is initiated usually when there is a tissue loss. Here, the healing process is more prolonged than the primary intention. Since there is an extensive open area, inflammatory reaction is also more widespread. Resulting granulation tissue is fragile and thus may bleed easily. As the
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granulation tissue matures, marginal epithelial cells move toward the centre of the wound and help in closing the defect. Meanwhile the surrounding skin also moves toward the center of the wound to close the defect.

Healing by tertiary intention (delayed primary closure) is characterized by gradual filling of a wound gap by granulations and cicatrix materials (Niessen et al., 1999).

2.6. Scar formation and maturation

Scar is the result of overproduction of collagen fibers during the wound healing process. It is an unavoidable consequence of wound repair resulting from tissue restoration by the repair mechanism. Scar tissue replaces the collagen of granulation tissue and newly healed wound. This process is dependent on factors like age of the individual, the topographic site of the wound as well as the duration taken for the wound to heal, in addition to the direction of the scar and the tension across it (Norman et al., 2008).

As body replaces and reorganizes the structure of collagen fibers after initial healing, scar undergoes a process of thickening and contraction. Once the reorganization process completes, scar tissue softens and fades in color. This process is termed as scar maturation process. Normal course of scar maturation involve 2 necessary factors; strength and appearance. As far as strength of the scar is concerned, scar tissue is not as strong as in normal uninjured skin. Though it gradually increases its ability to withstand injury, it fails to attain normal strength.
The scar appears red and slightly raised during the period of maximal production of collagen. As the change in the rate of collagen production is attained, degradation also occurs. Normal healing result is manifested with the normal type and amount of collagen production in the affected area. This process results in fading of redness and softening of the scar on the surface of affected area (Niessen et al., 1999).

Maturation of scar occurs as a sequence of distinct phases involving both microscopic and macroscopic stages over the course of one year with the varied rate of maturation. It has been identified that, in aged subjects (more than 55 years), the scar maturation takes place at an accelerated rate, while in the younger individuals (less than 30 years) the rate of scar maturation is observed to be lower (Bond et al., 2008).

Scar maturation also depends upon the behavior of new cell population and the quality of deposited connective tissue. Proper interaction between these events during the process of repair corrects collagen deposition pattern, which is a crucial event of wound healing for a better aesthetic result (Ehrlich, 1995).

The scar of a wound inflicted in the trunk of a young individual matures slowly and scars that have a lot of tension across them will take longer time to mature. Conversely, scars in older people, in thin skinned areas, those which heal rapidly by primary healing and have minimal tension across them mature quickly. Therefore the knowledge of variations in the nature of scar maturation for general surgeons or plastic surgeons is important to make the patient understand regarding the likely progress and outcome of their scar (Norman et al., 2008).
2.6.1. Types of scars

Varieties of scars have been identified in the clinical set up. Morphologically the scars can be atrophic or hypertrophic. Atrophic scars are in the form of sunken recess in the skin, which presents pitted appearance. Hypertrophic scars occur as a result of overproduction of collagen, which causes the scar to be raised above the surrounding skin. Though they are elevated but confined within the boundary of the site of injury or incision. They are amongst the most common problems after injury resulting from altered process of normal cutaneous wound healing. Hypertrophic scars are characterized by proliferation of dermal tissue with excessive deposition of fibroblast-derived extracellular matrix proteins, particularly collagen over long periods, and by persistent inflammation and fibrosis (Bishara, 2007). Keloids are more serious form of hypertrophic scarring as they grow beyond the boundary of the original injury or incision. Keloids are also regarded as dermal fibrotic disease in which elastic fiber assembly is disrupted as a result of lack of elastic fibers (Ikeda et al., 2009). Keloids occur when the body continues to produce masses of collagen after a wound has healed. It can also be caused by surgery, a wound due to an accident or even from body piercing.

2.6.2. Collagen and scar

Both hypertrophic scar and keloids express excess collagen. Hypertrophic scars usually contain predominantly type III collagen lying parallel to the epidermal surface (Slemp and Kirschner, 2006). Keloids are predominantly made up of disorganized type I and III collagen (Sephel and Woodward, 2001; Slemp and Kirschner, 2006). This shows, both scar types exhibit overproduction of multiple
fibroblast proteins leading to either pathological persistence of wound healing signals or failure of corresponding down regulators of wound healing cells (Sephel and Woodward, 2001). Excessive lay down of collagen and stretching of scar is high in more elastic skin as in children and young subjects (Berman et al, 2008).

A study by Lutz et al (1978), comparing the composition of collagen type between normal and pathological scars reported a slight increment of type III collagen in normal scar and its significant increase in case of a pathologic scar. Mechanical tension has been proposed to play a major role in the orientation of the bundles in scar tissue (Longacre et al., 1968).

Histopathological studies of a mature scar tissue is likely to show collagen bundle orientation parallel to the epidermis rather than randomly organized pattern as seen in normal dermis (Linares, 1996). Excessive over proliferation of fibroblasts and collagen production during prolonged process of inflammation results in unacceptable scars (Parlange, 2007). Nature of excessive lay down of collagen is often associated with the stretching of scar. It is more in the skin with abundant elastic content as seen in children or young adults (Berman et al 2008).

During scarring, the collagen bundles align themselves in a parallel manner and in the direction of mechanical tension. This factor is one of the major causes in onset of challenging scar formation that usually occurs at joint regions. However morphological difference of collagen in scar tissue and in normal skin between joints and control areas has not been established (van Zuijlen et al., 2003). Mechanical stimulus is known to induce changes in collagen in its organization, thickness, type
and in the process of synthesis (Guidry & Grinnel, 1985; Sawhney and Howard, 2002). Cell to matrix interaction directs the pattern of collagen fiber in the scar similar to that of dermis to provide better wound care result (Ehrlich and Kummel, 1996)

### 2.6.3. Elastic fibers and scar

Elastic fibers are not detected in the granulation tissue synthesized during early phases of wound healing, indicating that a certain differentiated status of fibroblasts is required to produce elastic tissue (Zheng et al., 2006). Decreased amount of elastic fibers is known to reduce the tensile strength in scar tissue (Levenson et al., 1965). Shuttleworth et al., (2005) however reiterate that, it is the abnormal elastic fiber network rather than decrease in its content, which causes reduced tensile strength of scar tissue. Thus, manipulation of elastic fiber synthesis, its deposition and organization could assist in better tissue restoration.

Excessive wound contraction and post grafting scarring usually occurs as a result of lack of organized elastic fiber network as observed in dermal component of artificial skin substitutes (Compton et al., 1989). Role of elastic fibers in the improvement of scar appearance, thence in better wound healing outcome has been demonstrated by the application of elastin based dermal substitutes (Rnjak et al., 2011). This is in fact a combined effect of its mechanical and signaling properties.
2.6.4. Collagen – elastic fiber co-relation with scar

The wound closure is also facilitated by stretching of adjacent skin. Though the exact mechanism of tissue adaptation to stretch is still not clear, role of collagen and elastic fibers in terms of their orientation and morphology in stretched and non-stretched skin is being correlated to wound healing related complications. A research study in this context reported the more parallel alignment of collagen fibers in stretched skin and stretched scar than in non-stretched skin and non-stretched scar. Similar pattern of alignment was also observed for elastic fibers after the stretch event (Verhaegen et al., 2012).

It has been also noted that, in response to stretch, both collagen and elastic fibers realign in a parallel fashion. Therefore in normal skin, thicker bundles and increased space between the bundles are found due to underlying mechanisms of adaptation to stretching (Verhaegen et al., 2012).

The fractional volume of collagen fibers is always higher in females than in males except for the 2nd and 3rd decades of life. Collagen fibers density also increases with age in both sexes up to 30-40 years. In reticular dermis of both sexes there is an increment of elastic fibers density in the first decade of life, followed by a marked drop in males (Vitellaro- Zuccarello et al., 1994). The clinical observation reveals that scar problem is more in children and young age group patients.
2.7 Skin lines

Wrinkles and skin creases are produced by muscular contraction. These skin lines lie mostly at right angles to the underlying muscular vector force. Relaxed skin tension lines (RSTL) are formed during relaxation of the skin and usually follow the different directions. RSTL are created by the natural stress on the skin from underlying structures (Fongo et al., 1966). Skin lines and creases are apparent at joints.

Lines of minimal tension (also termed as natural skin creases or wrinkles) are the lines that are visible externally and are formed by repeated bending of skin from muscular contraction until a permanent cutaneous crease has formed with adhesions between the dermis and deeper tissues (Tadros, 2007).

In certain regions of the body, surgical wounds heal with a better and less noticeable scar, if they are lying in a specific direction. This is because of a number of factors involving various skin lines and morphology of underlying collagen and elastic fiber content. Skin tension is due to protrusion of underlying structures and direction of underlying muscle, joint movements.

2.7.1. Relaxed Skin Tension Lines (RSTL)

RSTL are the skin tension lines which follow the furrows formed when the skin is relaxed. In living individual these lines frequently coincide with the wrinkle lines and therefore form a guide in planning elective incisions (Standring, 2005). They are defined by the micro-architecture of the skin, such as the alignment of collagen and elastic fibers, and with a minor role of influence of subjacent bone and soft tissue.
content. RSTL’s have the greatest cooperative influence on wound tension and final healing process. The RSTL’s generally lie parallel to external skin wrinkles but represent a distinct entity from the later and occasionally give rise to conflicts in some areas such as; at the lateral canthus, nasal tip and the glabella (Borges, 1984). If the resulting scar is to be aesthetically acceptable, it is suggested to make the incision parallel to the natural RSTLs (Standring, 2005).

2.7.2. Langer’s (cleavage) lines:

Langer’s lines were defined by Karl Langer by observing transformation of circular wound into an elliptical shape as rigor mortis developed in the cadavers. Langer’s postulation was that skin excisions directed along the long axis of these wounds results in a more favorable healing (Borges, 1984). Hence, the concept of Langer’s lines is based on minimum flexibility of skin along the cleavage lines (Figure 1a). These lines correspond to the coalition of most of the dermal collagen fibers. Understanding the direction of Langer’s lines in the specific area of the skin before surgical incision holds cosmetic importance. Hence, a surgical incision is carried out in the direction of Langer’s lines as the incisions made parallel to these lines generally heal better and produce minimum scarring. The exact direction of these lines often cannot be mapped in some regions of the body as there are differences among individuals. Clinical practice has also shown that these lines are not always precise. Therefore some surgeons opine that the concept of Langer’s lines is of historical interest (Ira et al., 2009).
Cleavage lines of the skin are often referred to as Langer’s line by the clinicians. Microscopic study on pattern of collagen fibers at cleavage lines revealed linear arrangement with regular and uniform pattern while in the area where these cleavage lines are ramified, a lack of regular direction was observed. This is achieved mainly by the collagen fibers in the reticular layer which are thus considered to be responsible for the morphology of cleavage lines (Namikawa et al., 1986).

2.7.3. Kraissl’s Lines

Kraissl’s lines are said to be exaggerated assumptions of normal wrinkles (Figure 1b). These lines are subjective as individual features vary due to the differences in contour or muscular development (Borges, 1984).

Figure-1: Schematic diagram to show relation of Langer’s cleavage lines (thick, continuous lines in a) and Kraissl’s anti - relaxed skin tension lines (thick lines in b) with respect to relaxed skin tension lines (broken lines) (Adopted from Borges, 1984).
Though anatomists and surgeons have attempted to produce a body map to indicate the best direction for elective incision to obtain most aesthetic scar, these maps frequently differ in regions, especially on face (Standring, 2005).

2.8. Importance of dermal collagen and elastic fiber content evaluation

Even though the importance of collagen for the strength and pliability of skin along with its role in the formation and behavior of scar related complications is well established, no uniform evaluation method is available for its quantification to understand pattern of its distribution. For research that focuses on wound healing and scar formation, polarized light method of evaluation is considered to be the most established method. According to some research studies, the accuracy of image analysis technique (such as Fourier image analysis) is said to be at par with observer ratings. Histomorphometric evaluation of dermal collagen by image analysis method is said to give promising result as compared to the subjective histological evaluation reported by several experts. Further, if observers perform an evaluation by conventional light microscopy, at least three observers are required to attain an acceptable inter-observer reliability (van Zijuljen et al., 2002). Although the collagen fiber density evaluation normally does not show changes with age, decrease in its content was reported with the observation in individual variations (Branchet et al., 1991).

The fractional area occupied by the selected tissue structure that is positively demonstrated by appropriate biological stain is suitable for measurement by image analysis using ‘TissueQuant’ software (Keerthana, 2012). This software works on the
principle of staining intensity quantification and outcome result expresses the percentage area occupied by the selected structure in the given area. The description of the software with the details of its calibration and validation are described by Keerthana (2012). Color based quantification and segmentation based analysis property of TissueQuant has been used in many studies (Chakravarthy et al., 2009; Sreenivasulu et al., 2011; Keerthana et al., 2012, Naveen et al., 2012 and Prabhu et al., 2014). This tool is proved to be superior in performance for color segmentation than commonly used commercial software such as Adobe Photoshop (Keerthana, 2012).

Based on their structure, both mature elastin and collagens are complex compounds. Due to this nature, the biochemical analysis on these biomolecules is a challenging tasks as analytical setbacks the relevant information (Samuel et al., 2009).

Morphometric assessment of collagen and elastic fibers of abdominal skin following post-bariatric abdominoplasty done on obese females for weight loss revealed the fact of presence of soft skin lacking sufficient collagen fiber network without damage to elastic fibers. The depletion of the collagen was seen more in epigastrium than hypogastrium (Orpheu et al., 2010).

According to a report by Gogly et al., (1997) the diameters of elastic fibers increased regularly in the skin among different age group. Similarly, collagen diameter in mid-dermis also increased strongly with age. But the area fraction occupied by the collagen bundles significantly decreased from 50 to 75 years. Since,
the clinical observation shows that the scar in children and young individuals does not settle well and most of the time they are unacceptable, above mentioned findings of the study indicate a strong correlation between scar appearance and behavior with dermal collagen and elastic fiber content (Naveen et al., 2012).

Clinical research on collagen and elastic fiber content of abdominal skin after surgical weight loss reported to be exhibiting undamaged elastic fibers but moderate increase in epigastrium. Conversely, pre-operative obesity was negatively correlated with hypogastric collagen concentration (Simone et al., 2010). This shows that skin stretching due to obesity (fat deposition under the skin) is opposed by elastic fibers. After losing the fat from subcutaneous tissue the skin becomes loose. Therefore, it may be indirectly inferred that the skin exerts a stretching force on the scar, so that its behavior and appearance is altered in proportion to the force which may vary depending upon the elastic fiber content and inherent property of elastic tissue (Naveen et al., 2012).
2.9. Lacunae in the literature

Much importance is attached to the appearance of skin, especially in the current day scenario. Medical conditions affecting the skin have marked effects, not only on our state of well being but also on the ways we interact with other people and on our suitability for certain occupations. In-spite of the existence of sophisticated treatment and availability of multi drug therapy, the consequences following the wound healing resulting in compromised scar behavior is still a mysterious task for both general and aesthetic surgeons.

Most of the studies on wound healing and scar formation reflect the qualitative features of collagen and elastic fiber structure, but none attempted to analyze the quantitative fraction of them.

- In our literature search we could not come across any data on histological observation of dermis in different regions of the human body and no study has attempted to quantify the dermal collagen and elastic fibers in two different orientations of the skin samples.

- Histomorphometric reports on quantification in terms of percentage area occupied by collagen and elastic fibers in different areas/regions and in different directions are also scanty.

- No study is reported in the literature about correlation evaluation between collagen and elastic fiber content as well as in their pattern of distribution between two different orientations.
Lacunae in the literature have also been noted with respect to the analysis of significance of differences in collagen and elastic fiber content of dermis in different parts of the human body with their clinical implications.

No measure and comparison has been attempted so far in the ratio values of dermal collagen and elastic fiber content between two different directions of skin sections.

Hence, the present study is an attempt to investigate and fill the lacuna found in the available literature.