UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES

LIGHT MICROSCOPIC STUDY OF INDIGENOUS GHANAIAN FEMALE
SCALP HAIR WITH RESPECT TO STYLING PROCEDURES

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DECLARATION BY THE CANDIDATE

I hereby declare that this thesis is the product of research I have personally undertaken under supervision and that neither the whole document nor part of it has been presented anywhere else for a degree.

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DECLARATION BY THE SUPERVISORS

We hereby declare that the practical work and presentation of this thesis were supervised in accordance with guidelines on supervision of thesis laid down by the University of Ghana.

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DEDICATION

This work is dedicated to the memory of my late sister Ms. Florence Aba Essel. I also dedicate this work to Mr. Paul Atiah and Mrs. Sethina Adjetey for the instrumental roles they played in getting this document completed in its current state and on time.
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ABSTRACT

**Background:** Microscopic examination of hair has been useful in personal identification in the forensic sciences for years. However, increasing artificial modification of scalp hair, especially by women for the purposes of beautification, threatens its relevance in this vein. Microscopic characterization of such hair could provide reference data that could help maintain its usefulness in personal identification in the forensic sciences and other areas.

**Aim:** The study determined the effect(s) of artificial modification on the microscopic structure of scalp hair.

**Methodology:** 480 hairs, obtained from five scalp areas of 96 indigenous Ghanaian females, were categorized into 3 groups (Natural unstyled, Natural styled and Relaxed styled) based on the modifications the hairs had been subjected to. The hairs were then prepared for light microscopy and microscopic features were examined.

**Results:** Shaft diameter decreased from Relaxed styled hair to Natural styled hair. The vertex had the largest shaft diameter and the right temporal, the smallest. The highest incidence of continuous medullation was recorded in the Natural styled group and the lowest, in the Relaxed styled group which also had the fragmentary type of medulla dominating. Both medullary diameters and indices increased from Relaxed styled to Natural unstyled. A positive Pearson’s correlation between shaft and medullary diameters existed for Natural unstyled (r=0.320, p=0.011) and Natural styled hair (r=0.235, p =0.022) but not Relaxed styled (r=0.122, p =0.2). The order of decreasing scale integrity was Natural unstyled, Natural styled and Relaxed styled. Proximal shafts had well preserved scale characteristics than distal shafts.

**Conclusion:** Hairs subjected to different styling procedures have microscopic characteristics with which they can be distinguished.
CHAPTER ONE

INTRODUCTION

1.0 Overview: Hair in human interactions

Physical appearance plays a vital role in human interactions (Knapp et al., 2014). It creates initial impressions and determines the course of relationships prior to any verbal exchanges (Reis & Sprecher, 2009). Following the establishment of a relationship, physical appearance again, provides cues that determine the ongoing attraction and perception of the interactants about the character of another person (Reis & Sprecher, 2009). It is thus little wonder that despite the adage ‘do not judge a book by its cover’, one falls victim to judging individuals by their appearance at one point or the other.

According to Trüeb (2006), hair constitutes an important part of the overall appearance of a person. The same face can make different impressions as the hairstyle of a person is varied, because human scalp hair frames the face (Choe & Ko, 2005; Rogers & Avram, 2008). Hair is thus a vital tool that influences the physical attractiveness of a person (Maffei et al., 1994; Patzer, 1988; Grimalt, 2001).

A study by Mercer (1987) showed that hair on the scalp has significant bearing on the self image of an individual. The condition and style of scalp hair influences the perception of others about the wearers because hair is one of the few characteristics that are manipulated in length, colour and style (Bolduc & Shapiro, 2001; Trüeb, 2006).
Hair also serves as a medium through which significant statements about ‘self’ and society and the codes of values that bind them and others are made (Mercer, 1987). Work by Paus and Cotsarelis (1999) revealed that, in most human populations, hair plays an important psychosocial role. Consequently, the growth characteristics and distribution of hair, for example hair loss and or excessive hair growth, have implications on social acceptability (Paus & Cotsarelis, 1999). It has been indicated by several studies (Cash, 1990; van der Donk et al., 1991; Cash et al., 1993) that hair loss (alopecia), for instance, creates emotional and psychological stress for millions of men and especially women. These stress, according to Hadshiew et al. (2004), lead to secondary morbidity (de Koning et al., 1990). There is evidence that stress due to hair loss is often akin to that due to severe chronic illness or life-threatening disease.

Hair is a characteristic attribute of mammals and except for the glabrous skin of the lips, palms and soles, the skin of mammals is covered with hair. Humans, however, compared with other primates, appear noticeably less hairy (Randall, 2007). According to Randall (2007), this relative hairlessness of humans is attributable to the reduction in structure of the body hair so that tiny, virtually colourless hair (vellus hair) covers many areas. Morgan (1982) opines that human hair must have become shorter and thinner since humans have about the same density of hair follicles expected of an ape of the same body size (Schwartz & Rosenblum, 1981; Rantala, 2007). Humans are therefore termed the ‘naked ape’ (Randall, 2007).

The scientific conundrum as to why humans lost the hair on the body remains to be answered. In spite of the numerous theories proposed, none has been generally accepted. Most of the existing theories, however, have explained human ‘nakedness’
on the assumption that it was an adaptation to the savannah environment (Rantala, 2007).

1.1 Women and hair

Women are known to make attempts at beautification by focussing on the skin and associated appendages (Olasode, 2009). Hinsz et al. (2001) however opine that many magazines of women present the conventional wisdom that the hair forms part of the identity and self-image of a woman. Consequently, a desire for a change in image in women is accompanied by a preparedness to change the hair in terms of style and length (Hinsz et al., 2001). According to Roseborough and McMichael (2009), women commit considerable time and expense to daily hair grooming, both at home and the hair salons, for special occasions and everyday appearances. This tradition of women could be explainable by the fact that beautiful and healthy scalp hair generally expresses femininity irrespective of culture of the individual (Aboagye, 2011). The value of hair to women seems to be succinctly expressed in the words of Martin Luther that ‘hair is the richest ornament of women’. This worth of hair could probably explain why women suffer more emotional and psychological stress for disorders of hair growth and distribution compared to men (Cash, 1990; van der Donk et al., 1991; Cash et al., 1993). According to Cash et al. (1993), the increased distress in women could emanate from both social and self-inflicted pressures for physical attractiveness. Besides, the increased distress could also originate from a concern about a deviation from what is considered normal female appearance (Cash et al., 1993).

It has been reported by Roseborough and McMichael (2009) that the practice of hair care among women is influenced by factors such as convenience, ease of styling, occupation, recreational activities and climate. In addition, the study found that the
practice of hair styling is determined by the characteristics of the hair and personal preferences. Moreover, prevailing cultural trends may influence styling practices (Roseborough & McMichael, 2009). The list of types of hairstyle is endless ranging from the very simple to sophisticated ones (Rigoletto et al., 2012). Rigoletto and colleagues explained the availability of these numerous hairstyles to be due to the creativity of an ever evolving salon industry and the technical advancements in the personal care industry. The creativity displayed by formulation chemists, as well as the everyday grooming behaviours of the average consumer, were the other reasons the study gave.

1.2 Hair care practices among Ghanaian females

The practices of hair care among Ghanaian women parallels those of the African-Americans women as described by Roseborough and McMichael (2009). These practices include cleansing (washing) of the hair often done with shampoos and conditioners. The frequency of washing varies depending on hair texture and style. Individuals with loose curly hair pattern may have several washes per week whereas those with more tightly coiled or complex hairstyles may wash more than once fortnightly. Hair emollients such as cream, oil and pomades are applied immediately after and in between washes to improve manageability and enhance lustre. Styling aids such as gels, moulding wax, mousse and sprays are also used to bind hair strands in place and enable a particular style to last for days to weeks. Hairstyles worn include short natural haircut or ‘afro’, long natural hair, dreadlocks, twists, braids/cornrows, chemically relaxed hair, heat-styled, braided extensions, sewn-in weaves and shaved hair.
Natural hair

Hair twisted using hair extension

Natural hair braided without hair extension

Weaved hairstyle

Corn row’ hairstyle with hair extension

Hair braided with hair extension

Figure 1: Popular hairstyles among Ghanaian females (www.google.com)
Again, hair grooming in Ghanaian females may be similarly organised into the model presented by Roseborough and McMichael (2009) for the African-American women: natural versus chemically relaxed hair. Hairstyles such as braiding, weaving and heat styling may overlap between these two categories as depicted below in Figure 1.

**Figure 2:** Line diagram of the typical styling techniques and how they overlap (Modified from Roseborough & McMicheal, 2009).

### 1.2.1 Natural Hair

According to Roseborough and McMichael (2009), natural hair refers to hair of Sub-Saharan African origin in its original state, without any chemical product applied to permanently alter the pattern of the hair. The study reported that the choice of natural hair is dependent on many factors including texture, personal style preferences, cultural trends and even spiritual beliefs. In the current study, the term ‘African hair’ would be used to denote hair of Sub-Saharan African origin.

The African hair consists of relatively low hair density in contrast to Caucasian and Asian hair but appears and feels denser (Loussouarn, 2001). Typically, African hair is
tightly curled and looks dry and matte (Johnson, 1997; Teri, 2010). The highly entangled nature of this type of hair makes combing difficult and therefore poses problems to the management of the hair (Epps & Wolfram, 1983). Work by Johnson (1997) has shown that a much higher force is required in grooming procedures for African hair than for hair of other ethnic origins. Consequently, the African hair is subjected to greater degrees of mechanical damage in comparison with hair of Caucasians and Asians. There is evidence that, cuticle cell loss and hair breakage, for instance, occur readily in African hair than in Caucasian or Asian hair during everyday grooming practices (Kamath et al., 1984; Kamath et al., 1985; Robbins, 1994). Moreover, a study by Khumalo (2006) suggested the likelihood that combing African hair on a daily basis, in some people, may be equivalent to a daily haircut.

1.2.2 Chemically Relaxed Hair

Relaxed hair is the result of the application of a chemical compound to the natural hair to permanently break hydrogen disulfide bonds along the hair shaft (Roseborough & McMichael, 2009; Miranda-Vilela et al., 2013). The consequence of the procedure is the release of the characteristics tight curls leaving the hair irreversibly straightened. This cosmetic alteration affects only the hair shaft and thus new emerging hair will grow in the natural (curly) shape (Miranda-Vilela et al., 2013). The chemical therefore has to be applied to new growth often between an interval of one to three months (Bolduc & Shapiro, 2001; Olasode, 2009). According to Roseborough and McMichael (2009), the time frame for the repeat of the procedure is dependent on the growth rate of the hair of the individual. A study by Olasode (2009), reported reasons for this practice to include beauty, social acceptability, convenience, ease of management, advice of friends and feeling of improved self-esteem. The active
ingredients contained in the hair straightening products include sodium hydroxide or ammonium thioglycate (lye relaxers) and guanidine hydroxide/carbonate (“no-lye” relaxers) (Olasode, 2009; Roseborough & McMichael, 2009; Miranda-Vilela et al., 2013).

### 1.2.3 Heat style

Heated tools may be used to change the hair characteristics (Roseborough & McMichael, 2009). The effect of heat is however temporary and the style is lost when the hair comes into contact with water (Roseborough & McMichael, 2009). Heat can also be used to either straighten hair for movement, lustre, and styling options, or it may be used to curl hair into attractive patterns. Heat is also used to dry, straighten, and smooth the hair shaft immediately after washing. Blow dryers use force heat to quickly evaporate excess water from the hair shaft (Roseborough & McMichael, 2009).

### 1.2.4 Braids and Twists

Braids are a hairstyle type that consists of sections of hair where segments (three) of each section are repeatedly twisted over each other to their very ends (Hughes, 2002). In ‘cornrow’ (a type of braids), the hair is braided onto the scalp in rows, usually all the way down to the nape of the neck, from where it then hangs (Samuels, 1995). Twists, on the other hand consist of two segments of hair repeatedly twisted over each other to the very ends (Hughes, 2002). For the purposes of this study, however, the term braids would encompass both braids and twist hairstyles. According to Roseborough and McMichael (2009), traditionally, the length of braids depended on
the length of the hair of the individual but current advances allow for the addition of
hair extensions. The hair extension (human or synthetic in origin) is braided-in
seamlessly with the scalp hair of the individual, helping stimulate the appearance of
longer or fuller hair (Roseborough & McMichael, 2009). The usage of hair extensions
makes it possible for simple braids to be modelled into several secondary and more
complex styles.

1.2.5 Weaves

Weaves are strands of human or synthetic hair attached to scalp hair to make long,
loose hair styles and give the appearance of glorious hair (Roseborough &
McMichael, 2009). Weaves come in different lengths, colours and styles. Several
methods for the attachment of weaves are available but the most popular method is as
described by Roseborough and McMichael (2009). The scalp hair is braided into
cornrows and then multi-strand wefts of hair are sewn to the braids with thread that
matches the hair colour. Alternatively, adhesives may be placed on the edge of a weft
of hair and then affixed to the scalp (Roseborough & McMichael, 2009).

Typically, braids and weaves could last for many weeks to months, depending on the
growth rate and texture of the hair of the individual. During this period, the hair is
given a break from chemical and heat styling as well as grooming practices. This
‘convenience’ attribute of braids and weaves accounts for the popularity of these
hairstyles among busy professional women and those in institutions of higher learning
in Ghana.
1.2.6 Effect of grooming and styling on hair

It has been established that the cuticle of a typical hair shaft undergoes a regular and progressive loss of the normal scale pattern in a proximodistal fashion (Bottoms et al., 1972; Robinson, 1976; Kelly & Robinson, 1982). These studies show that the part of the hair shaft nearest to the root, almost always has regularly edged cuticular scales whereas that further from the root, shows crenellate edges. Towards the end of long scalp hairs, there is increasing loss of cuticular scale and at the tip, scale pattern is almost completely absent so that the fusiform cells of the cortex are exposed. According to Garcia et al. (1977), the normal grooming processes of washing, drying with towel and combing could account for this patterned loss of the hair cuticle.

Continuous unidirectional traction and tight braids can lead to significant hair breakage (Roseborough & McMichael, 2009). Loss of hair due to traction (traction alopecia) has been reported to dominate among Africans compared to other populations with braids and weaves been implicated (Whiting, 1999; Harman, 1972). A study conducted by Kyei et al. (2011) also suggests that weaves and braids may contribute to the development of central centrifugal cicatricial alopecia (CCCA). Central centrifugal cicatricial alopecia is a type of scalp hair loss which was thought to be caused by the heat associated with the use of the hot comb for straightening hair. It can therefore be deduced from the two studies that braids and weaves could cause hair loss and so affect the quantity or density of hair on the scalp. How these hairstyles affect the microscopic characteristics of hair, however, is not yet known.
1.3 Problem statement

Morphological characterisation of scalp hair has been useful in the forensic sciences for personal identification purposes for years (Jaydip, 2010). This is because scalp hair possesses attributes that make it one of the most common physical evidences encountered at crime scenes (Oien, 2009). These attributes include: been readily available, ease of being shed, persistence in fabric, possibility of being transferred from one individual to another or a place, and good chemical stability and resistance to decomposition (Dachs et al., 2003; Bertrand et al., 2003).

Efforts at beautification have led to increasing artificial modifications to scalp hair. Literature on the morphological characteristics of artificially modified hairs is however scarce. Whether or not these modifications affect the normal hair morphology or the hair features relevant in personal identification is not yet known. This gap in knowledge poses a challenge to the usefulness of scalp hair in identification. For this reason, attention has been drawn to hairs from other body sites such as the face and chest in the determination of age, sex, ancestry, and race by forensic scientists and anthropologists (Aboagye et al., 2014). These androgenic hairs, however, may not find universal usefulness since they are sex specific; being traits of adult males (Marshall & Tanner, 1970; De Souza et al., 2003) but not females (Marshall & Tanner, 1969), except in hirsute conditions. This inadequacy associated with the use of androgenic hairs in forensic science underscores the need to morphologically characterise artificially modified scalp hair.
1.4 Justification

Mass fatalities, such as the World Trade Centre disaster in the United States of America (Sledzik et al., 2009; Mundudorff, et al., 2009), and the recent collapse of the Guest House of the Synagogue Church of All Nations (SCOAN) in Nigeria (Iguniwei, 2015) result in highly fragmented and commingled human remains. The identification of victims with different ethnic and geographic origins, in such instances, requires forensic anthropological data which can be contributed to by hair analyses.

As postulated by Aboagye et al. (2014) the increasing ease of international travel together with unpredictable natural and human-made disasters, present managers of catastrophe with the formidable task of identifying victims from body parts without the advantage of a manifest or other record(s) of the possible victims. Possible problems such as this demand detailed knowledge of anthropometric variants that distinguish people of disparate racial descent and gender. Human hairs survive calamities (Backwell et al., 2009) and could give clues to individual racial origin. For instance microscopic characteristics of scalp hair that suggests braiding and weaving could give clue of Sub-Saharan African origin.

Research on hair morphology in Ghana had been lacking until 2008, when Kalmoni (unpublished thesis) studied the morphological features of hairs from the scalp, eyebrow, pubic regions and axilla of Ghanaian adolescents. This was followed by another study by Aboagye and others (2014) which focused on the morphological features of androgenic hairs in Ghanaian males. Both studies employed light microscopy and obtained results comparable to the available evidences in the literature. The female subjects in the study conducted by Kalmoni by reason of being students of a Senior High School, had haircut as the standard hairstyle. The
morphological profiles of hairs of indigenous African hair subjected to other styling procedures are thus lacking. This is deemed needful because if hair styling alters microscopic profile this may lead to mis-identification in forensic cases. The present study therefore is aimed at microscopically profiling scalp hair of Ghanaian females subjected to popular styling methods.

The present study therefore attends to the paucity of data on the morphology of styled scalp hair.

1.5 Aim

The aim of this study is to determine the morphological profiles of scalp hairs of Ghanaian females.

1.6 Specific Objectives

1. To examine the morphological characteristics of hairs from cut natural hair (haircut hairstyle), natural braided and/or weaved-styled hair and relaxed braided and/or weaved-styled hair using light microscopy.

2. To compare morphological characteristics of hair within and between the three hairstyle groups.

1.7 Hypothesis

The styling of scalp hair has consequential effect(s) on hair morphology.
CHAPTER TWO

LITERATURE REVIEW

2.0 Structure of hair

Hair consists principally of keratin (about 65–95%) and other proteins, water, lipids (structural or free), pigments and trace elements (Robbins, 2002; Velasco et al., 2009). The three main components of hair shaft are: the cuticle, cortex, and medulla (Harrison & Sinclair, 2003).

Figure 3: Cross-section of hair shaft (Source: Saferstein, 2001)

2.0.1 The cuticle

Robbins (2002) stated that the cuticle, the outermost layer, comprises a protective layer of keratinized scales and can account for 10% of hair fibre by weight. The hair fibre achieves protection from environmental and chemical damages via this hair component (Draelos, 2000; Wolfram, 2003). According to Bhushan (2010), when the hair first emerges from the follicle, the cuticle consists of between 6 and 10
overlapping scales. These scales possess a proximal edge which rests against the cortex and a free edge which is directed outward (Draelos, 2000). The cell structure of the cuticle includes 3 major layers: the cystine-rich A-layer, the exocuticle, and the endocuticle (Dawber, 1996). The surface of the hair is covered in a covalently bounded, monomolecular layer of a unique branched fatty acid, 18-methyl eicosanoic acid (Sinclair, 2007). Studies have shown that the cuticle serves as the surface through which hair makes physical contact with its environment (Stamm et al., 1977a; Stamm et al., 1977b). These studies report that the structure and state of preservation of the cuticle determines the extent to which incident light is reflected, scattered and transmitted. This is because the cuticle is the medium through which light interacts with hair. It has been shown by Harrison and Sinclair (2003) that the cuticle classically has a smooth appearance which enables light reflection and limits friction between the hair shafts. The frictional properties of the cuticle also determine how hair responds to grooming (feels to touch, combs, handles and styles) (Stamm et al., 1977a; Stamm et al., 1977b). Consequently, the lustre and texture of hair are a responsibility of the cuticle (Harrison & Sinclair, 2003). In other words, the cuticle determines greatly, the pleasing or aesthetic appearance of human hair. It has been suggested by Wolfram and Lindeman (1971) that the cuticle may contribute to the mechanical properties of hair in human and its effect on the overall rates of chemical treatment of the hair.

Deedrick and Koch (2004a; 2004b) described three basic scale structures that make up the cuticle: coronal (crown-like), spinous (petal-like), and imbricate (flattened). The coronal or crown-like scale pattern is found in hairs of very fine diameter and resembles a stack of paper cups. Coronal scales are commonly found in the hairs of small rodents and bats but rarely in human hairs. Spinous or petal-like scales are
triangular in shape and protrude from the hair shaft. These are found at the proximal region of mink hairs and on the fur hairs of seals, cats, and some other animals but never in human hairs. The imbricate or flattened scales type consists of overlapping scales with narrow margins and is commonly found in human hairs and many animal hairs. Combinations and variations of these types are possible. Thus, human hair can be distinguished from animal hair based on the scale pattern. The scales of the hair of animals show many distinctions such as coronal and spinous patterns, whereas in the case of humans, the scale patterns are of the imbricate type.
Figure 4: Scale types and characteristics of scale margins (Source: http://www.microlabgallery.com/hair.aspx)
Figure 5: Scale features used in identification of hairs (Source: Backwell et al., 2009).
2.0.2 The cortex

The cortex is composed of crystallized α-keratin fibrils and is the component with the greatest fibre mass of the hair shaft. The cortex is remarkable mechanical property of the hair is the responsibility of the cortex and depends on time, temperature and humidity (Zuidema, 2003). Hair colour is established by the type, size and quantity of melanin granules in the cortex (about 3% by weight) (van der Mei, 2002).

2.0.3 The medulla

The medulla comprises only a small percentage of the fibre mass and may be continuous, discontinuous or completely absent along the fibre axis. Human hair is distinguishable from that of an animal by the medullary index (the ratio of the medulla’s width to the diameter of the hair) (Deedrick & Koch, 2004a; Deedrick & Koch, 2004b). The medullary index, is one-third (1/3) and below in humans compared to greater than one-third (1/3) in animal hair, due to the greater width of the medulla in animals (Deedrick & Koch, 2004a; Deedrick & Koch, 2004b).
2.1 Classification of human hair

On the bases of the length and diameter of hair follicles in the human skin, Sen (2010) described three different hair types: lanugo, vellus and terminal. Lanugo hairs which are fine and soft are produced in the very first cycle of hair growth by the hair follicle shortly after its development in the embryo. These hairs grow all over the body of the foetus, functioning mainly to retain body heat and are shed around the eighth month of development. A second generation of lanugo hairs grow and last until the first three months of post uterine life. Lanugo hairs are replaced by fine, short and unpigmented hairs, vellus hairs. Vellus hairs are softer than lanugo hairs and grow in most places
on the human body in both sexes. Vellus hairs are usually less than 2 cm long and follicles are not connected to sebaceous gland. Terminal hair is developed hair, which is generally longer, coarser, thicker, and darker than vellus hair. The phases of growth in terminal hair are more apparent than in vellus hair and the former generally has a longer anagen phase (Sen, 2010). Terminal hair contains a large hair follicle and sometimes, a medulla. During puberty, under the influence of androgens, vellus hairs in the pubic and axillary regions (in both sexes), as well as legs, chest and face (in case of males), transform to terminal hair (Randall, 2007). Terminal hairs include the eyebrows, eyelashes, beard, scalp, pubic and peri-anal hairs.

Human hair is also categorized according to ethnic origin, into Asian, Caucasian and African (Negroid) hairs. Asian hair has a greater diameter with circular geometry. African hair presents a high degree of irregularity in the diameter of hair along the hair shaft with an elliptic section. Caucasian hair has an intermediate diameter and section shape (Kamath et al., 1984; Menkart et al., 1996; Syed et al., 1995). According to Menkart et al. (1996), African hair has a physical shape resembling a twisted oval rod; whereas Caucasian and Asian hairs are more cylindrical African hair shows frequent twists, with random reversals in direction and pronounced flattening (Kamath et al., 1984). Works by Kamath et al. (1984) and Syed et al. (1995) have shown that African hair generally has less tensile strength and breaks more easily than Caucasian hair. African hair is more difficult to comb than Caucasian hair because of its extremely curly configuration (Syed et al., 1995). Studies show, however that, proteins and amino acids constituting keratin are similar in African, Asian, and Caucasian hairs (Dekio & Jidio, 1988; Dekio & Jidio, 1990; Menkart et al., 1996). African hair has less moisture content than Caucasian hair.
2.2 Morphogenesis of hair

The hair producing apparatus is a complex miniorgan of the skin known as the hair follicle. The hair follicle together with its associated structures (the sebaceous gland, the apocrine gland and the arrector pili muscle), form the pilosebaceous unit (Schneider et al., 2009). According to Schneider et al. (2009), hair follicle formation principally takes place during fetal and perinatal skin development. That is to say, no new follicles are made postnatally (Alonso & Fuchs, 2006). Several studies (Reynolds et al., 1999; Choung et al., 2007; Ito et al., 2007) have however demonstrated that de novo formation of hair follicle, may occur in adult mouse and rabbit skin, and even be induced in adult human skin, after wounding. It has been shown that different components of the follicle have different cell origins (Fuchs, 2007; Fuchs & Horsley, 2008). All epithelial components of the hair follicle as well as the sebaceous and apocrine glands are derived from stem cells of ectodermal origin. Mesoderm-derived cells give rise to the follicular dermal papilla and the connective tissue sheath. Alternatively, the pigmentary unit of the follicle arise from neural crest-derived melanocyte progenitors.

The human hair follicle is formed as a result of epithelio-mesenchymal interactions initiated around the third month of foetal development (Sen, 2010). It has been shown that when the skin begins as a single layer of epidermal stem cells in the embryo, specialized dermal cells organize in small clusters directly beneath the epidermal layer (Schmidt-Ullrich & Paus, 2005; Alonso & Fuchs, 2006). There are reports that reciprocal signalling occurs between a local epidermal thickening (placode) of the skin and the small clusters of specialized dermal cells (dermal condensate) directly beneath (Alonzo & Fuchs, 2006; Schneider et al., 2009). It is this communication between the two cell populations that leads to the consequent down growth of
epithelial stem cells, and hence, formation of the hair follicle (Alonzo & Fuchs, 2006; Schneider et al., 2009). The epithelial cells envelope the dermal condensate as the downward growth of the follicle progresses to form the mature dermal papilla (DP) (Alonso & Fuchs, 2006). According to Alonso and Fuchs (2006), a connection is therefore maintained between the follicle and the epithelium. The follicle and the epithelium are nonetheless, separated from the dermis by a basement membrane rich in extracellular matrix and growth factors synthesized and deposited principally but not solely by epithelial cells (Alonso & Fuchs, 2006). Hair is produced at maturity of the follicle, from the continuous division of the proliferative (matrix) cells and the subsequent terminal differentiation of the resulting progeny (Alonso & Fuchs, 2006). The growing hair (shaft) then emerges from the skin surface (Alonso & Fuchs, 2006).

According to Schneider et al. (2009), the mature (anagen) hair follicle is divided into an upper and a lower part. The ‘permanent’ upper part does not show any obvious cycling whereas the lower part is incessantly remodelled in each hair cycle. The infundibulum and the isthmus constitute the upper part of the hair follicle. The lower end of the infundibulum is discernible by the insertion of the sebaceous gland. The infundibulum is joined at the proximal end to the isthmus region of the outer root sheath (ORS), where the arrector pili muscle is inserted. Epithelial and melanocytic hair follicle stem cells are housed in the lower isthmus in a region called the ‘bulge’ (Schneider et al., 2009). It is from this niche that stem cells are obtained for the regeneration of the lower portion of the hair follicle (Taylor et al., 2000; Oshima et al., 2001). The bulge is the end of the permanent, non-cycling region of the hair follicle. A study by Schneider et al. (2009) showed that the lower cycling part of the follicle is the anagen bulb which represents the actual hair shaft plant. The bulge and anagen bulb (the bulbar end of the hair follicle) are separated by a long stretch of
suprabulbar hair follicle epithelium. Contained in the anagen bulb are the matrix cells (keratinocytes) and the pigmentary unit of the hair follicle. Matrix keratinocytes are activated bulge cells which have migrated to colonize the bulb (matrix area) (Schneider et al., 2009). These cells are rapidly reproducing and their number determines the size of the hair bulb and diameter of the hair shaft (Schneider et al., 2009).

Figure 7: Parts of the hair follicle (Source: Hoffmann & McElwee, 2013).

A recent study by Dhugga et al. (2014) established a correlation between skin colouration and melanin pigmentation on one hand, and hair size and follicle density on the other. From that study, individuals with a greater concentration of melanin
within the superficial layer of the skin had a lower follicle density and smaller sizes of hairs. Conversely, individuals with a lower melanin concentration and lighter skin colour had a full range of hairiness. Consequently, Dhugga et al. (2014) suggested that hair development may be related to skin colour. These authors infer that skin pigmentation may be functional in regulating the size of hair and the density of hair follicle. Increased concentrations of melanin in the basal layer of the epidermis may limit hairiness by negatively influencing the ability of the skin to produce hair.

2.3 Follicular Units

Headington (1984) pioneered a study that led to the revelation that human hair does not always grow singly. Human hair, under appropriate magnification, can be observed as emerging from the scalp in groupings, termed follicular units (FUs). These histological units consist of 1 - 3, and rarely, 4 or 5 hairs that form a distinct group delimited by a circumferential band of adventitial collagen (Headington, 1984). Each unit is composed of terminal and vellus hairs, the associated sebaceous glands and the arrector pili muscles, all surrounded by a band of collagen fibers known as the perifollicullum (Headington, 1984). Poblet and co-workers (2002) showed that the distribution of hair follicles into follicular units (FUs) is present in horizontal sections made at the infundibulum and isthmus, above the insertion of the arrector pili (AP) muscle. The study observed that, inferior to the isthmus, hair follicles tend to disperse and lose their orderly and growing distribution in the follicular units. Poblet and his colleagues, based on other findings of the same study, proposed an anatomical model in which a single AP muscular structure is shared by all follicles contained within the FU. This model has been confirmed by Song et al. (2006) through computer-based 3D reconstruction of serially sectioned images. Song et al. (2006) however are of the
opinion that, it would be more appropriate to state that a single AP muscle is associated with a follicular unit instead of a single AP muscular unit been shared by the unit as conveyed by Poblet and his colleagues.

The idea of follicular unit is being employed in a method of hair restoration surgery known as follicular unit transplantation (Jimenez & Ruifernández, 1999). Follicular unit transplantation recognises the follicular unit as the basic and exclusive element of tissue to be moved in the transplant. In this technique, follicular units are thoroughly dissected from the surrounding tissue and subsequently inserted into the recipient area. According to Jimenez and Ruifernández (1999), many surgeons deem follicular unit transplantation as the most reasonable way to accomplish a hair transplant procedure. This is because the hair is transplanted in a way that imitates its natural growth pattern. Seager (1997) suggested that the preservation of the whole morphology of the follicular unit could play a role in graft survival. The study by Seager (1997) established that single hair grafts split away from naturally occurring follicular clumps had a decreased survival rate compared with intact follicular units.

Jimenez and Ruifernández (1999) reported on the distribution of human hair in follicular units. The study found the number of follicular units per square centimetre in the occipital (donor) region of the human scalp to range between 65 and 85. The number of the follicular units had a slight propensity to increase with the hair density of the patient. Hair density ranged between 124 and 200 hair/cm². The 2-hair follicular unit was the commonest, followed by the 2-3 hair and one-hair unit respectively. An increase in the density of the hair of a patient corresponds to an increase in the number of two and three-hair follicular units. On the contrary, the amount of one hair follicular unit increased with a decrease in the hair density of the patient. The mean distance between follicular units ranged from 1.00 mm in patients
with high hair density to 1.40 mm in patients with low density. From these results, Jimenez and Ruifernández (1999) developed a mathematical model with which the number and the most likely distribution of 1, 2, and 3 hair groupings can be envisaged based on the hair density of the patient.

2.4 Hair cycle

Hair, in the opinion of Paus and Foitzik (2004), is unique among human body organs because of the capacity to regenerate throughout its existence. Hair regeneration is accomplished by the repetitive cycling of the hair follicle through successive periods of growth (anagen), regression (catagen) and rest (telogen) (Muller-Rover et al., 2001; Alonzo & Fuchs, 2006). According to Alonzo and Fuchs (2006), follicles produce an entire hair shaft from tip to root during each anagen. During catagen and telogen, follicles reset and prepare their stem cells in order to receive the signal to commence the next growth phase and make the new hair shaft (Alonzo & Fuchs, 2006).

During anagen, the matrix cells (undifferentiated keratinocytes in the hair bulb) proliferate; matrix cells have a cell cycle of approximately 18 hours (Lavker et al., 2003). Matrix cells are also known as transit-amplifying cells due to the limited number of cell divisions undergone before differentiating (Alonso & Fuchs, 2006). When differentiated, matrix cells ascend, adopting one of six lineages of the internal root sheath (IRS) and hair shaft (HS). These layers are from without to within; the henley, huxley and cuticle layers of the IRS and the cuticle, cortex and medulla layers of the HS (Alonso & Fuchs, 2006; Schneider et al., 2009). Terminally differentiated HS cells extrude their organelles and become tightly packed with bundles of filaments assembled from cysteine rich hair keratins. These bundles of filaments become physically cross-linked giving the HS high tensile strength and flexibility (Alonso &
The IRS also keratinizes so as to rigidly support and guide the differentiating HS upwards. The dead cells of the IRS however, degenerate as they reach the upper follicle, thereby releasing the HS which continues through the skin surface (Alonso & Fuchs, 2006). A decline in the supply of matrix cells slows HS and IRS differentiation resulting in the entry of the follicle into a destructive phase called catagen (Alonso & Fuchs, 2006).

Muller- Rover et al. (2001) described catagen as a dynamic transition between anagen and telogen. During catagen, the lower ‘cycling’ portion of each hair follicle regresses entirely in a process that includes apoptosis of epithelial cells in the bulb and outer root sheath (ORS), the outermost epithelial layer (Lindner et al., 1997). The bottom of the HS seals off into a rounded structure called club, due to the cessation of differentiation. The club moves upward until it reaches the permanent, non-cycling upper follicle, where it remains anchored during telogen (Lindner et al., 1997). The dermal papilla is preserved but pulled superiorly until it comes to rest next to the stem cells of the hair follicle bulge, persisting through telogen (Hsu et al., 2011; Driskell et al., 2011). During telogen, follicles lie dormant.

Alonso and Fuchs (2006) reported that activation by the dermal papilla of one or two quiescent stem cells near it at the base of the telogen follicle brings about a switch from telogen to anagen. The process leads to new downward growth of follicle to produce a new hair shaft (Blanpain et al., 2004; Tumbar et al., 2004; Driskell et al., 2011). The activated cells now begin to proliferate rapidly, and become the transit-amplifying daughter cells that are destined to form the new hair shaft. The new follicle forms adjacent to the old pocket that harbours the club hair, which will eventually be shed (exogen). The formation of the new follicle adjacent to the old
pocket creates the ‘bulge’ and adds a layer to the stem cell reservoir. The new hair emerges from the same upper orifice as the old hair (Alonso & Fuchs, 2006).

The mesenchymal component of the hair follicle aside being essential to follicular morphogenesis is also required for the success and sustenance of the follicle throughout its life (Panteleyev *et al.*, 1999; Chi *et al.*, 2013). The work by Panteleyev *et al.* (1999) established that contact between the DP and stem cells in the bulge is critical to the regeneration of the hair follicle and hence the formation of a new hair shaft. Lack of contact between DP and the bulge in the case of the hairless mutant mouse resulted in follicular degeneration rather than regeneration. Even when the ‘contact criterion’ has been met, Chi *et al.* (2013) demonstrated also using a mouse model that DP cell number is also crucial to hair regeneration. The study found that hair follicles with normal keratinocyte compartment failed to generate new hair when DP cell number declined below a critical threshold. Additionally, Chi and his colleagues (2013) found a correlation between DP cell number and the size and shape of the hair produce in mouse pelage. In the mouse, a single follicle produces different hair types in successive hair cycles from the same stem cell. This shift in the type of hair produced by a follicle is accompanied by a corresponding change in DP cell number.

In humans, the duration of the various stages of the hair cycle varies in different body parts. A study by Stene (2004) has shown that scalp follicles have the longest anagen phase which can last several years. The catagen and telogen phases for scalp hair last a few weeks and two months respectively. The majority of normal scalp follicles are in anagen (80–85%), with the rest either in catagen (2%) or in telogen (10–15%), though this varies with the time of the year for people living in temperate zones (Stene, 2004). Stene (2004) indicated that in other body regions such as the arms,
legs, thighs and fingers, anagen lasts about 2-4 months. According to Alonso and Fuchs (2006), the duration of anagen therefore determines the length of the hair and is in turn dependent on the continual proliferation of matrix cells at the base of the follicle. Conversely, the rate of hair growth varies much less over the body usually being close to 0.3–0.4 mm per day (Van Neste, 2004).

2.5 Control of the hair cycle

Mammalian hair cycle is a highly regulated system, and involves many factors important during development and morphogenesis (Porter, 2003). Bone morphogenetic proteins (Bmps) have been implicated in follicle (Botchkarev et al., 1999; Kulessa et al., 2000). Sonic hedgehog (Shh) and FGF7 are involved in the initiation of anagen; HGF, SGK3 and Msx2 maintain the growing hair follicles by protecting against apoptosis, effectively inhibiting the transition of follicles from anagen to catagen (Ma et al., 2003; Porter, 2003; Alonso et al., 2005). Signaling by Wnts proteins has also been shown to maintain follicle in anagen (Lo Celso et al., 2004; Lowry et al., 2005). Environmental factors, such as trauma or wounding, have been found by Stenn and Paus (2001) to initiate hair growth. Conversely, TGFα, TGFβ and TGF5 play a role in catagen initiation (Porter, 2003). Other molecules known to promote the transition to catagen include the growth factors FGF5 and EGF, neurotrophins such as BDNF and possibly the p75-neurotrophin receptor (Hebert et al., 1994; Hansen et al., 1997; Foitzik et al., 2000; Andl et al., 2004; Schmidt-Ullrich & Paus, 2005). Parathyroid hormone-related protein (PTHrP) also acts as a negative regulator of the hair cycle by promoting entry into catagen (Gonterman, 2008). It has been suggested by Diamond et al. (2006) that PTHrP may act as an anti-angiogenic
factor during late anagen. Together, all factors aforementioned help in the coordination and regulation of mammalian hair cycle (Porter, 2003).

2.6 Hair Pigmentation

The colour of hair relies on the presence or absence of melanins alone. The physical aspects of the hair fibre act only as a minor colour modifier (Tobin & Paus, 2001; Trüeb, 2006). This contrasts the situation in skin where the overall pigmentation is an admixture of oxidized/reduced hemoglobin (red/blue), carotenoids (yellow) and melanins (brown) (Tobin & Paus, 2001).

Melanin is produced by melanocytes through melanogenesis, the biochemical conversion of the amino acid tyrosine into eumelanin (brown-black pigment) and pheomelanin (yellow-red pigment) (Trüeb, 2006). The process which takes place within membrane-bound organelles called melanosomes, employs the enzyme tyrosinase, tyrosinase-related protein 1, and tyrosinase related protein 2 (Tobin et al., 2005). The melanosomes are transported from the cell body of the melanocytes to the precortical keratinocytes of the hair follicle (Irmak et al., 1995).

Follicular melanocytes are derived from epidermal melanocytes during hair follicle morphogenesis (Tobin et al., 2005). According to this study, follicular melanocytes could be allocated to five distinct anatomic compartments in the fully developed anagen hair follicle. These sections include the outer root sheath of the infundibulum, basal layer of sebaceous gland and upper dermal papilla. The others are mid-to-lower outer root sheath, the hair bulb and most proximal matrix. Production of pigment for the hair shaft is however the preserve of the melanocytes in the hair bulb (Tobin et al., 2005). Melanocytes in the hair bulb differ from their epidermal counterparts in been larger and possessing longer, more extensive dendrites. Again, bulbar melanocytes
have more developed golgi and rough endoplasmic reticulum and produce 2-4 fold larger melanosomes than epidermal melanocytes. Eumelanin granules transferred by bulbar melanocytes into cortical keratinocytes of hair stay minimally digested unlike in the epidermis where melanin degrades almost completely in the differentiating layers (Tobin et al., 2005).

In contrast to epidermal melanogenesis which appears to be continuous (Tobin et al., 2005), the activity of the hair bulb melanocyte is under cyclical control (Slominski et al., 2004a; Slominski et al., 2004b). Studies by Botchkareva et al. (2003) and Van Neste and Tobin (2004) have demonstrated that the establishment of the pigmentary unit and subsequent pigmentation of the hair is linked to the growth phase of the hair follicle. According to these studies, melanocytes are recruited during early anagen and the pigmentary unit formed in full growth phase. Pigment is then produced and transferred to the keratinocytes destined for the hair shaft. During late anagen, the pigmentary unit disassembles and eventually resolves. The resolution is depicted by the gradual reduction of melanocytes active in the pigmentary unit, during catagen. Tobin et al. (2005) have shown that keratinocytes continue to proliferate for some time after the pigmentary unit is resolved. This phenomenon explains why the most proximal part of a hair shaft in telogen remains unpigmented. Subsequent to the apoptosis of bulbar melanocytes during telogen, melanocytic stem cells from the outer root sheath of the hair follicle, replenish the hair bulb (Tobin et al., 2005). These stem cells however require the protein stem cell factor in order to attain melanin-producing status. The Stem cell factor, released from the dermal papilla, acts via the receptor tyrosine kinase c-kit to cause melanocyte proliferation and increase melanogenesis (Peters et al., 2002). Neuroendocrine factors also play a role in hair pigmentation. Adrenocorticotrophic hormone, beta endorphin, thyrotropin-releasing hormone and
the thyroid hormones triiodothyronine (T3 and T4) have been shown to promote melanogenesis. Work by Arck et al. (2006) has shown that Bcl-2, an anti-oxidant stress protein, is required for the maintenance of melanocytes of the hair follicles at the tip of the hair bulb. Conversely, the lack of Bcl-2 leads to disappearance of melanocyte precursors in the stem cell niche in mice (Nishimura et al., 2005).

According to Sherrow (2006), the darker the hair, the more the melanin granules contained therein. Darker shades of hair also tend to have more densely packed melanin cells. Lighter coloured hairs (for example, blond, and red) contain more phaeomelanin and less eumelanin whereas the opposite is true for hair colours ranging from brown to black. The study by Sherrow (2006) also found that hairs of different colours exhibit variation in thickness and in the quantity of hairs on the scalp. A negative correlation exists between hair thickness and hair quantity on the head (Sherrow, 2006). The highest amount of hairs on the scalp is found in natural blonds but individual hairs are thinner in diameter. This is followed by dark hair with intermediate thickness. Red hairs have the thickest diameter but fewer hairs overall on the scalp (Sherrow, 2006).

### 2.7 Functions of hair

Hair is significant to the survival of many mammals (Stenn & Paus, 2001; Randall, 2007). Hair contributes immensely to thermoregulation (Randall, 2007) and protects against heat loss by trapping a layer of air adjacent to the skin (Popescu & Hocker, 2007). Studies by Stenn and Paus (2001) and Tobin and Paus (2001) have indicated that camouflage provided by hair colours in some mammals like the arctic fox, have contributed to their survival and success ecologically. Accordingly, loss of hair (fur) or faulty colouration leads to death from cold or predation (Randall, 2007).
Conversely, hair is of little or no medical significance in humans because hair loss in humans is not life threatening (Randall, 2007). According to Mercer (1987), in comparison with other tissues, hair has been seen to play a passive role to the physical well-being of man. The loss of scalp hair in balding (androgenic alopecia) is neither a disease nor does the graying of hair induce any metabolic changes in the body (Mercer, 1987). In the view of Randall (2007), the relative hairlessness of humans has led to the virtual loss of the insulating and camouflaging roles. Consequently, the most relevant functions of hair in humans remain protection and communication (Randall, 2007).

All hairs form a protective barrier against ultraviolet (UV) radiations (Stenn & Paus, 2001; Randall, 2007; Robbins, 2012). Work by Tobin and Paus (2001) however shows that protection against harm caused by UV radiations is achieved largely by the amount of melanin in the skin, rather than in hair, a probable explanation for why humans have a lot of scalp hair (Aboagye, 2011).

The communicative and protective roles of human hair have also been documented in the eyebrow (Robbins, 2012). The study establishes that eyebrows restrain sweat and other extraneous matter from running into the eyes and protect the bony ridges of the eyes. There is evidence also that eyebrows serve other roles more visual in nature (Robbins, 2012; Sadr et al., 2003). These visual roles include the expression of emotions and facial recognition. Results from the study conducted by Sadr and colleagues have revealed that the absence of eyebrows in familiar faces leads to a very large and significant interference in recognition performance.

Eyelashes, like eyebrows have both protective and communicative functions in man (Robbins, 2012). Eyelashes protect the eyes from foreign particles (dust and debris)
and objects, and triggers the blink reflex (Sen, 2010). Eyelashes also play aesthetic and social roles and are important in the concept of physical beauty. Studies by Batchelor (2001) and Shaikh and Bodla (2006) found that in many cultures, long and thick eyelashes are considered a sign of beauty and often have a positive psychological consequence on women. As a result, women exploit a number of techniques to augment the overall prominence of the eyelashes (Draelos, 2001). These techniques include the application of mascara, which can provide increased eyelash volume, longer, darker lashes, and improved curl, however with a temporary effect (Draelos, 2001). Other lasting and sometimes permanent options such as eyelash extensions and transplants exist, however, with complications (O’Donoghue, 2000; Straub, 2008). According to Fagien (2010), the approval of the drug, bimatoprost for the treatment of hypotrichosis of eyelashes, seems to offer an additional option to the existing techniques.

According to Robbins (2012), nasal hairs also function protectively by filtering inspired air and retarding the flow of air in the respiratory system. This process allows the air to be warmed or cooled on entry into the body.

All hairs are endowed with sensory nerve endings and thus serve as sensory receptors. This feature supports the hair in the capacity of protecting the individual (Robbins, 2012).

Other human hairs such as found in the axillary, pubic and facial areas are implicated in sexual communication (De Souza et al., 2003). The growth of facial hairs and broadening of chin in the face of males at the onset of puberty is an indication of sexual maturity (De Souza et al., 2003). On the other hand, the development of pubic and axillary hairs signifies puberty in both sexes (Reynolds, 1951; Marshall &
Masculinity in sexually mature men is demonstrated with visible beard, chest and upper pubic diamond hair (Marshall & Tanner, 1970).

The communicative role of hair in man is also evident in the selection of mates (Kingsley, 1995). A study by Barber (2001) revealed that facial hair boosts the marriage prospects of a man by increasing physical attractiveness and perception of social status. Another study conducted by Freedman (1969), saw female subjects describe bearded men as more “masculine, matured and sophisticated”, while male subjects described them as “independent and extroverted”. Neave and Shields (2008) reported on the effect of facial hair manipulation on female perception of males. According to the study, males wearing full beard were considered the most masculine, aggressive, socially mature, and older. Those men who wore light beard were thought to be the most dominant. It was men with light stubble, who were considered to be the most attractive and preferred for both short and long-term relationships. According to Meskó et al. (2012) women increase physical attractiveness to potential mate by a change in ‘looks’ achieved through the application of different hairstyles. Consequently, Meskó and colleagues examined the effects of long scalp hair on the aesthetic evaluations of female facial attractiveness in four dimensions of perception (maturity, sexiness, femininity and health) related to mate choice. It was found that long hair more positively affected the evaluation of ‘less attractive’ faces compared to ‘more attractive’ faces (on the basis of their facial proportions). Work by Hinz et al. (2001) also found that hair length and quality may convey signs of youthfulness and health in a woman and hence signal reproductive potential.

Hairstyle has also been implicated in the communicative role of hair in humans. Men and women have at most times and in most cultures, worn hair in styles that differed
from each other. According to Weitz (2004), this difference in hairstyle between the sexes is the most widespread cultural rule about hair. The view of the Christian religion about hair that “Doth not even nature itself teach you, that, if a man have long hair it is a shame unto him? But if a woman have long hair, it is a glory to her…” (The Holy Bible King James Version, 2008), seems to support the difference in hairstyle among the sexes. Brebner et al. (2009) have established that hairstyle plays a significant role in perceiving men and women in the society and may be useful in separating the male and female identities. Subsequently, the adoption of a culturally-assigned hairstyle of one sex by the other poses a challenge to the society in the assignment of a sex to the individual.

It has been shown in a study by Winter (2003) that human has hair functioned communicatively as a social class signifier throughout history. According to the study, people of the upper-class always wore hair in styles that signal wealth and status. Such hairstyles, being at the cutting edge of fashion, make people of the upper-class, ‘hairstyle-pace setters’; setting hairstyle trends for the less wealthy. Hairstyles of individuals of the middle-class on the other hand, tend to be understated and professional (Ofek, 2009). People in this class aspire to have natural, healthy looking hair to communicate the possession of resources for good and healthy lifestyles (Ofek, 2009). The study by Ofek (2009) also showed that traditionally, people who belonged to the working-class tended to have practical and simple hairstyles. Contrarily, working-class individuals of today often have more elaborate and fashion-conscious hairstyles than the other social classes (Ofek, 2009).

The style in which hair is worn could also be used to communicate the religious affiliation, marital status, as well as life transition of the individual (Sherrow, 2006; Weitz, 2004). Shaving of the head is seen as a mark of worldly renunciation in
Christianity or Buddhism (Mercer, 1987). Conversely, growing of hair is a sign of inner spiritual strength for Sheikhs in Islam (Mercer, 1987). In Ghana, the only acceptable hairstyle for female adolescents in second cycle institutions is a ‘decent’ hair cut. For such persons, a transition from that level to the next is communicated often via a change from the haircut to a chemically relax and or braided hairstyle. Some female students even relax or braid the hair during vacation to illustrate the change in environment. That is to say that the individual is not subject to the authority of the school during that period.

2.8 Androgens and hair growth

Work by Griffin and Wilson (1989) using males with androgen insensitivity, established that without androgens or their activity, scalp hair grows constitutively whereas growth of body hair is repressed. On the contrary, androgen action in genetically susceptible individuals, result in scalp alopecia, characterised by the miniaturisation of hair follicles of the scalp in a defined pattern. During sexual maturation, vellus hairs transform into terminal ones in the axilla and pubis (in both sexes) and on the face, chest and extremities (in men) under the influence of androgen (De Souza, 2003). Excess androgen action is known to produce unwanted hair growth (hirsutism) in women. How one and the same hormone induces varying responses in the same organ in different body regions remains an enigma. According to Kaufman (2002), factors such as increased number of androgen receptors, increased local production of high-potency androgens, and/or reduced degradation of androgens may be involved in this phenomenon. The observation that transplanted scalp hair follicles retain the donor site balding response, suggests that the varying responses are an
intrinsic quality unique to the particular follicles and do not depend on regional environment (Oslen, 2001).

2.9 Hair aging

Trüeb (2005) described aging of hair in three scopes. These comprise weathering of the hair shaft (progressive degeneration of the hair fiber from the root to the tip), aging of the hair follicle (which manifests as decrease of melanocyte function or greying) and decrease in hair production (in androgenetic and senescent alopecia).

2.9.1 Hair weathering

The free end of a normal growing hair fiber generally undergoes wear and tear (Trüeb, 2005). The degree of this deterioration is dependent on the extent of environmental and cosmetic insults. It is for this reason that scalp hair, with the longest growth phase, is often subjected to more damage than hairs of other body sites. Studies (Bottoms et al., 1972; Wei et al., 2005) have demonstrated that usually the damage is most prominent only near the tip of scalp hair. Often the tip appears lustreless and paler than the more proximal growth, with varying degrees of split ends. The overlapping cuticular cells on the surface of the hair fiber are potentially susceptible to friction damage from excessive combing and brushing, particularly when wet (Trüeb, 2005). The application of excessive heat or chemicals (for the purposes of bleaching, straightening, etc) to the hair may cause additional damage or make the hair more prone to mechanical abrasion (Trüeb, 2005). Subsequently, longitudinal fissures arise between exposed cortical cells of hair with loss of cuticle and ultimately result in the fracturing of hair at these sites. Expectedly, abnormal
hairs with inherent weakness are more susceptible to mechanical abrasion (Trüeb, 2005).

### 2.9.2 Hair graying

Hair graying in humans (canities) is a natural age-related phenomenon (Damon & Roen, 1973). The rate of graying in individuals is however genetically influenced for which reason early onset of graying in kinships is a common occurrence (Trüeb, 2005). Trüeb (2005) indicated a variation in the pattern of graying between men and women. According to the study, the process begins at the temples and in the sideburns in men and around the perimeter of the hairline in women. The graying then gradually works its way back through the top, sides, and back of the hair.

It has been established that graying and eventually whitening of hair is the consequence of a reduction in the melanin content of hair follicles (Trüeb, 2005). This loss of melanin has been linked to a number of events. These include: a decline in the number of melanogenically-active melanocytes in the hair follicle (Nishimura et al., 2002); a defect in the transfer of melanosomes; a decrease in the activity of enzymes involved in melanogenesis (Commo et al., 2004).

Studies by Tobin & Paus (2001) and Slominski et al. (2004a) have attributed the reduction of melanogenically-active melanocytes in the follicle with age to the ectopic differentiation of melanocyte stem cell into melanogenic ones. The above studies report that the ectopic differentiation of melanocyte stem cell is advanced by both endogenous and exogenous oxidative species, accruing in the follicles with age. As a result of the loss of stem cells, the hair bulb melanocytes are unreplenished after apotosis. Moreover, Nishimura et al. (2002) has confirmed that oxidative stress also
causes premature apoptosis of melanogenic melanocytes in the hair bulb. In effect, fewer melanosomes are incorporated into cortical keratinocytes of the hair shaft. Accordingly, there is a decrease in pigmentation in succeeding anagen phase of the follicular cycle (Botchkareva et al., 2003) until ultimately the hair bulb is without any melanogenic melanocytes. In addition to the above, there seems to be a defect in the transfer of melanosomes because in spite of the proximity between keratinocytes and the melanocytes with the remaining melanosomes, the keratinocytes may not contain melanin (Slominski et al., 2004a). Tobin et al. (2005) states that this anomaly is caused by either defective melanosomal transfer to the cortical keratinocytes or melanin incontinence as a result of melanocyte degeneration.

Work by Peters et al. (2011) distinguished two types of hair graying in humans: senile and premature graying (canities). Senile canities is thought to result from the exhaustion of the regenerative capacity of the hair pigmentation and through programmed events during aging. Conversely, premature canities is seen as caused by environmental factors, inflammation or psycho-emotional stress (Peters et al., 2011). Trüeb (2005) states that hair graying has been associated with certain autoimmune disorders such as autoimmune thyroid disease and pernicious anaemia as well as several rare premature aging syndromes (as Werner’s syndrome).

The age at which hair graying is considered a normal incidence is pegged at 34 ± 9.6 years in Caucasians and 43.9 ± 10.3 years in Africans (Trüeb, 2005). According to a study by Trüeb (2005), by the age of 50 years, 50% of people have 50% gray hair regardless of sex and hair colour. Contrary to senile canities, premature graying seems ill-defined as several researchers have given different definitions. Gould et al. (1978) and Glasser (1991) defined premature graying as 50% of hair turning gray before the age of 50. Graying is premature when it occurs before age 20 for Caucasians, 25 for
Asians and 30 for Africans (Tobin & Paus, 2001; Trüeb, 2005). Morton et al. (2007) defined premature graying as all or most of the hair is gray before the age 40 and Zayed et al. (2013) defined it as first appearance of gray hair before the age of 30.

An association between smoking and premature graying has been suggested by Zayed et al. (2013). The study explained this observation in line with a study by Trüeb (2003) that smoking could generate huge amounts of reactive oxygen species (ROS). The ROS would lead to increased oxidative stress which could damage the melanocytes. The presence, often of highly vacuolated melanocytes in the bulb of gray hair follicles, appears to suggest a response to increased oxidative stress. There is evidence that oxidative stress triggers the selective apoptosis of melanocytes in the bulb of the hair follicle but not in the outer root sheath (Arck et al., 2006).

2.9.3 Hair loss (Androgenic alopecia)

Androgenic alopecia has been the term used to generally describe the patterned loss of scalp hair in genetically susceptible individuals (Kaufman, 2002). The condition is also termed male patterned hair loss (MPHL) and female patterned hair loss (FPHL) in men and women respectively (Kaufman, 2002). Studies have indicated that androgenic alopecia is characterised at least in the early stages by the thinning of hair as opposed to follicular loss (Price, 1975; Whiting, 1993). This hair thinning is the results of the progressive transformation of thick, pigmented terminal hairs into short, fine, hypopigmented vellus-like hairs.

MPHL and FPHL differ from each other in several ways. According to Kaufman (2002), MPHL typically begins with bitemporal recession, then progressive thinning in the frontal and vertex areas of the scalp. Recession of the frontal hairline is a
frequent occurrence. The frontal and vertex thinning areas may eventually merge, culminating in near complete visible hair loss over the top of the scalp. On the contrary, FPHL is characterised by diffuse thinning in the frontal and parietal areas of the scalp, the frontal hairline being preserved (Kaufman, 2002). Unlike in men, complete baldness in affected scalp regions is rarely observed in premenopausal women with FPHL. Studies by Venning and Dawber (1998) indicated however that, postmenopausal women may develop or advance to a pattern of hair loss more characteristic of men with MPHL. Hair over the occipital region of the scalp is conserved in both sexes.

A study by Hamilton (1942) indicated that MPHL presents after puberty, usually becoming apparent in the third decade and in almost all cases by the fourth decade of life. On the other hand, FPHL may present as late as the sixth decade of life (Oslen, 2001). In men with MPHL, miniaturisation of the follicle originates from an inherited sensitivity of the hair follicles to normal levels of circulating androgens. Furthermore, a genetic predisposition is a requirement for the development of MPHL but not FPHL (Kaufman, 2002). In spite of records of women presenting with scalp hair loss and virilisation due to androgen-secreting tumours, there has been no evidence to prove a genetic and/or hormonal basis for common FPHL in which virilisation is not commonly observed (Kaufman, 2002).

The implication of androgen in the pathophysiology of alopecia came to light from the studies of Hamilton (1942; 1951). On the other hand, the demonstration that dihydrotestosterone (DHT) is the specific androgen involved in MPHL came from the work of Walsh et al. (1974) using subjects with genetic deficiency of type 2, 5 alpha reductase (5αR). Clinical trials with finasteride, a selective inhibitor of type 2 5αR further corroborated the role of DHT in MPHL (Leyden et al., 1999; Finasteride Male
Pattern Hair Loss Group, 2002). Whereas finasteride produced significant improvements in hair growth in men with MPHL, it failed to achieve same in females with FPHL (Roberts et al., 1998; Finasteride Male Pattern Hair Loss Group, 2002). It is this finding that served as an eye-opener and led to the suggestion that other players may be involved in the pathophysiology of FPHL. Formerly, both MPHL and FPHL were thought to result from an abnormal sensitivity of scalp hair follicles to circulating androgens (Kaufman, 2002).

According to Zhang et al. (2014), it has been the focus of the management strategy of alopecia to achieve two main goals. These objectives are: to stimulate existing follicles with topical and oral medication, to grow new hair and recreate a fuller head of hair via transplantation. Work by Draelos (2011b) reveals that, in addition to the above treatment options, others such as laser therapy, dietary supplementation among others also exist. Of the numerous drug therapies available the most widely used have been minoxidil and finasteride (Yazdabadi & Sinclair, 2011; Higgins & Christiano, 2014; Zhang et al., 2014). These treatment options often are associated with problems of cost, side effects and inadequate long term coverage (Zhang et al., 2014). Consequently, attention is being shifted towards innovative cell based therapies involving the dermal papilla cell (DPC) as a means of growing new hair in previously bald area (Weber & Chuong, 2013). Regeneration of hair follicles using DPCs has been shown to be successful in animal (rodent) models (Inasmatsu et al., 1998; Kishimoto et al., 2000). In humans, however, two main challenges exist. These include the retention of the hair inducing ability of the dermal papilla as well as the maintenance of dermal papilla productivity after several passages of culture. A study by Kang et al. (2012) showed that human dermal papilla spheres could induce hair follicles from mouse epidermal cells. Subsequently, Higgins et al. (2013) also
indicated that dermal papilla cells cultured in three dimensional spheroids could partially restore the inductive ability and are capable of inducing de novo hair follicles in human skin. Moreover, it has been demonstrated by Thangapazham et al. (2014) that inserting adult DPC into dermal-epidermal composites could improve hair-inducing properties and then achieve human hair reconstitution.

2.10 Significance of human hair

Human hair has found usefulness in several areas including archaeology, forensics and health (Thompson et al., 2014). According to Thompson et al. (2014), the hairs obtained from archaeological excavations and forensic investigations are capable of providing record of the geochemical environment of an individual. These hairs therefore provide an exceptional opportunity to rebuild the environment, diet and residential location of individuals. Gupta (2014) revealed the application of human hair in a variety of fields in different geographical locations (Table 1 & 2). Hair on the scalp (Wurst et al., 1999; Pianta et al., 2013) and body (chest, arm and leg) (Pianta et al., 2013) have been found valuable in the quantitative determination of ethyl glucuronide (EtG) as a direct alcohol marker in clinical and forensic toxicology. Other studies have analyzed hair at the molecular level for exposure to drugs (Balíková 2005; Sen, 2010) and environmental pollutants (Wei et al., 2013). Microscopic examination of hairs is relevant in the cosmetic industries in the assessment of the impact of hair-care products on consumers (Kharin et al., 2009). Similarly, in the medical field, the analysis of hair microscopically is utilised in the determination of nutritional effects (Rasmussen & Børsting, 2000) and toxic-element levels (Kinova et al., 1988). Other medical usefulness of microscopic studies of hair include the determination of dermatological diseases (Plozheimer et al., 2000; Oien, 2009),
osteoopenia (low bone mineral density), familial osteoporosis and cardiovascular diseases (Rosen et al., 1994; Orr-Walker et al., 1997; Tobin & Paus, 2001; Mistry et al., 2010). Primarily, however, the microscopic characterization and comparison of human hairs is functional in the area of forensics to associate crime with perpetrators and witnesses.

Table 1: Geographical spread, historical age, and scale of human hair uses in practice (Adopted from Gutpa, 2014)

<table>
<thead>
<tr>
<th>Use</th>
<th>Countries in which present</th>
<th>Age and scale(^a) of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wigs, hair extensions, eyebrows, beard, and so forth</td>
<td>Production: India, China, Korea, Tunisia, Italy, Russia, Bangladesh, and Pakistan Market: USA, UK, Africa, Japan, China, and Italy (almost every country)</td>
<td>Centuries old; very large scale</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>China, India, and USA</td>
<td>Centuries old (recent in USA); medium scale (few villages/towns)</td>
</tr>
<tr>
<td>Pest repellent</td>
<td>India, USA, and Mauritius</td>
<td>Centuries old; small scale</td>
</tr>
<tr>
<td>Clay reinforcement</td>
<td>India, Bangladesh, Syria, and Europe</td>
<td>Centuries old; medium scale</td>
</tr>
<tr>
<td>Oil-water separation</td>
<td>USA, Philippines</td>
<td>15 years; medium scale</td>
</tr>
<tr>
<td>Stuffing toys, furniture, mattresses, and so forth</td>
<td>India, USA, Hawaii, and few European countries</td>
<td>A century old; medium scale</td>
</tr>
<tr>
<td>Fabric making</td>
<td>China, India</td>
<td>Few centuries old; medium scale</td>
</tr>
<tr>
<td>Artwork</td>
<td>Past: China, England, USA, Prussia, France, Italy, and Scandinavian countries. Present: China, USA,</td>
<td>China, 1000 years, Europe, 200 years, and recent revival, 20 years; small scale</td>
</tr>
<tr>
<td>Hydrolyzed protein (HHKP)</td>
<td>USA, Europe</td>
<td>20 years; small scale</td>
</tr>
<tr>
<td>Extracting amino acids</td>
<td>India, China, Korea, and Europe</td>
<td>40 years; medium scale</td>
</tr>
<tr>
<td>Ethnomedicinal uses</td>
<td>China, India</td>
<td>Centuries old; small scale, carbonized hair medium scale</td>
</tr>
<tr>
<td>Suturing material</td>
<td>Europe, India, China, and Turkey Europe,</td>
<td>5 centuries, others, 50 years; very small scale</td>
</tr>
<tr>
<td>Testing material for hair care products</td>
<td>Europe, USA</td>
<td>100 years; small-medium scale</td>
</tr>
<tr>
<td>Cosmetic brushes</td>
<td>India, USA</td>
<td>40 years; small-medium scale</td>
</tr>
<tr>
<td>Hygroscope</td>
<td>India, China, USA, and Romania</td>
<td>200 years; very small scale</td>
</tr>
<tr>
<td>Nesting material for birds</td>
<td>USA, Europe</td>
<td>In nature, centuries, by humans, 50 years; small scale</td>
</tr>
<tr>
<td>Ropes</td>
<td>America, Japan</td>
<td>100 years, small scale</td>
</tr>
<tr>
<td>Musical instrument</td>
<td>Philippines</td>
<td>Centuries old, very small scale</td>
</tr>
<tr>
<td>Oil filter</td>
<td>China, Europe</td>
<td>100–50 years ago, medium scale</td>
</tr>
</tbody>
</table>

\(^a\)Large scale: millions of kg; medium scale: thousands of Kg; small scale: \(\sim\) 100 kg.
Table 2: Countries undertaking new research on human hair uses. (Adopted from Gutpa, 2014)

<table>
<thead>
<tr>
<th>New uses/areas of research</th>
<th>Countries where research is undergoing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid fertilizers</td>
<td>India, USA, Korea, and Bangladesh</td>
</tr>
<tr>
<td>Concrete reinforcement</td>
<td>Canada, India</td>
</tr>
<tr>
<td>Pollution control</td>
<td>Canada, Singapore, India, Iran, Korea, Egypt, and Jordan</td>
</tr>
<tr>
<td>Molded furniture and objects</td>
<td>UK</td>
</tr>
<tr>
<td>Engineering polymers</td>
<td>Singapore, China, Japan, and India</td>
</tr>
<tr>
<td>Follicle cell cultures/tissue regeneration</td>
<td>Switzerland, UK, Korea, and France</td>
</tr>
<tr>
<td>Composites for superconducting systems</td>
<td>India, Greece, and The Netherlands</td>
</tr>
<tr>
<td>Flexible microelectrodes</td>
<td>China</td>
</tr>
</tbody>
</table>

2.11 Microscopic examination of hair

Classically, hair is examined for investigative and associative information through light microscopy (Scientific Working Group on Material Analysis (SWGMAT), 2009). Hair examination in such instances involves two main practices: the identification of an unknown (questioned) hair and its subsequent association with hair of known origin (Deedrick, 2000). The primary step in the procedure is the authentication that the sample in question is hair rather than fibre. Subsequently, attempts are made to obtain other relevant information such as organismal (human or animal), body as well as racial origins. Other details include the assessment for artificial alteration (hair dyeing), whether the hair was crushed or cut and means by which the hair was obtained (pulled or fell naturally). Characteristics of hair upon which comparison are mainly based include diameter, medullation, pigmentation, scale characteristics, shape and form (Sen, 2010). Hairs are first examined macroscopically to determine features resolvable by the naked eye such as colour, form and curl. This assessment is then followed by microscopic examination of the other characteristics (Deedrick, 2000). There is evidence that hair of animal origin
could be characterised to the species level, beyond which discrimination becomes impossible (Deedrick, 2000; Oien, 2009).

Hair examination is performed to establish the possibility of contact between two or more persons or an individual and an object. Such evidence is valuable in violent incidences where there may have been physical contact like homicide and sexual assault, burglary and armed robbery. Hairs gleaned from clothing and debris in the instances above could be of immense help in providing information for the detection of suspects (Deedrick, 2000). The advent of mitochondrial DNA (mtDNA) sequencing has augmented the range of tests available for assessing the association between a questioned hair and an individual, in the absence of nuclear material.

Some concerns have been raised regarding the employment of microscopic examination in the association of questioned sample to victims. Houck and Budowle (2002) opine that both microscopic and molecular analyses of hairs are useful in forensic investigations and recommend the use of both methods in the analysis of hair evidence where possible. According to the study, both methods depend on independent types of information and neither alone allows absolute positive identification. Moreover, microscopic examination has additional worth of reducing the duration and cost of mtDNA analysis since a large number of questioned hairs can be dealt with quickly. Also valuable information can be obtained from microscopic examination of features unrelated to the issue of identity (e.g. forceful hair removal) which is not possible with mtDNA analysis (Houck & Budowle, 2002).
2.12 Morphological studies on hair in Ghana

Kalmoni (2008) examined by light microscopy, hairs from the scalp, eyebrow, axilla and pubic areas of sixty (60) Ghanaian male and female adolescents. Results showed that hair diameter increase in the order eyebrow, scalp, axilla and pubic hair. Axilla and scalp hair were close in diameter. Generally, hairs from males recorded higher scale counts per unit area than hairs from females. The study also revealed that regular waved scale pattern dominated in males whereas irregular mosaic pattern dominated in females.

Aboagye (2011) studied the microscopic morphology of some selected and androgenic hairs (facial and chest hairs) in Ghanaian male cadavers. Both qualitative and quantitative features of hair such as cuticular scales and hair shaft and medullary diameters were determined and compared between five body regions. The results indicated both macroscopic and microscopic regional differences indicating hair from these regions were morphologically distinguishable from each other. Grey hairs were distinguishable from black hairs obtained from the same location of an individual. The study also recorded for the first time, a greater shaft diameter for grey hair from the sideburn and chest compared to their black hair counterparts.
CHAPTER THREE

MATERIALS AND METHODS

3.0 General

3.0.1 Study design
The study was a cross-sectional study which employed standard laboratory procedures in the light microscopic examination of scalp hair from Ghanaian female subjects (volunteers).

3.0.2 Ethical consideration
Approval for the study was obtained from the Ethical and Protocol Review Committee, College of Health Sciences, University of Ghana, Korle-Bu (Protocol ID. No.: MS-Et/M.7 – P 4.4/2014-2015). Permission was also sought from appropriate institutional authorities where necessary. In addition, subjects were made to consent to their participation in the study via the completion of informed consent form (see Appendix 2), before hair samples were taken.

3.0.3 Study site
The study was conducted at three main sites: the St Mary’s Senior High School, Korle Gonno, the Greenhills School of Health Sciences (GSHS), Mallam and the School of Biomedical and Allied Health Sciences (SBAHS), College of Health Sciences (CHS), Korle Bu. The St Mary’s Senior High School contributed subjects with natural unbraided hair to the study. Together, the GHSH and SBAHS contributed subjects with natural or chemically treated scalp hair which were braided / weaved to the study. Preparation of hair samples and the subsequent examination under the light
microscope was carried out in the laboratory of the Department of Anatomy, SBAHS, CHS, Korle Bu.

3.0.4 Subject/Study population

Ninety six (96) Ghanaian female adolescents and adults, who had haircuts, wore braids and or weaves were recruited for the study upon satisfying all inclusion criteria through the completion of the research questionnaire (Appendix 1). Selection of subjects was done by convenient sampling. This sampling method was employed because of the nature of the study and also due to the fact that convincing people to participate in such research was a major challenge. Males, non-Ghanaian females and Ghanaian female minors as well as Ghanaian female adolescents and adults who wear hairstyles not included in the aforementioned categories (eg. dreadlocks) were excluded.

3.0.5 Sample size determination

The sample size was determined using this formula based on the nature of the study design and statistical consideration of the study population;

\[ n = \frac{z^2(p)(1-p)}{d^2} \]  

(Klufio, 2003).

Where, \( z \) is standard score at 95% confidence interval = 1.96

\( p \) is the expected prevalence = 0.5

Since the prevalence was not known, 0.5

\( d \) is precision( corresponding to effect size) = 0.1

\( n \) is the sample size = 96
3.0.6 Sample collection procedures

One hair strand was plucked randomly from each of five scalp areas namely the frontal, left temporal, right temporal, vertex and occipital, of each subject with a pair of cosmetologist’s tweezer. These strands of hair were kept in five different envelopes, labelled appropriately, according to the scalp area from where the hairs were plucked. Each envelope also had the identification (ID) number allocated to the donor of the hairs. All five envelopes containing the hairs from the five scalp regions were in turn kept in an envelope bearing the ID number of the subject. These measures were employed to ensure the regional as well as individual identity of the hair fibres. A total of four hundred and eighty (480) hairs strands were obtained from the ninety six (96) subjects categorised into three major groups on the bases of their hair care practices and styling preferences thus: 32 Natural unstyled (non-chemically treated hair that has been cut); 32 Natural styled (non-chemically treated, braided and /weaved hair); 32 Relaxed styled (chemically treated, braided and/ weaved hair).

3.1 Light microscopy

3.1.1 Hair sample preparation

Prior to the preparation of these hairs, their lengths were determined by a direct method using a 300 mm (30 cm) metric rule. This measurement was done by holding the free ends of each hair fibre with two pairs of cosmetologist’s tweezers and extending the fibre parallel to the metric rule.

The hairs were cleaned using the method described by Aboagye et al. (2014). Briefly, hairs were washed using alcohol (5% v/v) for 35 minutes in sterilin plastic dishes, blotted clean and allowed to dry at room temperature.
3.1.2 Whole mount, scale preparations and microscopy

A thin layer of clear nail polish (Bichun Manicure Supernail Hardener, Korea) was brushed onto a standard microscope glass slide (25.4 x 76.2 mm, 1 mm - 1.2 mm thick, frosted microscope slides, Surgifriend Medicals Middlessex England) and allowed to stand for a few minutes to partially dry. A pair of cosmetologist’s tweezers (Stella, Ailin cosmetic corporation, China) was used to carefully place fibres individually onto the tacky nail polish to adhere to the nail polish and then left to dry. Long hairs were divided into three sections, proximal (portion closest to the scalp), middle and distal (farthest from the scalp) and mounted. The hairs were examined by scanning from the root to tip with the x10 objective lens under the optical light microscope (Leica Galen III, Leica, Buffalo, USA) to observe the morphological characteristics of the hair. One eyepiece lens of the microscope was then replaced with a digital microscope eyepiece (Lenovo Q350 USB PC Camera) connected to a computer (HP Compaq dx2300 Microtower), and supported by the imaging software, CyberLink YouCam (2.0.0.2519, Cyberlink Corporation, Taipei, Taiwan) and digital images captured at the same magnification (x10). Subsequently, a pair of cosmetologist’s tweezers was used to carefully pull the hairs from the slide in one smooth motion, leaving an impression of the scales in the dried nail polish. The scale casts of the proximal and distal regions of the hair were then examined with the same microscope under higher magnification (x40 objective) and digital images captured in the manner earlier described.

3.2 Structural features of hair examined

Structural features of the hair examined included both qualitative and quantitative features.
3.2.1 Quantitative features

Quantitative characteristics measured included hair shaft (HS) and medullary (MD) diameters, as well as medullary index (MI).

3.2.1.1 Hair shaft and medullary diameters

Hair shaft diameters were computed as averages from the diameters of three whole mount images per portion (proximal, middle and distal) of the hair shaft (Figure 4). Medullary diameters were measured for the middle portion of the hair only. This was done by measuring the medulla diameters at three different randomly chosen sites on each of three selected middle shaft images. Microsoft publisher (2007 software) ruler was calibrated with a micrometer stage graticule (100 x 0.01 mm, Graticules Limited, Tonbridge, Kent England) so that all measurements in publisher units were converted to micrometer (μm) (Kalmoni, 2008; Aboagye, 2011)(see appendix 3). Medullary indices were also computed for the middle hair shafts only from the corresponding shaft and medullary diameters thus:

\[
\text{Medullary Index} = \frac{\text{Medullary diameter (μm)}}{\text{Hair shaft diameter (μm)}}
\]

3.2.1.2 Mean scale count and interval between scale margins

Three randomly selected scale cast images each for the proximal and distal portions of the hair were also assessed quantitatively for the interval between scale margins and mean scale count. The Microsoft publisher ruler was used to measure the interval between the margins of two scales at six (6) randomly selected points parallel to the longitudinal direction of each micrograph and an average calculated (Figure 5). From across the width of the same images, the number of scale edges was counted at two different sites (Figure 5). The mean calculated from the six (6) results obtained per
portion of the hair shaft became the mean scale count for that region of the hair shaft. According to Wyatt and Rigott (1980), the mean scale count is a reciprocal function of the linear size of the cuticular scale exposed.

3.2.2 Qualitative features

Three whole mount images were randomly selected from a series of captured images of each of the three parts of the hair shaft (proximal, middle and distal) and assessed for qualitative features. Such characteristics include the presence or absence of medulla, type of medulla present, type of hair root and nature of hair tip. Two randomly selected scale cast images for the proximal and distal portions of the hair were also assessed for qualitative descriptions of scale pattern, type of scale edge, as well as separation between scales margins.
**Figure 8:** Micrograph of a cut natural hair depicting quantitative characteristics measured: HD represents hair shaft diameter and MD, medullary diameter. Also shown are the medulla (M) and cortex (C) of the hair fibre.

**Figure 9:** Micrograph of scale cast of a cut natural hair illustrating qualitative features considered. ISM = Interval between scale margins, SC = Scale edges counted across the transverse width of micrograph.
3.3 Statistical analyses of results

Data obtained were entered into Minitab 15 English and analyzed accordingly. All parametric data results were expressed as means and standard deviations (SD). Statistical significance of the difference within and between group means (averages) were performed by one-way analysis of variance (ANOVA) and the independent t test.

Nonparametric data results were expressed as median and range (R) and statistical significance of the difference within and between group medians verified using Krauskal-Wallis and Mann Whitney tests. Test for association was done using Chi square and correlation established using Pearson’s correlation (r). The statistical differences between groups were determined at a 95% confidence interval. Consequently, all differences with probability (p) value less than α = 0.05 were considered statistically significant.

Results obtained were presented as tables and appropriate graphs were drawn using Microsoft Office Excel, 2007, to pictorially illustrate differences between group means or median.
CHAPTER FOUR

RESULTS

4.0 General

The overall mean age of subjects was 21.96 yrs (SD = 6.832). The mean ages for the individual groups were: 15.34 yrs (SD = 0.865), 24.58 yrs (SD = 6.88 years) and 26.03 yrs (SD = 5.08 yrs) for Natural unstyled hair, Natural styled hair and Relaxed styled hair respectively (Table 2). The overall mean hair length was 79.74 mm (SD = 60.88). The mean hair length for the three hairstyle groups were 25.09 mm (SD = 9.653), 88.92 mm (43.32), and 125.50 mm (62.80) for the Natural unstyled, Natural styled and Relaxed styled hair groups respectively (Table 3).

Table 3: Mean age and hair length of the various hairstyle groups

<table>
<thead>
<tr>
<th>Hairstyle</th>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>SE</th>
<th>StDev</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural unstyled</td>
<td>Age (yrs)</td>
<td>32</td>
<td>15.34</td>
<td>0.15</td>
<td>0.87</td>
<td>14.00-17.00</td>
</tr>
<tr>
<td></td>
<td>Hair length (mm)</td>
<td>160</td>
<td>25.09</td>
<td>0.76</td>
<td>9.65</td>
<td>4.00 – 62.00</td>
</tr>
<tr>
<td>Natural styled</td>
<td>Age (yrs)</td>
<td>32</td>
<td>24.58</td>
<td>1.24</td>
<td>6.88</td>
<td>19.00-55.00</td>
</tr>
<tr>
<td></td>
<td>Hair length (mm)</td>
<td>160</td>
<td>88.92</td>
<td>3.48</td>
<td>43.32</td>
<td>27.00 – 209.00</td>
</tr>
<tr>
<td>Relaxed styled</td>
<td>Age (yrs)</td>
<td>32</td>
<td>26.03</td>
<td>0.90</td>
<td>5.08</td>
<td>19.00-39.00</td>
</tr>
<tr>
<td></td>
<td>Hair length (mm)</td>
<td>160</td>
<td>125.50</td>
<td>4.96</td>
<td>62.80</td>
<td>10.00 – 369.00</td>
</tr>
<tr>
<td>Overall</td>
<td>Age (yrs)</td>
<td>96</td>
<td>21.96</td>
<td>0.70</td>
<td>6.83</td>
<td>14.00 – 55.00</td>
</tr>
<tr>
<td></td>
<td>Hair length (mm)</td>
<td>480</td>
<td>79.74</td>
<td>2.79</td>
<td>60.88</td>
<td>4.00 – 369.00</td>
</tr>
</tbody>
</table>
4.1 Hair shaft diameter

The overall mean shaft diameter was 68.29 μm (SD= 16.88). The increasing order of shaft diameter was Natural styled hair (64.31 μm, SD = 14.30), Natural unstyled hair (64.48 μm, SD = 18.45) and Relaxed styled hair (76.08 μm, SD = 14.83). Figure 10 compares the overall and group mean shaft diameters of all hairstyle groups. The regional mean shaft diameters of hairs, obtained from the collation of diameters of the three portions (proximal, middle and distal) of each hair shaft for the hairstyle groups are shown in Table 4. For the Natural unstyled hair group, the median of the hair diameter (Z score) from the sampled areas were as follows: Right temporal (R), 58.81 μm (-2.05), Occipital (O), 58.81 μm (-1.86), Left temporal (L), 56.43 μm (-1.74), Frontal (F), 58.81 μm (-1.01) and Vertex (V), 76.67 μm (6.65) (Table 4). The median diameters (Z Score) for the Natural styled hair group were Right temporal (R) - 61.67 μm (-2.15), Left temporal (R)- 61.19 μm (-1.09), Frontal (F)- 62.29 μm (-0.06), Occipital (O) - 64.29 μm (0.25) and Vertex (V)- 68.90 μm (3.06) (Table 4). That for the Relaxed styled group are Right temporal (R) - 74.29 (-2.06), Occipital (O) - 74.29 (-1.73), Left temporal- 77.14 (0.35), Frontal (F)- 76.43 (0.39) and Vertex- 81.67 (3.04) (Table 4). Comparison of the median shaft diameters between the five scalp regions within each hairstyle group showed significant differences, for the Natural unstyled (p = 0.000), Natural styled (p = 0.016) and Relaxed styled (p = 0.009) groups respectively. Inter-group regional comparison of the median shaft diameter also showed significant differences (Table 5).
Figure 10: Bar chart of the overall and group median shaft diameters.
Table 4: Regional shaft diameters of the hairstyle groups.

<table>
<thead>
<tr>
<th>Hairstyle of Scalp</th>
<th>Region of Scalp</th>
<th>No. of fibres (n)</th>
<th>Diameter of Shaft Mean (μm)</th>
<th>SE. Mean</th>
<th>SD</th>
<th>Range (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural unstyled hair</td>
<td>F</td>
<td>96</td>
<td>62.55</td>
<td>1.72</td>
<td>16.83</td>
<td>27.14 - 106.67</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>96</td>
<td>62.17</td>
<td>1.92</td>
<td>18.57</td>
<td>18.57 - 119.05</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>96</td>
<td>61.27</td>
<td>1.70</td>
<td>21.78</td>
<td>21.78 – 105.71</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>96</td>
<td>60.39</td>
<td>1.60</td>
<td>23.81</td>
<td>23.81 – 97.62</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>96</td>
<td>76.01</td>
<td>2.01</td>
<td>31.90</td>
<td>31.90 – 127.14</td>
</tr>
<tr>
<td>Natural styled hair</td>
<td>F</td>
<td>96</td>
<td>64.16</td>
<td>1.31</td>
<td>12.83</td>
<td>30.95 – 96.19</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>96</td>
<td>62.74</td>
<td>1.49</td>
<td>14.59</td>
<td>30.95 – 58.57</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>96</td>
<td>64.41</td>
<td>1.45</td>
<td>14.23</td>
<td>29.05 – 99.52</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>96</td>
<td>61.49</td>
<td>1.43</td>
<td>14.05</td>
<td>30.48 – 104.29</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>96</td>
<td>68.75</td>
<td>1.52</td>
<td>14.93</td>
<td>38.57 – 111.43</td>
</tr>
<tr>
<td>Relaxed styled hair</td>
<td>F</td>
<td>96</td>
<td>76.69</td>
<td>1.30</td>
<td>12.76</td>
<td>45.24 - 114.29</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>96</td>
<td>75.99</td>
<td>1.20</td>
<td>11.78</td>
<td>45.24 – 102.38</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>96</td>
<td>73.56</td>
<td>1.47</td>
<td>14.38</td>
<td>35.71 – 103.81</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>96</td>
<td>72.61</td>
<td>1.58</td>
<td>15.51</td>
<td>31.43 – 107.14</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>96</td>
<td>81.54</td>
<td>1.80</td>
<td>17.61</td>
<td>50.95 – 138.57</td>
</tr>
</tbody>
</table>
Table 5: Regional comparison of shaft diameters between the three hairstyle groups.

<table>
<thead>
<tr>
<th>Scalp Region</th>
<th>No. of fibers (n)</th>
<th>Natural unstyled</th>
<th>Natural styled</th>
<th>Relaxed styled</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (μm)</td>
<td>Z Score</td>
<td>Median (μm)</td>
<td>Z Score</td>
<td>Median (μm)</td>
</tr>
<tr>
<td>F</td>
<td>288</td>
<td>58.81</td>
<td>-4.61</td>
<td>64.29</td>
<td>-2.67</td>
</tr>
<tr>
<td>L</td>
<td>288</td>
<td>56.43</td>
<td>-4.01</td>
<td>61.19</td>
<td>-3.00</td>
</tr>
<tr>
<td>O</td>
<td>288</td>
<td>58.81</td>
<td>-4.10</td>
<td>64.29</td>
<td>-1.33</td>
</tr>
<tr>
<td>R</td>
<td>288</td>
<td>58.81</td>
<td>-3.39</td>
<td>61.67</td>
<td>-2.50</td>
</tr>
<tr>
<td>V</td>
<td>288</td>
<td>76.67</td>
<td>0.84</td>
<td>68.90</td>
<td>-4.60</td>
</tr>
</tbody>
</table>

4.2. Type and form of medulla

The type and form of the medulla was found to vary along the length of some hair shafts in all three groups (Figures 11-22). Hairs classified as having absent medulla included those in which no medulla was visible and those in which a distinction could not be made of the areas of the cortex and medulla due to heavy pigmentation of the fibre. The Natural unstyled hair group had 75.63 % (363) and 24.38% (117) of hairs with the absent and present types of medulla respectively (Figure 23). Eighteen point seven one percent (87) of hairs of the Natural styled hair group had absent medulla whereas 81.29% (378) had medulla present (Figure 23). The proportion of hairs in the Relaxed styled hair group with absent and present types of the medulla were 10.29% (49) and 89.71% (427) respectively (Figure 23). Chi square established a significant association between the type of medulla (absent or present) and the type of hairstyle.
Additionally, there was a significant association \( p = 0.000 \) between the form of the medulla when present and hairstyle. Overall, the Natural unstyled hair group has the greatest proportion of hairs with a continuous medulla, 7.5\% (36), followed by the Natural styled hair group, 5.81\% (27) and then the Relaxed styled hair group, 2.10\% (10) (Figure 24). The greatest proportion of hairs with both the discontinuous (Di) (48.11\%) and fragmentary (F) (39.50\%) forms of medulla were seen in the Relaxed styled group (Figure 24) and was followed by the Natural styled hair group (Di- 43.66\% and F- 31.83\%) and then the Natural unstyled hair group (Di- 11.88\%) and F- 4.79\%) (Figure 24). Figure 25 shows the proportion of hairs exhibiting the various medullary types and forms in the proximal, middle and distal portions of the hair shaft for all three hairstyle groups.
Figure 11: ‘Absent-Absent-Continuous’ medullary variation along a single shaft of Natural styled hair (Tone, contrast and colour was enhanced to make medulla more visible).
Proximal

Absent Medulla

Middle

Absent Medulla

Distal

Discontinuous Medulla

Figure 12: ‘Absent-Absent-Discontinuous’ medullary variation along a single shaft of Natural styled hair (Tone, contrast and colour was enhanced to make medulla more visible).
Figure 13: ‘Discontinuous-Discontinuous-Continuous’ medullary variation along a single shaft of Natural styled hair (Tone, contrast and colour was enhanced to make medulla more visible).
Proximal

Continuous Medulla

Middle

Continuous Medulla

Distal

Discontinuous Medulla

Figure 14: ‘Continuous-Continuous- Discontinuous’ medullary variation along a single shaft of Natural styled hair (*Tone, contrast and colour was enhanced to make medulla more visible*).
Proximal

Absent Medulla

Middle

Continuous Medulla

Distal

Continuous Medulla

**Figure 15:** ‘Absent-Continuous-Continuous’ medullary variation along a single shaft of Natural styled hair (*Tone, contrast and colour was enhanced to make medulla more visible*).
Proximal

Absent Medulla

Middle

Absent Medulla

Distal

Fragmentary Medulla

Figure 16: ‘Absent-Absent-Fragmentary’ medullary variation along a single shaft of Natural styled hair (*Tone, contrast and colour was enhanced to make medulla more visible*).
Figure 17: ‘Absent-Fragmentary-Absent’ medullary variation along a single shaft of Natural unstyled hair (*Tone, contrast and colour was enhanced to make medulla more visible*).
Proximal

Absent Medulla

Middle

Discontinuous Medulla

Distal

Absent Medulla

**Figure 18:** ‘Absent-Discontinuous-Absent’ medullary variation along a single shaft of Natural unstyled hair (*Tone, contrast and colour was enhanced to make medulla more visible*).
Figure 19: ‘Continuous-Continuous-Absent’ medullary variation along a single shaft of Natural unstyled hair (*Tone, contrast and colour was enhanced to make medulla more visible*).
Figure 20: ‘Continuous-Fragmentary-Fragmentary’ medullary variation along a single shaft of Natural unstyled hair *(Tone, contrast and colour was enhanced to make medulla more visible).*
Proximal

Continuous Medulla

Middle

Fragmentary Medulla

Distal

Absent Medulla

**Figure 21:** ‘Continuous-Fragmentary-Absent’ medullary variation along a single shaft of Relaxed styled hair (*Tone, contrast and colour was enhanced to make medulla more visible*).
Figure 22: ‘Fragmentary-Fragmentary-Continuous’ medullary variation along a single shaft of Relaxed styled hair (*Tone, contrast and colour was enhanced to make medulla more visible*).
**Figure 23:** A bar chart showing the number of hairs with (Present) and without (Absent) medulla in all hairstyle groups.

**Figure 24:** A bar chart comparing the number of hairs with the various types of medulla between the hairstyle groups.
4.3 Medullary diameter and medullary index

Medullary diameters were measured for the middle segments of the hair shaft only. The proportions of middle hair shafts that showed visible medulla were 20% (63), 58.75% (94) and 58.12% (93) for the Natural unstyled, Natural styled and Relaxed styled respectively. Tables 6-8 show the means of the medullary and shaft diameters (MD & SD) as well as medullary index (MI) for all five scalp regions for the three hairstyle groups. There were no significant regional intra-group variations of the medullary and corresponding shaft diameter, as well as medullary index for the Natural unstyled group (MD - p = 0.153, SD - p = 0.352, MI - p = 0.153), Natural unstyled group (MD - p = 0.940, SD - p = 0.136, MD - p = 0.803), and the Relaxed styled group (MD - p = 0. 174, SD - p = 0.431, and MI - p = 0.2.97). There was however a significant inter-group variation for MD (p = 0.000), SD (p = 0.000), and
MI (p = 0.000). The median (Z score) medullary diameter, was lowest in the Relaxed styled (12.86 μm (-3.61), followed by the Natural styled (14.68 μm (-0.15) and the Natural unstyled (17.00 μm. (4.18). The mean Shaft diameter, mean (St. Dev) increased in the order, Natural unstyled, 69.76 μm (12.61), Natural unstyled, 73.93 μm (16.09) and Relaxed styled, 82.36 μm (13.76). Medullary index, median (Z score), was largest in the Natural unstyled group- 0.210 (4.33), followed by the Natural styled- 0.238 (1.86) and then the Relaxed styled group- 0.151 (-5.76). A significantly positive Pearson’s correlation of medullary and shaft diameters were recorded for the Natural unstyled (0.320, p = 0.011) and Natural styled (0.235, p = 0.022) groups but not the Relaxed styled group (0.122, p = 0.2). The overall mean medullary diameter and mean medullary index (mean, SD) were 15.052 μm (6.579) and 0.205 (0.091) respectively.
Table 6: Medullary and shaft diameters of the middle shafts and the resulting medullary indices of Natural unstyled hair of female Ghanaians.

<table>
<thead>
<tr>
<th>Region of Scalp</th>
<th>No. of fibres (n)</th>
<th>MEDULLARY DIAMETER</th>
<th>SHAFT DIAMETER</th>
<th>MEDULLARY INDEX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (μm)</td>
<td>SE Mean</td>
<td>SD</td>
</tr>
<tr>
<td>F</td>
<td>7</td>
<td>19.50</td>
<td>1.36</td>
<td>3.59</td>
</tr>
<tr>
<td>L</td>
<td>17</td>
<td>19.08</td>
<td>1.53</td>
<td>6.32</td>
</tr>
<tr>
<td>O</td>
<td>15</td>
<td>15.48</td>
<td>1.04</td>
<td>4.01</td>
</tr>
<tr>
<td>R</td>
<td>16</td>
<td>16.99</td>
<td>1.15</td>
<td>4.06</td>
</tr>
<tr>
<td>V</td>
<td>8</td>
<td>15.47</td>
<td>1.48</td>
<td>4.19</td>
</tr>
</tbody>
</table>
Table 7: Medullary and shaft diameters of the middle shafts and the resulting medullary indices of Natural styled hair of female Ghanaians.

<table>
<thead>
<tr>
<th>Region of Scalp</th>
<th>No. of fibres (n)</th>
<th>MEAN (μm)</th>
<th>SE</th>
<th>RANGE (μm)</th>
<th>MEAN (μm)</th>
<th>SE</th>
<th>RANGE (μm)</th>
<th>MEAN</th>
<th>SE</th>
<th>SD</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>24</td>
<td>14.95</td>
<td>0.97</td>
<td>4.75</td>
<td>8.57-30.86</td>
<td>67.33</td>
<td>1.73</td>
<td>8.46</td>
<td>55.24-87.62</td>
<td>0.224</td>
<td>0.014</td>
</tr>
<tr>
<td>L</td>
<td>16</td>
<td>14.85</td>
<td>1.23</td>
<td>4.92</td>
<td>7.14-27.50</td>
<td>66.43</td>
<td>4.14</td>
<td>16.58</td>
<td>44.29-98.57</td>
<td>0.236</td>
<td>0.028</td>
</tr>
<tr>
<td>O</td>
<td>19</td>
<td>14.32</td>
<td>1.42</td>
<td>6.20</td>
<td>4.71-33.29</td>
<td>68.50</td>
<td>2.72</td>
<td>11.85</td>
<td>53.33-99.52</td>
<td>0.210</td>
<td>0.019</td>
</tr>
<tr>
<td>R</td>
<td>18</td>
<td>14.71</td>
<td>1.03</td>
<td>4.39</td>
<td>8.57-25.00</td>
<td>70.02</td>
<td>2.90</td>
<td>12.32</td>
<td>54.76-104.29</td>
<td>0.213</td>
<td>0.014</td>
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<td>V</td>
<td>17</td>
<td>15.23</td>
<td>1.11</td>
<td>4.59</td>
<td>9.29-22.64</td>
<td>77.48</td>
<td>3.06</td>
<td>12.63</td>
<td>56.19-94.76</td>
<td>0.197</td>
<td>0.013</td>
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</tbody>
</table>
Table 8: Medullary and shaft diameters of the middle shafts and the resulting medullary indices of Relaxed styled hair of female Ghanaians.

<table>
<thead>
<tr>
<th>Region of Scalp</th>
<th>No. of fibres (n)</th>
<th>Mean (μm)</th>
<th>SE Mean</th>
<th>SD</th>
<th>Range (μm)</th>
<th>Mean (μm)</th>
<th>SE Mean</th>
<th>SD</th>
<th>Range (μm)</th>
<th>Mean</th>
<th>SE Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>17</td>
<td>16.99</td>
<td>2.80</td>
<td>11.53</td>
<td>3.59-50.50</td>
<td>82.04</td>
<td>3.40</td>
<td>14.00</td>
<td>64.29-114.29</td>
<td>0.206</td>
<td>0.031</td>
<td>0.129</td>
<td>0.049-0.530</td>
</tr>
<tr>
<td>L</td>
<td>18</td>
<td>9.916</td>
<td>0.961</td>
<td>4.08</td>
<td>4.03-17.14</td>
<td>80.54</td>
<td>2.76</td>
<td>11.69</td>
<td>46.67-95.95</td>
<td>0.128</td>
<td>0.015</td>
<td>0.062</td>
<td>0.046-0.245</td>
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<td>O</td>
<td>21</td>
<td>13.02</td>
<td>1.29</td>
<td>5.89</td>
<td>3.59-22.86</td>
<td>79.19</td>
<td>3.21</td>
<td>14.71</td>
<td>56.19-105.81</td>
<td>0.171</td>
<td>0.018</td>
<td>0.084</td>
<td>0.035-0.347</td>
</tr>
<tr>
<td>R</td>
<td>17</td>
<td>14.27</td>
<td>2.32</td>
<td>9.56</td>
<td>4.03-42.50</td>
<td>82.94</td>
<td>2.34</td>
<td>9.65</td>
<td>60.00-104.29</td>
<td>0.169</td>
<td>0.026</td>
<td>0.105</td>
<td>0.057-0.482</td>
</tr>
<tr>
<td>V</td>
<td>20</td>
<td>14.94</td>
<td>1.96</td>
<td>8.78</td>
<td>4.47-40.00</td>
<td>87.12</td>
<td>3.77</td>
<td>16.86</td>
<td>60.48-114.76</td>
<td>0.178</td>
<td>0.025</td>
<td>0.110</td>
<td>0.049-0.416</td>
</tr>
</tbody>
</table>
4.4 Scale characteristics

Scale characteristics of only the proximal and distal segments of the hair shaft were considered (Figures 28-30). The characteristics of the cuticular scales studied included quantitative counts of the number of scales per unit length and measurement of interval between scales. Qualitative descriptions of scale pattern, nature of scale margins and distance between scales were also made.

4.4.1 Mean scale count (MSC)

In general a significant difference (p= 0.00) in the mean count per 1 cm was recorded between the proximal (1.721, SD = 0.246) and distal (1.579, SD = 0.412) portions of the hair at a 95% CI of (0.1853-0.0988). Significant intra group variation (Natural unstyled, p= 0.00; Natural styled, p= 0.026; Relaxed styled, p=0.00) also existed between the mean scale counts of the proximal and distal shafts of the hair with the proximal portions of the hair recording the higher mean scale count. In the Natural styled group, the mean scale count was 1.615 (SD = 0.244) for the proximal shaft and 1.486 (SD = 0.364) for the distal (Table 9). The Natural styled group had a mean scale count of 1.819 (SD = 0.252) and 1.757 (SD = 0.240) for the proximal and distal shafts respectively (Table 9). The mean scale count for the proximal shaft for the Relaxed styled group was 1.731 (SD = 0.196) and that for the distal shaft was 1.498 (SD = 0.522) (Table 9). Inter group variation of mean scale count was significant at p = 0.000 for both the proximal shaft and distal shafts. The order of decreasing mean scale count for the proximal and distal shafts were Natural styled, Relaxed styled and Natural unstyled. Figure 26 shows the mean scale count of the proximal and distal shafts for all hairstyle groups.
Table 9: Mean scale count per 1 cm for all hairstyle groups.

<table>
<thead>
<tr>
<th>Hairstyle</th>
<th>Part of shaft</th>
<th>No. of fibres</th>
<th>Mean</th>
<th>SE Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural unstyled</td>
<td>Proximal</td>
<td>160</td>
<td>1.61</td>
<td>0.019</td>
<td>0.244</td>
<td>0.00-2.51</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>160</td>
<td>1.49</td>
<td>0.029</td>
<td>0.364</td>
<td>0.00-2.66</td>
</tr>
<tr>
<td>Natural styled</td>
<td>Proximal</td>
<td>155</td>
<td>1.82</td>
<td>0.020</td>
<td>0.252</td>
<td>0.94-2.48</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>155</td>
<td>1.76</td>
<td>0.019</td>
<td>0.240</td>
<td>0.71-2.75</td>
</tr>
<tr>
<td>Relaxed styled</td>
<td>Proximal</td>
<td>160</td>
<td>1.73</td>
<td>0.016</td>
<td>0.196</td>
<td>1.24-2.66</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>159</td>
<td>1.50</td>
<td>0.041</td>
<td>0.522</td>
<td>0.00-3.19</td>
</tr>
</tbody>
</table>

Figure 26: A bar chart comparing the mean scale count of the proximal and distal shafts within and between hairstyle groups. Error bars represent standard error of the means.
4.4.2 Interval between scale margins (ISM)

Table 10 shows the interval between scale margins for the proximal and distal shafts for all three hairstyle groups. Generally, the difference between the median interval between scale margins (ISM) for the proximal (8.626 µm) and distal shaft (8.930 µm) was significant at \( p = 0.000 \). Intra group difference between the median IMS for the proximal and distal portions of the hair was significant for the Relaxed styled group (\( p=0.000 \)) but not for the Natural unstyled (\( p = 0.726 \)) and Natural styled (\( p=0.188 \)) groups. Inter-group comparisons of both the proximal ISM and distal ISM were significant at \( p = 0.000 \). The proximal median ISM was lowest in the Natural unstyled (7.98 µm, \( z= -3.57 \)), followed by the Relaxed styled (8.55 µm, \( z= -1.26 \)) and Natural styled (8.92 µm, \( z= 4.87 \)). The distal median ISM trend was as for the proximal shaft; Natural unstyled (8.01 µm, \( z= -4.81 \)), Relaxed styled (9.22 µm, \( z= 1.83 \)) and Natural styled (9.01 µm, \( z= 3.01 \)). Figure 27 shows the median ISM of the proximal and distal portions of the shaft for groups.
Table 10: Interval between scale margins for all hairstyle groups.

<table>
<thead>
<tr>
<th>Hairstyle</th>
<th>Part of shaft</th>
<th>No. of fibres</th>
<th>Mean (µm)</th>
<th>SE Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural unstyled</td>
<td>Proximal</td>
<td>160</td>
<td>8.41</td>
<td>0.211</td>
<td>2.605</td>
<td>0.00-32.45</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>160</td>
<td>8.12</td>
<td>0.197</td>
<td>2.489</td>
<td>0.00-14.85</td>
</tr>
<tr>
<td>Natural styled</td>
<td>Proximal</td>
<td>155</td>
<td>9.08</td>
<td>0.100</td>
<td>1.240</td>
<td>6.66-16.32</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>155</td>
<td>9.26</td>
<td>0.107</td>
<td>1.340</td>
<td>6.10-12.87</td>
</tr>
<tr>
<td>Relaxed styled</td>
<td>Proximal</td>
<td>160</td>
<td>8.35</td>
<td>0.132</td>
<td>1.666</td>
<td>0.00-12.48</td>
</tr>
<tr>
<td></td>
<td>Distal</td>
<td>159</td>
<td>8.65</td>
<td>0.232</td>
<td>2.920</td>
<td>0.00-14.32</td>
</tr>
</tbody>
</table>
4.4.3. Scale patterns

Scales patterns were described as regular mosaic (RM), irregular mosaic (IM), regular wave (RW) and irregular wave (IW) for both the proximal and distal portions of the hair shaft (Figures 28-30). In general, the sampled scalp hairs depicted more waved scale pattern (52.2\% (482)) than the mosaic scale pattern 47.73\% (442) (Figure 31). Chi square results also showed that there is a significant association between the type of hairstyle and the type of scale patterns (p = 0.002). Generally, the Natural unstyled group showed a higher proportion of the mosaic scale pattern (50.12\%) than the waved pattern (48.88\%) while the natural styled and relaxed styled hairs had greater proportions of the waved pattern than the mosaic pattern (Figure 31).

Figure 32 compares the overall proportions of hairs with the various scale patterns for all groups. The Natural unstyled group showed the greatest proportion, 16.93\% (53)
of the RM pattern, followed by the Relaxed styled group, 9.21% (28) and then the Natural styled group, 7.77% (24). The highest proportion of the IW scale pattern was seen in the Relaxed styled group (39.80% (121), followed by the Natural styled (35.28% (109), and then the Natural unstyled group, 34.19% (107). The irregular wave scale pattern was seen most in the Natural styled hair group (40.13%), followed by the Relaxed hair group (31.25%), and Natural unstyled had the least (29.71%).

The proportions of hairs of the proximal and distal portions of the hair shaft with the various scale patterns both within and between all the three hairstyle groups are shown in Figure 33. In the proximal segment of the hairs of the three groups the predominant scale pattern is irregular mosaic while in the distal segment, the predominant scale pattern is irregular wave for all three hair groups (Fig. 33).
A1: Proximal

Smooth, near, regular wave

A2: Distal

Rippled, close, irregular wave

B1: Proximal

Smooth, distant, irregular mosaic

B2: Distal

Rippled, near, irregular wave

Figure 28-A: Variation of scale characteristics at the proximal and distal ends of shafts of Natural unstyled hair (*Tone, contrast and colour was enhanced to make scales more visible*).
C1: Proximal

Smooth, distant, regular mosaic

C2: Distal

Ripped, distant, irregular mosaic

Figure 28-B: Variation of scale characteristics at the proximal and distal ends of shafts of Natural unstyled hair (*Tone, contrast and colour was enhanced to make scales more visible*).
A1: Proximal

Smooth, near, regular mosaic

A2: Distal

Crenate, distant, irregular mosaic

B1: Proximal

Smooth, distant, irregular mosaic

B2: Distal

Crenate, distant, irregular mosaic

Figure 29-A: Variation of scale characteristics at the proximal and distal ends of shafts of Natural styled hair (*Tone, contrast and colour was enhanced to make scales more visible*).
Figure 29-B: Variation of scale characteristics at the proximal and distal ends of shafts of Natural styled hair (Tone, contrast and colour was enhanced to make scales more visible).
A1: Proximal

![Image of smooth, near, regular wave]

Smooth, near, regular wave

A2: Distal

![Image of rippled, distant, irregular mosaic]

Rippled, distant, irregular mosaic

B1: Proximal

![Image of crenate, distant, irregular mosaic]

Crenate, distant, irregular mosaic

B2: Distal

![Image of no scales]

No scales

**Figure 30-A:** Variation of scale characteristics at the proximal and distal ends of shafts of Relaxed styled hair (*Tone, contrast and colour was enhanced to make scales more visible*).
C1: Proximal

Crenate, close, regular wave

C2: Distal

Rippled, near, irregular wave

Figure 30-B: Variation of scale characteristics at the proximal and distal ends of shafts of Relaxed styled hair (Tone, contrast and colour was enhanced to make scales more visible.)
D1: Proximal

Smooth, close, regular wave

D2: Distal

Rippled, distant, irregular wave

**Figure 30-C:** Variation of scale characteristics at the proximal and distal ends of shafts of Relaxed styled hair (*Tone, contrast and colour was enhanced to make scales more visible*).
**Figure 31:** A bar chart of the number of hairs showing the mosaic versus waved scale patterns for all hairstyle groups.

**Figure 32:** A bar chart comparing the number of hairs showing the various scale patterns (regular mosaic (RM); irregular mosaic (IM); regular wave (RW); irregular wave (IW)) between hairstyle groups.
Figure 33: A bar chart depicting inter- and intra-group comparison of scale patterns of the proximal (P) and distal (D) hair shafts.

4.4.4 Nature of scale margins

The smooth (S), crenate (Cr) and rippled (R) scale margin types were observed (Figures 28-30). The portions of the hair shaft considered for this description were the proximal (P) and distal (D) portions. Figure 34 compares the overall proportions of hairs with the various scale margins for all groups. The smooth scale margin type is commonly found in the Natural unstyled (34.94%) group, followed by the Relaxed styled group (9.55%), and then the Natural styled group (6.15%) (Figure 34). The greatest proportion of the Cr margin was again seen in the Natural unstyled group (21.15%), followed by the Natural styled (16.50%), and then the Relaxed styled group (11.24%) (Figure 34). The proportions of hair with the R margin type were 79.21% in
Relaxed styled, 77.35% in the Natural unstyled, and 43.91% in the Natural styled. A test for association between scale margins and hairstyle was significant at $p = 0.043$. Figure 35 shows the proportion of scale margin types found in the proximal and distal portions of the hairs in the three hair style groups. The rippled type of scale margin is the predominant type found in the proximal and distal portions of all the hair style types except in the proximal portions of the Natural unstyled group where the smooth scale margin was the commonest (Figure 35).

**Figure 34:** A bar chart comparing the number of hairs with the various scale margins between hairstyle groups.
Figure 35: A bar chart illustrating intra- and inter-group comparisons of the number of proximal and distal shafts of the various hairstyle groups exhibiting the different types of scale margins.

4.4.5 Scale Separation

This qualitative scale characteristic was described for the proximal and distal portions of the hair shaft using the terms, close, near and distant. Figure 36 shows the overall proportions of hairs with the various scale separation types for all groups. The Natural unstyled group showed the greatest proportion (3.26%) of the close type, followed by the Natural styled group (2.47%) and then the Relaxed styled group (1.00 %) (Figure 36). The greatest proportion of the near type was again shown by the Natural unstyled group (65.47%), followed by the Natural styled (59.26%), and then the Relaxed styled group (57.48%) (Figure 36). The order of decreasing proportion of the distant type was Relaxed styled (41.53%), Natural styled (38.27%) and Natural unstyled (31.27%) (Figure 36).
The proportion of scale separation types found in the proximal and distal segments of the sampled hair from the three hair style groups are shown in Figure 37. For the Natural unstyled type of hair, the near type of scale separation is the commonest in both the proximal and distal portions of the hair. Similarly, the near type is commonest in the proximal portions of the Natural styled and Relaxed styled hairs (Figure 37). In the distal portions of the relaxed styled hair, the commonest separation is the distant types. In all segments of all hair style types, the near type of scale separation is the commonest followed by the distant except in the Relaxed styled group where the distal portion recorded a higher distant type of scale separation than the near type (Figure 37).

**Figure 36:** A bar chart comparing the number of hairs with the various scale separation types between hairstyle groups.
4.5. Hair root and tip morphology

Hair roots were classified as anagen, telogen and catagen roots whereas the tips were classified as finely pointed, razor cut, blunt tip or fribillated (Figures 38-39). Most of the roots of hairs of the various hairstyle groups were anagen roots with the number of catagen and telogen hairs just about equal. A greater number of hairs of the Natural unstyled and Relaxed styled groups showed razor or blunted tips whereas the Natural styled hair group mostly showed finely pointed tips.
Figure 38: Morphology of roots found in all three groups of hairstyles (Tone, contrast and colour was enhanced to make images more visible).
Figure 39-A: Nature of hair tips found in all three groups of hairstyles (*Tone, contrast and colour was enhanced to make images more visible*).
**Figure 39-B:** Nature of hair tips found in all three groups of hairstyles (*Tone, contrast and colour was enhanced to make images more visible*).
CHAPTER FIVE

DISCUSSION

5.0 Hair Diameter

This study has found a statistically significant difference in the median diameters of hair shafts from the various scalp regions for the entire study population. Similarly, the hair shaft diameters of the various scalp regions sampled showed statistically significant variation within and between hairstyle groups. Similar findings have been reported in earlier studies (Garn, 1951; Van Neste, 2004; Jasuja & Minakshi, 2002) although there are variations in the regions of focus of each of these studies.

According to Garn (1951), on a normal scalp (pre-alopecia), hair is finest at the temples and most coarse at the sideburns (of which the lower parts are actually beard hair). Van Neste (2004) recorded a larger shaft diameter for the occipital hairs than the temporal regions hairs. In this study, that is only true when the occipital region is compared with the right temporal region but not the left which has the same median hair shaft diameter. Jasuja and Minakshi (2002) from the study of 25 males and females demonstrated not only regional scalp variations but also intra-regional variation within the same individual.

The hair shaft is derived from the progeny of keratinocyte stem cells in the follicular epithelium whose growth and differentiation is directed by the dermal papilla embedded in the hair bulb. Hair is known to have a growth rate of 0.3-0.4 mm / day (Van Neste, 2004) with a slightly lower rate for Africans compared to other races (Loussouarn et al., 2005; Nonhlanhla, 2005). Ibrahim and Wright (1982) observed that in addition to a constant equal increase in length per unit time during the growth period of hair, there is also a constant increase in width such that there is an increase
by a factor of hundreds or even thousands between the tip and the bottom of the fully
grown hair. This could be translated to mean that whereas the length of hair depends
on the growth rate and the duration of anagen (Alonzo & Fuchs, 2006), the thickness
or diameter of hair is dependent on the growth rate of the hair only. Thicker hairs
would therefore be expected to have faster growth rates than finer hairs. A study by
Baque et al. (2012) confirmed that a thicker hair fibre corresponds to a faster growth
rate. Since the size of hair produced depends on the size of the follicle which also is
dependent on the size of the dermal papilla (Ibrahim & Wright, 1982; Chi et al.,
2013), faster growing follicles would be expected to be larger to produce thicker hairs
and vice versa. The regional difference in the diameter of hairs observed in this study
therefore suggests a variation in the growth rate and hence size of follicles on
different regions of the scalp. Follicles on the vertex are the fastest growing and
largest whereas those on the right temporal region are slowest growing and the
smallest.

The regional difference in the growth rate of scalp hair is supported by several studies
(Loussouarn et al., 2005; Van Neste, 2004; Robbins, 2012). The study by Loussouarn
et al. (2005) on the rates of hair growth of different geo-racial groups showed that the
growth rate of African hairs increases from the occipital, through the temples to the
vertex. Van Neste (2004) demonstrated a faster growth rate for hairs at the occipital
region than for temples. Robbins (2012) found that hair grows faster at the vertex and
slower at the temples, (0.44 mm / day vs 0.39 mm / day) and (14 cm / year vs 13 cm /
year) for Caucasian males and females respectively. The findings of this study are
thus more consistent with the findings of Robbins (2012); hairs grow slowest on the
right temple and fastest on the vertex.
On the contrary, the suggestion of a regional follicular size variation is not supported by the study done by Mulinari-Brenner et al. (2006) which found no significant difference in the size of the hair follicle distributed in several scalp regions (mid-frontal, vertex, occipital and right temporal areas). The study was carried out using follicles from cadavers. The hair follicle is the only living portion of hair; the shaft being a dead tissue. It is possible that the post-mortem changes that are associated with tissues at death, could account for the no variation in follicular size the study obtained.

Inter-group regional comparison showed the lowest median shaft diameter for the Natural unstyled hair, followed by the Natural styled hair and then, the Relaxed styled hair for all scalp regions considered except in the vertex where a higher median shaft diameter for the Natural unstyled hair than the Natural styled hair was observed.

The growth of hair in humans is affected by age, sex, race nutrition, hormonal levels and location on the body (Hamilton, 1958; Saitoh et al., 1969). The diameter of hair has been shown to increase with age. Gaur et al. (2007) studied the age, gender and caste variations in scalp hair micro-morphological variables among Brahmmins and Banias of Punjab, India and found that averagely, hair shaft diameters increased up to 30 years in females. Other studies (Tajima et al., 2007; Nagase et al., 2009) have reported an increase in scalp hair with age with the maximum diameter attained at age 40. The inter group variation in shaft diameters in this study could therefore be accounted for by the variation in the age of the individuals in the various group. Individuals in the Natural unstyled group were the youngest, followed by the Natural styled group and then the Relaxed styled group. Accordingly, shaft diameter increased in the same manner. The larger shaft diameter for the vertex in the Natural unstyled group compared to the Natural styled group is difficult to explain with the age
phenomenon. The only possible explanation could be that before puberty, hair growth is most rapid on the vertex of the scalp. After puberty however, the growth rate of hair on the vertex slows and becomes less rapid as compared to the hair growth on the occiput in both males and females (Blume-Peytavi et al., 2008).

The mean shaft diameter obtained for the Natural unstyled hair group (64.31 µm) was lower than that (71.15 µm) obtained by Kalmoni (2008) for scalp hair of Ghanaian female adolescents. Bearing in mind the changes in shaft diameter with age, the lower age (mean, range) of the individuals in the Natural unstyled hair group for this study (15.34 yrs, 14.00 yrs -17.00 yrs) compared to that of the group (18 yrs, 15 yrs - 20 yrs) in the study by Kalmoni (2008) could account for this difference. Moreover, the mean for the Natural unstyled hair group is the average of the diameters of the three hair segments of all hairs obtained from the five scalp regions under consideration. Kalmoni’s work failed to indicate from which region(s) of the scalp the hairs were obtained and the segment(s) of the hair shaft from which the diameters were measured are also not known. Considering the significant regional differences in shaft diameter recorded here, knowledge of the scalp region from where hair shafts were obtained in the study by Kalmoni (2008) would have been helpful in making a meaningful comparison. The finding that the overall mean diameter obtained for this study was also lower than what was obtained by Kalmoni is somewhat surprising considering the age range of the subjects in this study and the fact that hair diameter increases with age. The only plausible explanation for this finding is as given above for the Natural unstyled hair group; possible difference in the region from where hair samples were obtained and in part(s) of the hair from which measurements were made.
5.1 Medulla

5.1.1 Occurrence of medulla

The current study recorded a significantly higher proportion of hairs with a medulla than hairs without a medulla. The same was observed for all the three hairstyle groups. The Natural unstyled group showed the greatest percentage of hairs without a medulla, followed by the Natural styled group and then the Relaxed styled group. This decreasing order of absence of medulla paralleled the increasing order of shaft diameter recorded in this study suggesting an association between the diameter of hair and the occurrence of a medulla. The finding is corroborated by the studies by Wynkoop (1929) and Hausman (1930) that thicker hairs are more likely to have a medulla than finer hairs. Banerjee (1962) reported that frizzy and woolly hairs show the highest incidence of absence of medulla whereas straight hair, the highest incidence of presence of medulla. Tolgyesi et al. (1983) demonstrated that beard hair which is coarser than scalp hair contain a higher percentage of medulla than scalp hair. Kalmoni (2008) confirmed that the medulla if it present, conformed to the diameter of the hair.

The absence or presence of medulla in the hair shaft might be due to the characteristics of the fibre itself (Banerjee 1962). Non medullation may mean no medulla or that melanosome density of medullary and cortical cells are similar such that the medulla would be inconspicuous (Irmak et al., 1995). The degree of pigmentation of the hair influences the conspicuousness of medulla under the microscope (Aboagye, 2011). Hairs classified as having no medulla in this study included those without an obvious medulla in the cortex and those in which the areas of the cortex and medulla were indistinguishable due to the high degree of pigmentation. Khumalo et al. (2005) observed large numbers of melanin granules in
the cortex and rarely within the cells of the cuticle of scalp hairs of Africans which is not seen in Caucasian or Asian hair. A greater number of hairs in the Natural unstyled hair group were intensely pigmented than the Natural styled hair group which also had more hairs with intense pigmentation than the Relaxed styled hair group. This could account for the observed differences in the hairs with or without medulla. The decreased pigmentation of the Relaxed styled group made the medulla more visible when present than the other groups.

The difference in pigmentation between the Natural unstyled and Natural styled groups would be difficult to explain. Considering, however, that tropical regions have sunshine for most part of the year and the effect of sunlight on hair (loss of colour) (Jeon et al., 2008; Sebetic et al., 2008), it is likely that the hairs of the Natural styled group have had longer exposure to sunlight (due to the longer hair length) compared to the Natural unstyled hair (regularly cut short hair) culminating in the difference in extent of pigmentation. In addition to the effect of sunlight on hair, the difference in pigmentation between, the two Natural hair groups and the Relaxed group could be ascribed to an additional effect of the relaxers on the hair shaft. One of the complaints of patrons of hair relaxers is depigmentation (loss of colour) of the hair shaft (Olasode, 2009). It is possible that the effect of the relaxer makes the hair more susceptive to the effect of the sunlight than the other groups, increasing the loss of pigmentation by hairs of this group.

5.1.2 Form of the medulla

The medulla when present was found to vary in form at the proximal, middle and distal parts of some hair shaft and could be continuous, discontinuous or fragmentary. The variation of the form of medulla along the hair shaft observed in this study is
consistent with the work of Hutchinson and Thompson (1999). According to the study, the form of the medulla in scalp hair is affected by the hair cycle, being continuous for the first 50% of anagen, discontinuous for the next 25% and virtually absent or absent for the final 25%.

The greatest proportion of hairs with the continuous form of the medulla was recorded by the Natural unstyled group, followed by the Natural styled and Relaxed styled groups respectively. This order is parallel to the increasing order of discontinuous and fragmentary medulla forms. The record of a higher continuous medulla by the Natural unstyled than the Natural styled groups which was also higher than the Relaxed group is somewhat surprising bearing in mind the work by Wynkoop (1929). The study by Wynkoop showed that the amount of medulla in hair is related to the fibre diameter, establishing a strong positive relationship between hair fiber diameter and the amount of medulla. Consequently, finer hairs do not contain a medulla, medium sized hairs generally contain a broken (discontinuous) medulla and the thickest hairs generally contain a continuous medulla. Judging from the mean shaft diameters of the hairs recorded for the various groups, the Relaxed styled hair should have had the greatest proportion of hairs with a continuous medulla and the least with fragmentary medulla. It is possible that the styling procedures that the hairs in the Natural styled and Relaxed styled groups have been subjected to could have been the reason for the above results.

5.1.3 Medullary diameter and index

The medullary diameter and index were measured only for the middle segment of the hair shaft. The Natural unstyled hair group had the lowest proportion, of middle
portion of hairs with a medulla which is to be expected due to significantly low presence of medulla the group recorded. The about equal proportion of middle hair shafts with a medulla in the Natural styled and Relaxed styled groups is unexpected since the latter had more occurrence of medulla than the latter. Coming however from the point that the occurrence of medulla is correlated with the phase of the hair cycle could offer some explanation but weakly. The diameter of the medulla was significantly higher in the Natural unstyled group, followed by the Natural unstyled and then the Relaxed styled hair. The value of medullary diameter recorded for the Natural unstyled group was however higher than was recorded by Kalmoni (2008) in Ghanaian female adolescents. The mean diameters of the hair shafts that contributed to the medullar diameter however increased from Natural unstyled to the Relaxed styled group. These results are a bit unexpected considering that an association has been established between hair shaft diameter and the medullary size. Hairs with larger diameters have correspondingly large medulla (Hutchinson & Thompson, 1999; Kalmoni, 2008; Van Neste, 2004). It is possible that the styling (braiding and weaving) that these hairs are subjected to affects the medulla in thickness and form. Expectedly, the medullary index was largest in the Natural unstyled hair group followed by the Natural styled and then the Relaxed styled groups. The medullary indices recorded in each hairstyle group and the overall medullary index were less than 1/3 of the hair shaft. This result is consistent with the value of medullary index recorded for human hair in literature (Kalmoni, 2008; Kshiragar et al., 2009).
5.2 Scale characteristics

Significant differences in both qualitative and quantitative scale characteristics were observed in the proximal and distal portions of the shaft both within and between groups. This finding is in agreement with the study by Khenniche et al. (2003) which observed that cuticular features of scalp hair vary along the shaft at the proximal, middle and distal ends.

5.2.1 Quantitative characteristics

5.2.1.1 Mean scale count (MSC) and interval between scale margins (ISM)

Within all groups, the proximal shafts recorded higher MSCs than the distal shaft. The distal shafts also recorded higher ISMs than the proximal shafts for all groups except the Natural unstyled group where the ISM of the proximal shaft was higher than the ISM for the distal shaft. This suggests a relationship between the MSC and the ISM of a hair shaft. The higher the MSC, the lower the ISM and vice versa. Several studies (Tolgyesi et al., 1983; Takahashi et al., 2006; Aboagye, 2011) have found a negative association between the MSC and the ISM. The higher MSC values verses the lower ISM values for the proximal shafts compared to the distal shaft could also be attributed to the wear and tear phenomenon mentioned earlier contrary to the study of Aboagye (2011). The scale characteristics of the proximal shaft are closer in appearance to the scale characteristics of the hair when it first emerged from the scalp than the distal shafts on the basis of exposure to trauma (Bottoms et al., 1972; Robinson, 1976). Therefore, the number of scales of the shaft decreases whereas the distance between scale margins (Figure 15) increases in a proximo-distal fashion due to attrition.
The increasing order of both MSC and ISM for the proximal and distal parts of the hair shaft was Natural styled, Relaxed styled and Natural unstyled. These results seem to suggest a positive association between MSC and ISM for both the proximal and distal shaft within groups, contrary to the findings of other researchers (Tolgyesi et al., 1983; Takahashi et al., 2006; Aboagye, 2011).

An association has been established between fibre size and ISM (Baque et al., 2012); thicker fibres had shorter inter-scale distance and finer fibres had larger inter-scale distance. The studies by Tolgyesi et al. (1983), Takahashi et al. (2006) and Aboagye (2011) also found that hairs with higher MSC and lower ISM were thicker than hairs with lower MSC and higher ISM. These findings suggest an association between hair size on one hand, and MSC and ISM on the other. Fibre size alone, however, cannot be used to explain the results obtained for the inter-group variation observed in this study since hairs in the Relaxed styled group were the largest in diameter followed by the Natural styled and Natural unstyled groups respectively. The only plausible explanation could a combined effect of fibre size and the phenomenon of wear and tear. The Natural unstyled group with the smallest shaft diameter is expected to have the largest ISM and smallest MSC. However, because this hair group is less traumatized (shortest fibre length) than the other groups, the ISM would be comparatively smaller than the other styled groups which are so traumatized, due to attrition of the scales. The diameter of fibres in the Natural styled group is smaller than the Relaxed styled group and so the former should have lower MSC and higher ISM compared to the latter. As a result of the potentiated effect of chemical treatment and styling procedures on the Relaxed styled hair as well as the duration of such exposure (longest fibre length), must have lost some scales hence the lower MSC than the Natural styled group.
5.2.2 Qualitative assessment of scales

5.2.2.1 Scale pattern

Overall, a greater number of the wave pattern was observed than mosaic and irregular than regular. The Natural unstyled group recorded a higher number of hairs with mosaic patterns than the wave pattern whereas the reverse was true for hairs of the two styled group. The order of decreasing regular scale pattern was Natural unstyled, followed by the Relaxed styled and then Natural styled. The decreasing order of the irregular scale pattern was also Natural styled, Relaxed styled and Natural unstyle. The proximal shaft showed higher regular scale patterns than the distal shaft.

The finding of more mosaic than wave scale patterns in the Natural unstyled group is in consonance with a study by Kalmoni (2008) on the scalp hair of Ghanaian female adolescents.

It has been established from studies on scalp hair (Kelly & Robinson, 1982; Khenniche et al., 2003) that when hair first emerges from the scalp, the edges are smooth and regular. With an increase in distance from the scalp and mechanical contact with other hairs and surfaces, the regular nature is altered to irregular (Kelly & Robinson, 1982; Khenniche et al., 2003). This explains why generally the Natural unstyled group recorded a higher proportion of regular than irregular scale patterns compared to the two styled groups. Hairs of the Natural unstyled group by nature of their cut tips (appendix) and length are not allowed to grow long. These hairs are therefore not subjected to the extent of mechanical and chemical insults experienced by hairs of the styled groups and therefore are more likely to retain the regular nature of the scale patterns. Garcia et al. (1977) discussed the effect of cutting on the scale pattern of the cuticle. The study stated that keeping the length of hair constant by cutting will enhance the condition of the cuticle than allowing hair of the same length
to grow freely. Aboagye (2011) found that facial hairs which are prevented from growing long by cutting are not subjected to aggressions that could result in attrition of the cuticles edges.

Between the two styled groups (Natural and Relaxed), the Natural styled group recorded significantly lower regular patterns and higher irregular patterns compared to the Relaxed styled group. This results is a bit surprising considering the fact that hairs in the Relaxed styled group were longer and in addition to the trauma experienced from the styling practices (braiding and weaving), have been weakened by continuous regular application of chemical relaxers (Trüeb, 2005, Olasode, 2009). It was thus expected that the Natural styled group would present a higher regular scale pattern than the Relaxed styled group. Garcia et al. (1977), however, noted that the normal weathering of the hair shaft as described by Bottoms et al. (1972) could be attributable to the normal process of grooming; washing towel drying and combing. Robbins and Robbins (2003) have shown that the natural (kinky) hair is difficult to comb and require higher forces in grooming procedures compared to hair of other races. Consequently, natural hair suffers greater mechanical injury than hairs of individuals of other races during everyday grooming procedures (Draelos, 2011a; Khumalo, 2006). Chemical relaxing of the kinky hair releases the tight curls in the natural hair and makes it easier to manage (Roseborough & McMichael, 2009). As a result, although relaxed hair is weaker than natural hair (Etemesi, 2007; Shetty et al., 2013) the ease with which it is managed is greater than for the natural hair. This means that even though relaxed hair is more prone to cuticular damage, the lower combing force required in its grooming decreases the rate of attrition of the cuticle. The regular nature of the scale pattern is thus more preserved especially at the proximal ends, than the natural styled hair. On the contrary, the higher force required
in grooming the natural hair results in a comparatively greater degree of weathering of the hair thus changing the regular pattern to the irregular one.

Based on the same reasons given above regarding the alteration of the regular pattern to the irregular pattern with aging of the hair shaft and the calibre of hairs in the various groups, the record of a higher number of hairs with the regular pattern at the proximal than the distal parts of the hair shaft in all groups is expected. This finding is in line with animal study by Kitpipit and Thanakiatkrai (2013) which found 100% regular (wave) scale pattern for the proximal part of the hair shaft and none for the distal part.

5.2.2.2 Scale margins

The nature of the cuticular scale margin has a relation with the amount and degree of trauma that the hair has been subjected to resulting in the wear and tear of the cuticle margins (Bottoms et al., 1972; Kelly & Robinson, 1982). It is also influenced by the age of the hair. Smooth scale margins indicate a better state of the cuticle than crenate margins which also represents a better state than rippled margins. The proximal part of the hair shaft is younger (in terms of time of emergence from the scalp) than the distal part and would therefore have been less exposed to mechanical or chemical (relaxed hair) insults compared to the distal part. Expectedly, the proportions of hairs with the smooth scale margin were always significantly higher in the proximal portions of the hair shaft than the distal portions in all hairstyle groups. Conversely, the distal portions of the hair shaft always recorded significantly higher number of the rippled scale margin than the proximal portions as the case should be. This is similar to results obtained by Kitpipit and Thanakiatkrai (2013) who obtained 76.00% vs
0.00% hairs with smooth scale margins compared to 1.00 % vs 67.70 % of rippled scale margin for the proximal vs distal shaft portions respectively in the study of tiger hair morphology and its variations. Natural unstyle hair again showed the greatest number of hairs with the smooth scale margin, followed by the Natural styled hair and then the Relaxed styled hair.

5.2.2.3 Scale separation
In general the assessment of scale separation revealed a significantly higher proportion of the ‘near’ form, followed by the ‘distant’ and ‘close’ in all groups. The proximal and distal ends of the hair shaft also showed significant variation in the proportion of hair with the various scale separation types in all hairstyle groups. The Natural styled group showed no close scale separation type at the proximal shafts but the Natural unstyled recorded a greater proportion than the Relaxed styled group. The high combing force used in grooming the Natural styled hair compared to the Relaxed styled hair could account for the absence of and the reduced proportion of the close type scale separation in these hair style types respectively. The proximal portion of the hair shaft showed an increasing near separation type in the order, Natural styled, followed by the Relaxed styled and then Natural unstyled may also be attributed to the length and management of the hair. Hairs of the Natural unstyled and Relaxed styled groups were longer and thus required higher grooming (combing) forces than the Natural unstyled group increasing attrition in these two groups. The increased attrition in the Natural styled and Relaxed styled hairs increase the distance between adjacent scales more than it does in the Natural unstyled group.

The finding that the Natural unstyled group showed the greatest proportion of proximal hair with the distant separation type followed by the Relaxed styled group
and then the Natural styled group is consistent with the report by Wyatt and Riggott (1980) that hairs with smaller diameter have large scales and larger scale separations while those with bigger diameter have small scales and near scale separations. This is confirmed by Baque et al. (2012) who found that thicker hair fibers corresponded to a shorter inter-scale distance. This is because the proximal shafts by reason of their closeness to the scalp are expected to maintain as much as possible the morphological characteristics with which the hair emerged from the scalp. For this reason, the Natural unstyled hair having the smallest diameter in this study is expected to show the minimum number of hair with the close and near types of scale separation but the highest number of the distant type compared to the other groups. Similarly, the Relaxed styled group with the largest shaft diameter is expected to show the near and or close scale separation types more than the others.

A different situation was observed in the distal portion of the hair shaft. The Natural unstyled group recorded the highest proportion of hairs with the close separation type and was followed by the Natural styled and Relaxed in that order. The highest proportion of the close type was recorded by the Natural styled, followed by the Natural unstyled and then the Relaxed styled. The distant type was recorded in the decreasing order, Relaxed styled, Natural unstyled and Natural styled. The phenomenon of trauma and wear and tear explained earlier for the other scale features could account for these findings. It appears that with wear and tear, a decrease in the distance between scales results. This incidence probably explains why the proportion of hairs with the near and distant separation types respectively decrease and increase in the distal portion of the hair for all hairstyle groups. For the same reason, the proportions of hair with the close scale type increased in the distal part of the hair shaft for all but the Relaxed hair group where there was a decrease. The only probable
explanation is that due to the extensive wear and tear in this group due to the length of the hair and the intensity of trauma (both chemical and mechanical from braiding and weaving) the higher proportion of the distal hairs compared to the other groups had lost all scales, assuming that scales became close to each prior to their loss entirely. Generally in all groups, the near scale separation type was the most recorded, followed by the distant type and then the close type. The study by Kalmoni (2008) also recorded a higher near separation type than the distant and near for Ghanaian female adolescents.

Though most of the findings on scale characteristics have been attributed to the phenomenon of wear and tear and cosmetic influences, it must be stressed that other factors have immense influences on scale development in hair.

5.3 Types of root and nature of tip.

A greater number of hairs exhibited anagen roots compared to catagen and telogen roots. This finding could be explained by the growth pattern of human hair, the region of the body under consideration and the method of hair sampling the study employed. Human hair is known to occur in a mosaic pattern compared to the synchronous pattern of hair growth exhibited by animals. Consequently, the phase of the hair cycle of a follicle at any one point in time is independent of the growth phase of adjacent follicles. Additionally, it has been established that a greater proportion of scalp hair (80-85%) are in the growth phase (anagen) of the hair cycle whereas only a few are in catagen (2%) and telogen (10-15%) (Stene, 2004). Since hairs from all five scalp regions of interest in the current study were obtained from subjects through random
plucking with a pair of tweezers of a cosmetologist, the probability of picking up a lot of hairs in anagen is very high.

The greater proportion of hairs of the Natural unstyled hair group exhibiting the blunt razor cut tips is expected. This is because the standard hair style for individuals in the group (adolescents in Second cycle Institutions in Ghana) is a haircut. The shorter length at which the hairs are always maintained prevents the kind of weathering experienced by hairs of very long length (fribillation of hairs). The Relaxed styled hair group also showed an appreciable number of hairs with blunted cut ends in addition to the fribillation of the tips. The fact that relaxed hairs have their weakened tips occasionally trimmed to avoid fribillation and exposure of the cortex accounts for this observation. Natural unstyled hairs have not been chemically straightened and so do not need the tips trim hence the absence of razor of blunt cut ends. The finely pointed nature of the hair tips are as a result of the reduction of the hair diameter with increasing distance from the scalp due to the normal regular and progressive removal of the cuticle (Bottoms et al., 1972). All hairs of the Natural unstyled hair group showed only finely pointed tips because such hairs are neither cut nor trimmed as are the cases in the two other hairstyle group. The tip becomes pointed by reason of the normal patterned loss of the cuticle associated with normal grooming.

**5.4 Conclusion**

This study has established that hair shaft diameter was smallest in the Natural styled group, followed by the Natural unstyled and then Relaxed styled groups. The study also found that scalp hair is finest at the right temporal region and thickest at the vertex. The medulla when present in a hair shaft varies in form at the proximal,
middle and distal portions. Shaft diameter correlated with medullary diameter for the Natural unstyled and Natural styled hair groups but not the Relaxed styled group. The least number of hairs with medulla was recorded in the Natural unstyled hair and the most, in the Relaxed styled group. The fragmentary and discontinuous medullas were the predominant types found in the styled groups whereas the continuous type was dominant in the Natural unstyled group. There exists significant association between hairstyle and scale characteristics of hair. Proximal shafts had well preserved scale characteristics than distal shafts. Therefore hairstyle must be considered when using scalp hair in personal identification in the forensic sciences.

5.5 Recommendation

From the findings in this study it is recommended that:

- Future studies should consider the microscopic characteristics of other hairstyles such as dreadlocks and hair plaiting with thread.
- Further studies should be carried out to ascertain the individual effect(s) of braids and weaves on the microscopic structure of hair.
- Future studies be carried out that compare the morphological characteristics of styled scalp hair among children and adults.
- Comparison should be made between the microscopic morphology of styled scalp hair of pre and post menopausal women in future studies
- Subsequent studies should determine the micro-morphological characteristics of gray styled hair.
5.6 Limitations

- There was no means of authenticating the claim of study participants to be indigenous Ghanaians.
- Other hairstyles such as dreadlocks were not considered.
- The high degree of weathering in some hairs made the assessment of certain scale characteristics very challenging.
REFERENCES


Sledzik, P., Dirkmaat, D., Mann, R., Holland, T., Mundorff, A. Z., Adams, B., …


APPENDIX ONE

DEPARTMENT OF ANATOMY
SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES
COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF GHANA

IMPACT OF STYLING ON SCALP HAIR STRUCTURE IN GHANAIAN FEMALES

RESEARCH QUESTIONNAIRE

This questionnaire solicits for information, and subsequently, samples of scalp hair to be used in a research that seeks to determine the effect of styling on the structure of scalp hair of Ghanaian females. It is purely for academic and health research purposes and nothing else. Your identity would be kept anonymous (never be disclosed) as we do not require your name on the form and the information treated confidential as much as possible. Please answer as accurately as possible.

Part A: Personal Details
Please Tick the appropriate option

[A1] What is your age range (in years)?
☐ 61 – Above

[A2] What is your highest level of formal education?
☐ No education ☐ Primary ☐ SHS ☐ Post secondary
☐ Tertiary ☐ Other (please specify) ..........................

[A3] What is your Occupation?
☐ Student ☐ Civil Servant ☐ Public Servant ☐ Farmer
☐ Housewife ☐ Other (please specify) ..........................

[A4] Are you a Ghanaian? ☐ Yes ☐ No

[A5] Are both of your parents Ghanaians? ☐ Yes ☐ No

[A6] Are your immediate grandparents Ghanaians? ☐ Yes ☐ No

[A7] What is the current state of your scalp hair?
☐ Virgin (untreated) ☐ Chemically treated (relaxed)

If your hair is chemically treated (relaxed) jump to Part C.
Part B: For Virgin (untreated) Hair only
Please Tick as many as applies to you where necessary.

[B1] Why is your hair virgin (untreated) now?
☐ Religious beliefs  ☐ Personal reasons  ☐ Medical reasons
☐ Adverse effects  ☐ Others (please state) ..........................................

[B2] Have you ever relaxed your hair?  ☐ Yes  ☐ No

[B3] If your answer is ‘Yes’ in [B2], when was the last time you relaxed your hair?
☐ about 3 months ago  ☐ about 6 months ago
☐ More than 1 year ago  ☐ Others (please state) ..................................

[B4] If you answered [B3], why did you stop treating your hair at that time?
☐ Religious beliefs  ☐ Personal beliefs  ☐ Medical reasons
☐ Adverse effects  ☐ Others (please state) .................................. 

Part C: For Currently Relaxed (treated) Hair only
Please Tick or fill in where appropriate.

[C1] For how long has your hair been relaxed
☐ 0–12 months  ☐ 1–2 yrs  ☐ 2–3 yrs  ☐ 3–4 yrs
☐ 4–5 yrs  ☐ more than 5 yrs?

[C2] How often do you relax your hair (new growth)?
☐ Every 2 weeks  ☐ every month  ☐ every 2 months
☐ every 3 months

[C3] Which hair relaxer do you use currently? _____________________________

[C4] Have you stacked to the same hair relaxer for the past 3 years?
☐ Yes  ☐ No

[C5] If you answered ‘No’ in [C4], how many different relaxers have you used within that period? _____________________________

[C6] Have you ever experienced any adverse effects attributable to the hair relaxers?  
☐ Yes  ☐ No
If ‘Yes’, please state the adverse effect(s)?  ____________________________________________
Part D: For Both Virgin and Relaxed Hair

Please **Tick** or **fill in** where appropriate.

[D1] How often do you wash your hair?
- Once every week
- Twice every week
- Once every two weeks
- Once in a month

[D2] Where do you wash your hair?
- At home (by myself)
- At the salon (by a hairdresser)
- Both at home & salon

[D3] If you answered ‘Both ...’ in [D2] above, rate in terms of percentage (e.g. 50% of the time), how often you wash your hair at: (A) home ______ and (B) the salon _______________

[D4] How do you dry your hair?
- Air dry
- Heat blow
- Both (sometimes air drying, other times heat blowing)

[D5] If you answered ‘Both’ in [D4] above, rate in terms of percentage (e.g. 50% of the time), how often you dry your hair by: (A) air drying _______ and (B) heat blowing _________________

[D6] Have you ever dyed your hair?  [ ] Yes  [ ] No

[D7] Give any reason for dyeing your hair __________________________________________

[D8] When last did you dye your hair?
- 0-12 months ago
- 1-2 yrs ago
- 2-3 yrs ago
- 3-4 yrs ago
- 4-5 yrs ago
- More than 5 yrs ago

[D9] How often do you dye your hair?
- Once every six months
- Once every year
- Twice every year
- Other (please state) ________________________________

[D10] Do you braid your hair?  [ ] Yes  [ ] No

[D11] When braiding my hair,
- I use hair extensions all the time
- I use my own hair all the time
- I use my own hair at one time and extensions at another

[D12] How often do you braid your hair?
- Every week
- Once in two weeks
- Once every month
- Once every 2 months
- Once every three months
- Once every 6 months
- Once a year

[D13] How long do the braids stay on your head?
- 2 weeks
- 4 weeks
- 6 weeks
- 8 weeks
- 10 weeks
- 12 weeks
- More than 12 weeks

[D14] What is the time frame between one braid and the next? _____________
[D15] In between consecutive braids, what do you do with your hair? ___________

[D16] Do you put on weaves?  □ Yes  □ No

[D17] Give reasons for your answer above _______________________________

[D18] How often do you fix weaves?
□ Every 2 weeks  □ every month  □ every 2 months
□ every 3 months

[D19] How long do each fixed weave stay on your head?
□ 2 weeks  □ 4 weeks  □ 6 weeks  □ eight weeks
□ ten weeks  □ 12 weeks  □ More than 12 weeks

[D20] Have you noticed any adverse effects of weaves on your hair or scalp?
□ Yes  □ No
If ‘Yes’ give the adverse effect you have noticed __________________________

[D21] What is the time frame between one weave and the next?___________

[D22] In between consecutive weaves, what do you do with your hair?__________________________

[D23] Do you go from braids to weaves and vice versa?  □ Yes  □ No

[D24] For how long (averagely) is the hair relieved from one hairstyle before the next (braids–braids, weave–weave, braid–weave, weave–braid) _____________

[D25] For how long (in total) within a year, is your hair free from weaves or braids?
APPENDIX TWO

INFORMED CONSENT FORM

Name of principal investigator: Esther Adjoa Essel
Name of organisation: Department of Anatomy, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, Korle-Bu, Accra.
Research title: Scalp hair morphology after styling procedures: A light and electron microscopic study.

I ................................................................. have been invited to take part in the above titled research. The purpose of the research I have been told is: to assess the effect of styling on hair structure.

I am informed that the nature of procedure such as the method of sample collection chosen and its subsequent analysis will not pose any risks or hazards neither will it cause undue pain to me. My only involvement in the research will be to allow hairs on my scalp to be plucked using a pair of cosmetic tweezers. The hairs will be examined under the light and scanning electron microscopes for its morphological characteristics. I have been told that the essence of the research is to generate data which would be useful in the area of forensics and cosmetic industry.

I understand that I will not by my participation in this study, derive any personal benefits but help in generating data that will be useful to researchers. I do have the right to refuse participation in this research if I wish to without being affected in any way. I may ask questions now or later for clarification on issues pertaining to the research.

I may contact:
1. The Supervisors (Prof. F. K. Addai, Dr. S. K. Adjenti, and Dr. B. Hottor) of the Department of Anatomy, School of Biomedical and Allied Health Sciences, Korle-Bu.
   Tel: 0302672020
2. The Ethical and Protocol Review Committee, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, Korle-Bu, Accra, who have the task to ensure that no harm is made to research participants.

3. Esther Adjoa Essel, to ask questions or make complaint later using the address below:

Department of Anatomy, School of Biomedical and Allied Health Sciences, College of Health Sciences, Korle-Bu, Accra.
Tel: 0302672020/ 0242939352

I have read the information contained herein, or it has been read to me. I have had the opportunity to ask questions about it and have received satisfactory answers to them. I give my voluntary consent to partake in this study as a subject with the understanding that I can redraw anytime from the study without been affected any way.

Signed: ………………………………
Date: ………………………………
Place: ………………………………
APPENDIX THREE

CALIBRATION OF PUBLISHER RULE WITH STAGE GRATICULE

X40 objective lens

6.5 cm ≡ 7 graticule units (GUs) 9.3 cm ≡ 10 GUs
Each graticule unit (GU) = 0.01 mm Each GU = 0.01 mm
Therefore, 6.5 cm ≡ 7 x 0.01 mm Therefore, 9.3 cm ≡ 10 x 0.01 mm
6.5 cm ≡ 0.07 mm 9.3 cm ≡ 0.1 mm
1 cm ≡ \( \frac{0.07 \text{ mm}}{6.5} \) 1 cm ≡ \( \frac{0.1 \text{ mm}}{9.3} \)
1 cm ≡ 0.010769 mm 1 cm ≡ 0.010753 mm

Therefore, 1 cm ≡ \( \frac{0.010769 \text{ mm} + 0.010753 \text{ mm}}{2} \)
1 cm ≡ 0.010761 mm

1 mm = 1000 μm
Therefore, 1 cm ≡ 0.010761 x 1000 μm
1 cm ≡ 10.761 μm

X10 objective lens

3.5 cm ≡ 15 GUs 4.9 cm ≡ 21 GUs
Each GU = 0.01 mm Each GU = 0.01 mm
Therefore, 3.5 cm ≡ 15 x 0.01 mm Therefore, 4.9 cm ≡ 21 x 0.01 mm
3.5 cm ≡ 0.15 mm 4.9 cm ≡ 0.21 mm
1 cm ≡ \( \frac{0.15 \text{ mm}}{3.5} \) 1 cm ≡ \( \frac{0.21 \text{ mm}}{4.9} \)
1 cm ≡ 0.042857 mm 1 cm ≡ 0.042857 mm

Therefore, 1 cm ≡ 0.042857 mm
1 mm = 1000 μm

Therefore, 1 cm ≡ 0.042857 x 1000 μm

≡ 42.857 μm