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REGENERATIVE STEM CELLS IN AESTHETIC MEDICINE AND ITS EFFECT ON SKIN AGEING

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Abstract

Skin aging is a complicated biological process that depends on a combination of intrinsic and extrinsic factors. Aging skin has wrinkles, an unequal complexion, loss of elasticity, and thinness. Skin health is a major factor representing overall well-being and perception of human health. As a result, strategies to address aging and dysfunction have been developed over the last few decades. Understanding the mechanism behind skin ageing is necessary to elucidate the mechanism of action and, therefore, the potential advantages of the claimed anti-aging products. In this research, the types of aesthetic procedures that help the skin in generating new cells through the healing process mechanism (like hifu, Needling collagen induction therapy, skin peels, etc.) are analyzed. In this study, the effectiveness of topical pharmaceutical agents like cell regulators (botanicals, retinols, hormones, and peptides) and antioxidants (flavonoids, polyphenols, and vitamins) is presented along with the mechanism of each procedure for the production of stem cells and their mechanisms of action.

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Chapter One

Background

Aging is an unavoidable but extendable process (Sharpless and DePinho, 2007). Aging brings several diseases together causing devastating effects on human society and overloads the entire health care system and economy (Sharpless and DePinho, 2007; Lopez-Otin et al., 2013). Arthritis, cardiovascular disease, cancer, dementia, osteoporosis, diabetes, hypertension, degeneration of tissues, neuropathy, stroke, obesity, and depression are examples of age-induced diseases (Sharpless and DePinho, 2007; Lopez-Otin et al., 2013). Aging is a vital trigger of diseases and extends their shocks to affect vision, hearing, muscular strength, bone mass, immunity, nerve function, and metabolic disorders (Artandi et al., 2015). Aging is a fundamental threat to life itself, posing significant challenges to the entire system, demanding a pressing need to treat these health issues (Ullah and Sun, 2018).

"It is generally recognized that the ageing process has an impact on the natural course of wound healing." (Bonifant and Holloway, 2019). Age-related differences in wound healing have been clearly documented. There is a consensus that wounds heal more slowly in the elderly population and that all phases of the wound healing process are affected (Ratsinis et al., 2019; David et al., 1993) In other words, the population over the age of 65 years is increasing globally, and this may be accompanied by an increase in the number of individuals experiencing delayed wound healing (Bonifant and Holloway, 2019) There is a breadth of research to show that age-related changes in the epidermis and dermis change the skin's ability to resist damage and injury (Robert et al., 1993).

Wound healing necessitates the coordinated activation and interaction of many different cell types. Adipocytes have been found as a novel participant in wound healing, in addition to the traditional important players such as macrophages, endothelial cells, and (myo-)fibroblasts (Ploner et al., 2021). Certain injuries, particularly deep epidermal wounds, tend to be able to overwhelm endogenous repair processes, resulting in pathological scarring (Kwan et al., 2017; Finnerty et al., 2016).

Regenerative medicine has a lot of potential for repairing and restoring damaged tissue. Regenerative medicine researchers examine the molecular, cellular, and developmental processes that govern the growth of new, healthy tissue. To give a clear idea of what Regenerative Medicine entails, it is indeed helpful to proceed beyond the field's goals, which are to "understand the molecular and cellular mechanisms of regeneration, which occurs naturally, and how these mechanisms differ from the mechanisms of scarring," as well as to "use the knowledge gained from this mechanistic understanding to develop therapies that will stimulate functional regeneration of damaged human tissues that lack the capacity to regenerate" (David, 2006).

Regenerative medicine aims to treat and cure diseases by discovering out how nature restores the form and composition of damaged or diseased organs and tissues. Regenerative Medicine is evolving to help alter the world of science and medicine by integrating numerous fields such as biology, chemistry, engineering, mathematics, and computer science (UNMC, 2020).

According to Stocum (2006), "Most tissues are in a constant battle between tissue regeneration and repair by fibrosis (scarring)." He also notes a fundamental question scientist in the field of Regenerative Medicine ask: "Why do we see scar tissue form (fibrosis) when an organ or body part is damaged or lost (for example, a finger or toe is severed) instead of regrowth or regeneration of the tissue?" In this study, the effect of aesthetics on skin ageing will be examined.

The Human Cells

There are trillions of cells in the human body. They support the body's structure, absorb nutrients from food, transform those nutrients to energy, and perform specialized functions. Cells also contain the body's genetic material and can replicate themselves. Cells are made up of several sections, each of which serves a different purpose. Organelles, for example, are specialized structures that perform specific jobs within the cell (NLM, 2021). Human cells are organized into three main regions: Nucleus, Plasma Membrane, and Cytoplasm.

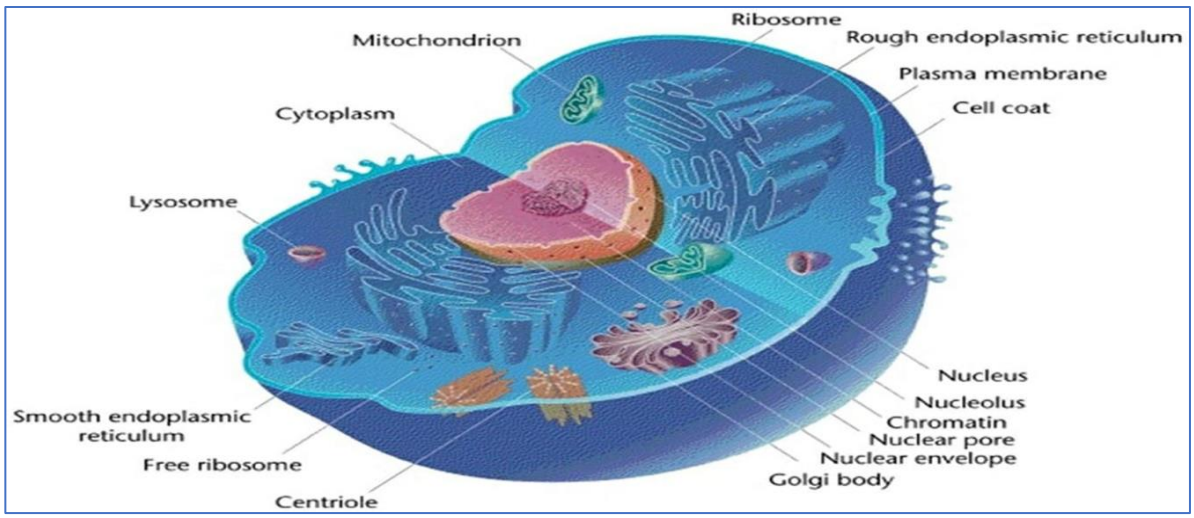


Figure 1: Parts of the human cell

Nucleus

The nucleus is the cell's control center and contains genetic material (DNA). The nucleus is a specialized structure found in most cells (with the exception of bacteria and blue-green algae) that is separated from the rest of the cell by a double layer known as the nuclear membrane. This membrane appears to be connected to the cell's endoplasmic reticulum (a membranous network) and features pores that allow big

molecules to pass through. The nucleus directs and regulates the cell's functions (such as development and metabolism) and houses the genes, which hold the cell's genetic information. Small entities found within the nucleus are known as nucleoli. The nucleoplasm is a gel-like framework where the nuclear components are suspended. The cell's command center is the nucleus, which sends instructions to the cell on how to grow, mature, divide, or die. It also contains the cell's genetic material, DNA (deoxyribonucleic acid). The nuclear envelope surrounds the nucleus and protects the DNA while also separating the nucleus from the remainder of the cell (Britannica, 2019).

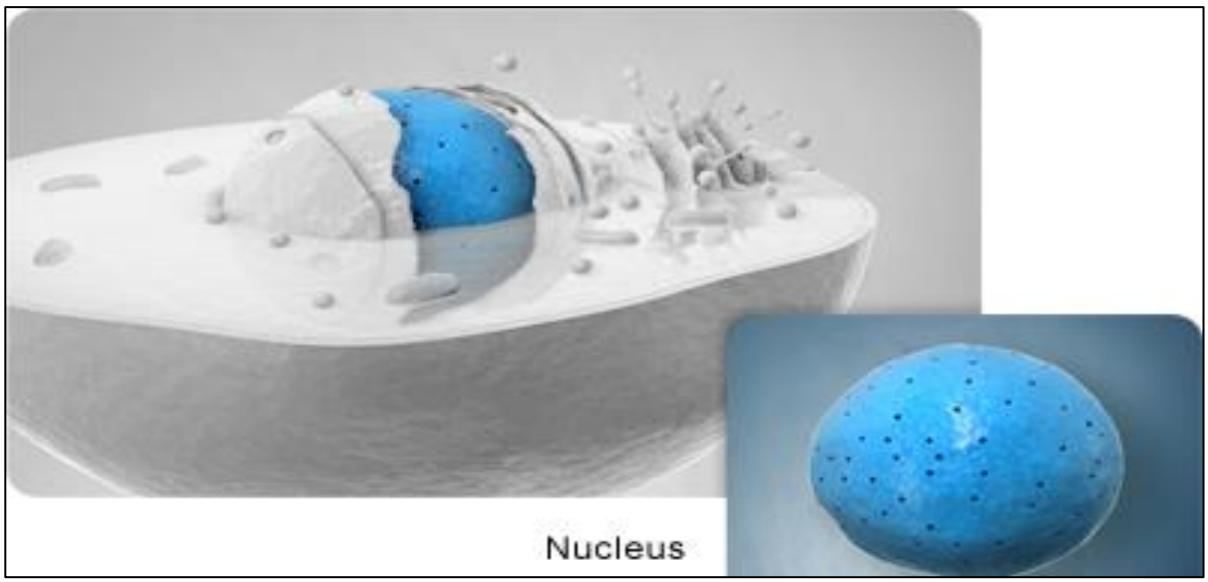


Figure 2: Nucleus

Source: US National Library of Medicine

The Nucleus consists of the nuclear membrane, nucleolus, and chromatin. Each will be discussed in the following subsections.

The Nuclear Membrane

A nuclear membrane is a double membrane that encloses the cell nucleus. It separates the chromosomes from the rest of the cell (NIH, 2021). The nuclear membrane includes an array of small holes or pores that permit the passage of certain materials, such as nucleic acids and proteins, between the nucleus and cytoplasm. We distinguish between eukaryotes (organisms with a nucleus) and prokaryotes (organisms without a nucleus) when dividing the species that exist on this planet. The nucleus holds all of a eukaryotic cell's genetic material, yet this genetic material must be preserved. The nuclear membrane, a double membrane that encloses all of the nuclear genetic material as well as all of the other components of the nucleus, protects it. There are some small holes or pores that are in the nuclear membrane that allow the messenger RNA and the proteins to move between the nucleus and the cytoplasm. However, the nuclear

membrane regulates which material should be in the nucleus as opposed to which material should be in the cytoplasm.

Nucleolus

The nucleolus is a structure within the cell nucleus that is responsible for ribosome production and assembly. Ribosomes are assembled and transported to the cell cytoplasm, where they act as protein synthesis sites. The nucleolus does not contain the chromosomes, rather, it contains the machinery necessary to assemble the cell's ribosomal RNAs. Ribosomal RNAs are then transferred into the cytoplasm through nuclear pores, where they meet the ribosome, which is the protein machinery. These ribosomal RNAs contribute in protein processing by guiding messenger RNAs through the ribosomes; nevertheless, these RNAs do not produce proteins. They are non-coding RNAs that aid in the protein translation process of messenger RNAs. These RNAs are created in the nucleus, just like other messenger RNAs, but ribosomal RNAs are made in the nucleolus, a highly particular portion of the cell nucleus.

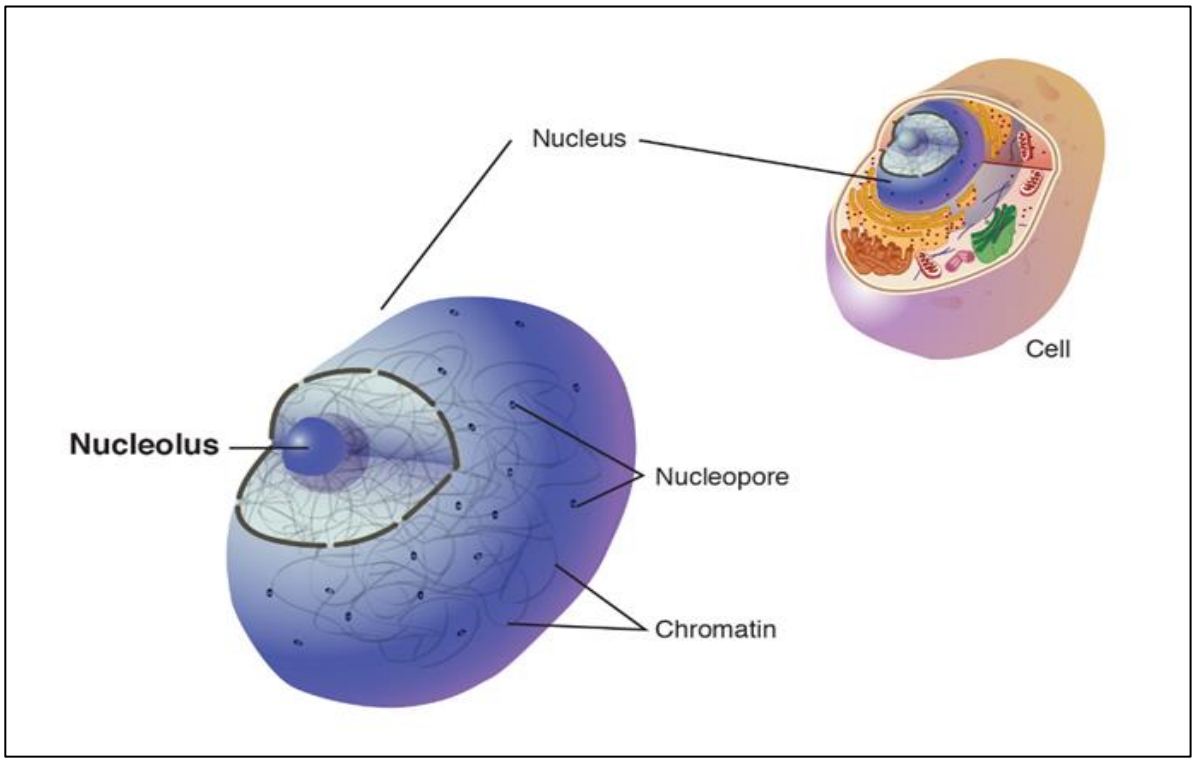


Figure 3: Nucleolus

Chromatin

Chromatin is a DNA and protein-based material found within chromosomes. The genetic instructions for the cell are carried by DNA. Histones are the main proteins in chromatin, and they help bundle DNA into

a compact form that fits inside the cell nucleus. DNA replication and gene expression are linked to changes in chromatin shape.

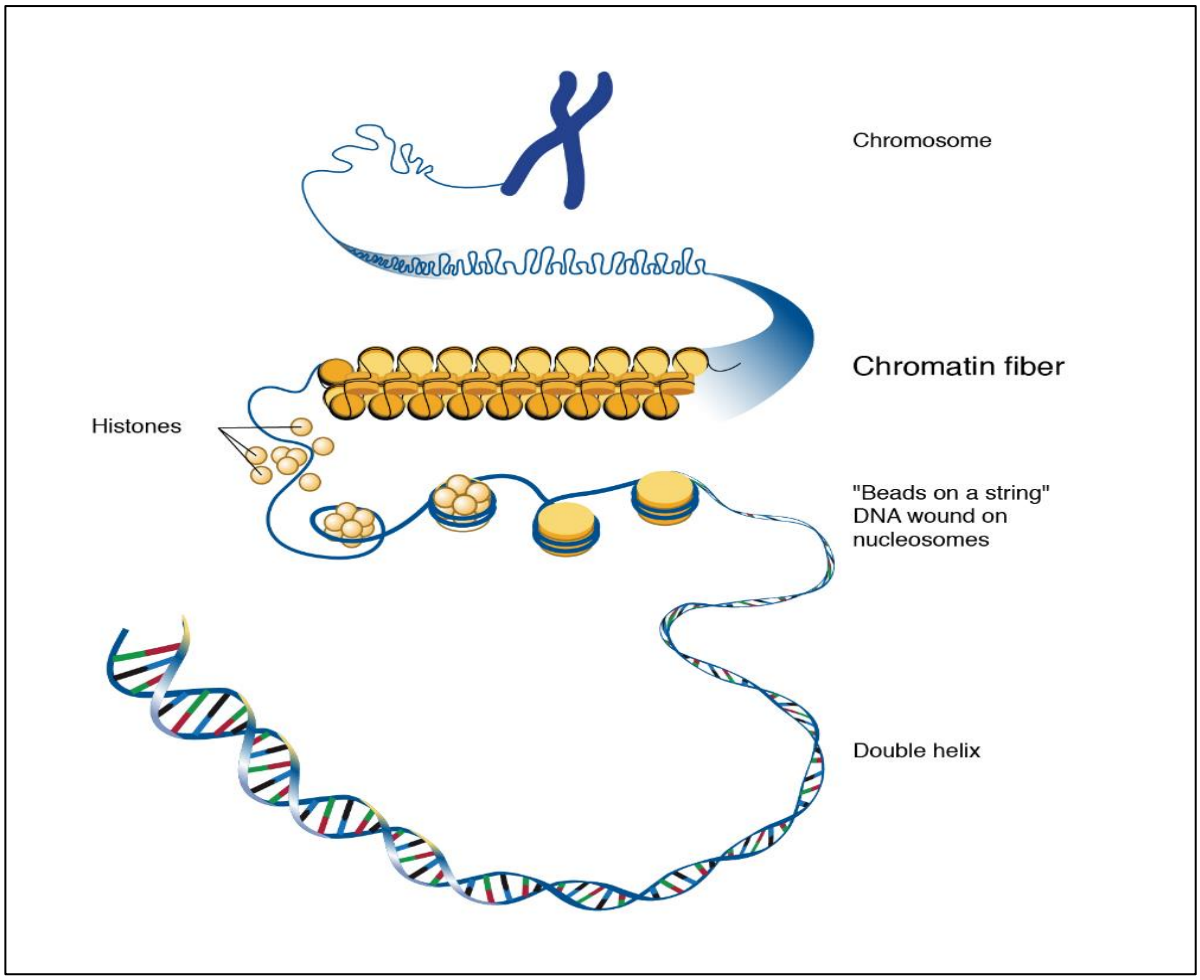


Figure 4: Chromatin

Histones are the most important proteins in chromatin. They serve as DNA packaging components. The importance of chromatin stems from the fact it is a reasonably impressive packing method for getting all of the DNA inside a cell. If the DNA inside a single cell is stretched end to end, it would be about a yard long. Since each cell is around a hundred millimeters wide, this is an excellent packing job for the DNA core in something with a diameter of a hundredth of a millimetre. The chromatin does this by wrapping and unwrapping the DNA in an extremely tight coil known as chromati.

The Plasma Membrane

The plasma membrane, also called the cell membrane, is the membrane found in all cells that separates the interior of the cell from the outside environment. In bacterial and plant cells, a cell wall is attached to

the plasma membrane on its outer surface. The plasma membrane consists of a semi-permeable lipid bilayer. The plasma membrane regulates the transport of materials entering and exiting the cell.

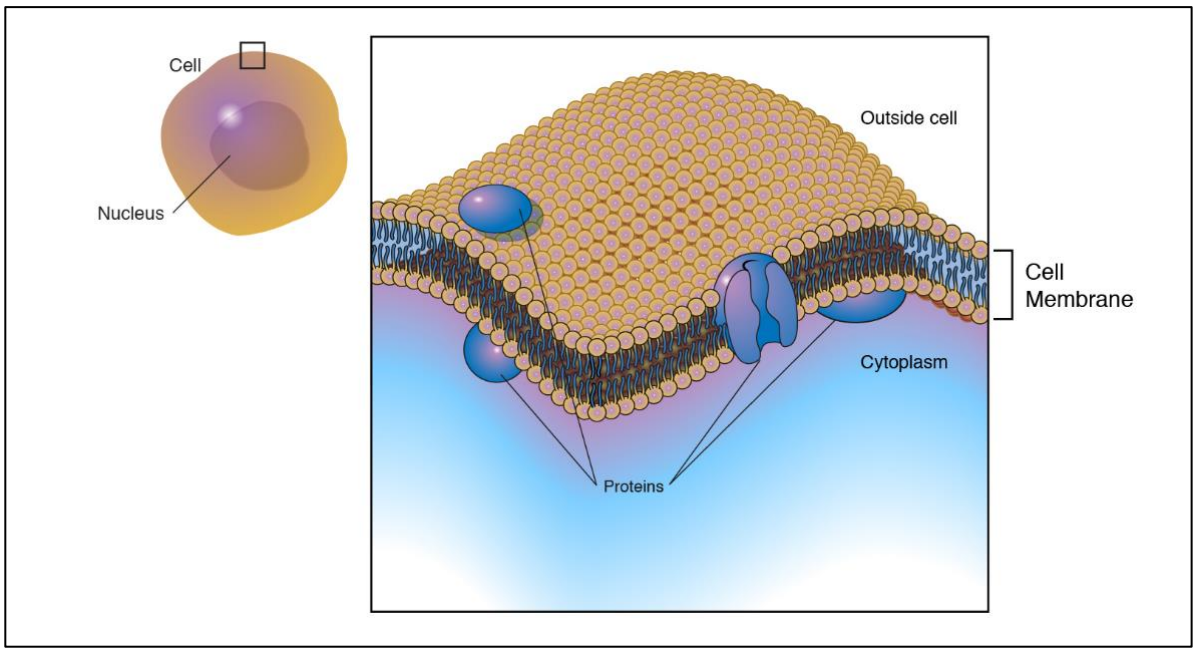


Figure 5(a): The Plasma Membrane

The plasma membrane provides protection for a cell. It also provides a fixed environment inside the cell. The plasma membrane has several different functions. One is to transport nutrients into the cell and to transport toxic substances out of the cell. Another is that the membrane of the cell, which would be the plasma membrane, will have proteins on it which interact with other cells. Those proteins can be glycoprotein, meaning there is a sugar and a protein moiety, or they could be lipid proteins, meaning there is a fat and a protein. The proteins that stick outside of the plasma membrane will allow interaction among cells. The cell membrane also provides some structural support for a cell. There are different types of plasma membranes in different types of cells; generally, the plasma membrane has a lot of cholesterol in it as its lipid component. This is different from certain other membranes within the cell. There are various plants and microbes, such as bacteria and algae, that have different protective mechanisms. In fact, they have a cell wall outside of them, and that cell wall is tougher and structurally sounder than a plasma membrane.

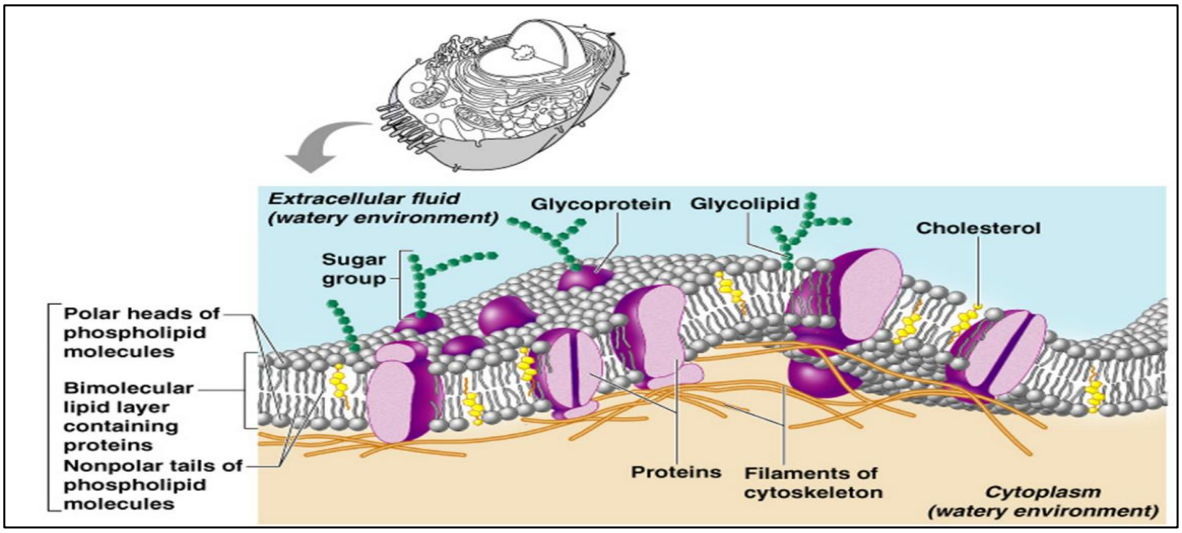


Figure 6(b): The Plasma Membrane structure

Cytoplasm

Within cells, the cytoplasm is made up of a jelly-like fluid (called the cytosol) and other structures that surround the nucleus. Cytoplasm is a thick solution that fills each cell and is enclosed by the cell membrane. It is mainly composed of water, salts, and proteins. In eukaryotic cells, the cytoplasm includes all the material inside the cell and outside of the nucleus. All the organelles in eukaryotic cells, such as the nucleus, endoplasmic reticulum, and mitochondria, are in the cytoplasm. Organelles are the metabolic machineries of the cell. The portion of the cytoplasm that is not contained in the organelles is called the cytosol; the cytosol is a fluid that suspends other elements of the cell. Although cytoplasm may appear to have no form or structure, it is highly organized. A framework of protein scaffolds called the cytoskeleton provides the cytoplasm and the cell with their structure.

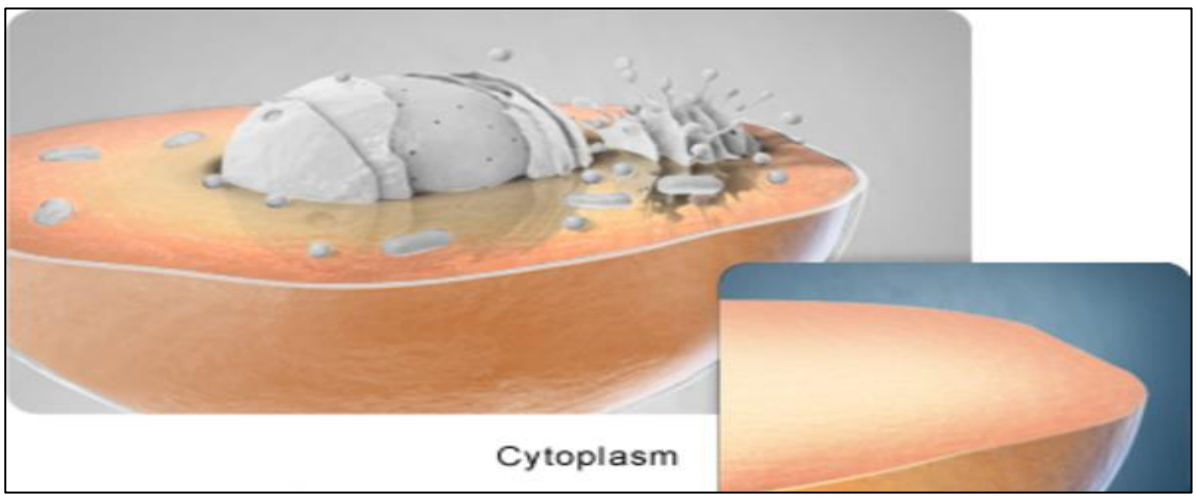


Figure 7: Cytoplasm

Source: US National Library of Medicine

Cytoplasmic Organelles

Cytoplasmic organelles are "little organs" that are suspended in the cytoplasm of the cell. Each type of organelle has a definite structure and a specific role in the function of the cell. Examples of cytoplasmic organelles are ribosomes, mitochondrion, Cytoskeleton, endoplasmic reticulum, Lysosomes and Peroxisomes golgi apparatus, and lysosomes.

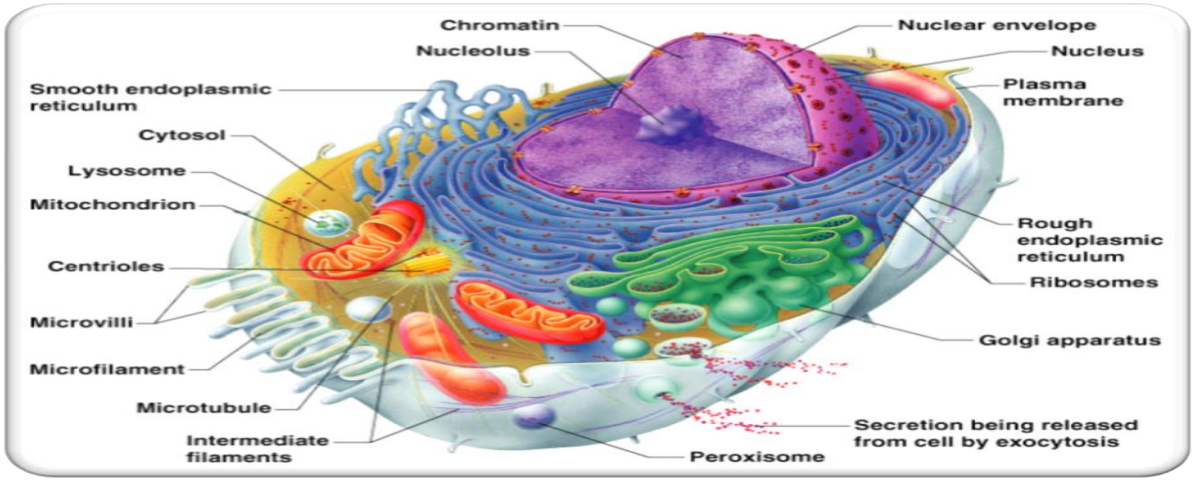


Figure 8: Cytoplasmic Organelles

Ribosome

A ribosome is a cellular particle made of RNA and protein that serves as the site for protein synthesis in the cell. The ribosome reads the sequence of the messenger RNA (mRNA) and, using the genetic code, translates the sequence of RNA bases into a sequence of amino acids.

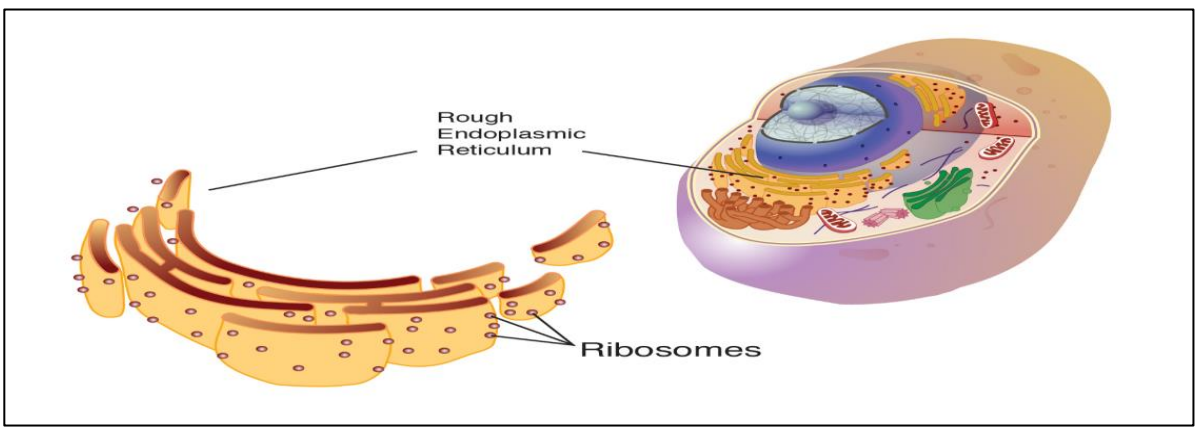


Figure 9: Ribosome

Ribosomes are a part of the protein-generating factory in the cell. The ribosome itself is a two-subunit structure that binds to messenger RNA. This structure acts as a docking station for the transfer RNA that

contains the amino acid that will then become part of the growing polypeptide chain, which eventually becomes the protein.

Mitochondria

Mitochondria are membrane-bound cell organelles (singular, mitochondrion) that generate most of the chemical energy needed to power the cell's biochemical reactions. Chemical energy produced by the mitochondria is stored in a small molecule called adenosine triphosphate (ATP). Mitochondria contain their own small chromosomes. Generally, mitochondria, and therefore mitochondrial DNA, are inherited only from the mother.

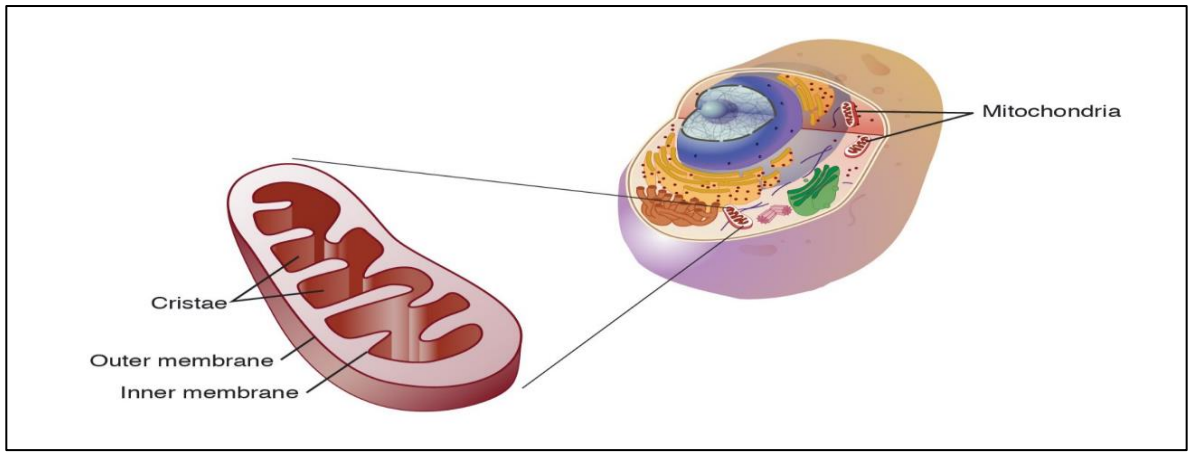


Figure 10: Mitochondria

Mitochondria organelles bound with two different membranes; this unusual for an intercellular organelle. These membranes function for the purpose of mitochondria, which is basically to generate energy by passing the chemicals within the cell through a conversion process. This conversion process produces energy in the form of ATP, since the phosphate is a high-energy bond and provides energy for other reactions within the cell. So, the function of the mitochondria is to produce energy. Some different cells have different amounts of mitochondria because they need more energy. For example, the muscle, the liver, the kidney, and the brain have a lot of mitochondria which lives from the energy those mitochondria produce. If there is a defect in the pathways involved in the mitochondrial functions, many different types of symptoms will arise in the muscle, brain, and sometimes in the kidneys as well.

Cytoskeleton

Cytoskeleton is a system of filaments or fibres present in the cytoplasm of eukaryotic cells (cells containing a nucleus). The cytoskeleton has several critical functions, including determining cell shape, participating in cell division, and allowing cells to move. It also provides a track-like system that directs

the movement of organelles and other substances within cells. The cytoskeleton organizes other constituents of the cell, maintains the cell's shape, and is responsible for the locomotion of the cell itself and the movement of the various organelles within it.

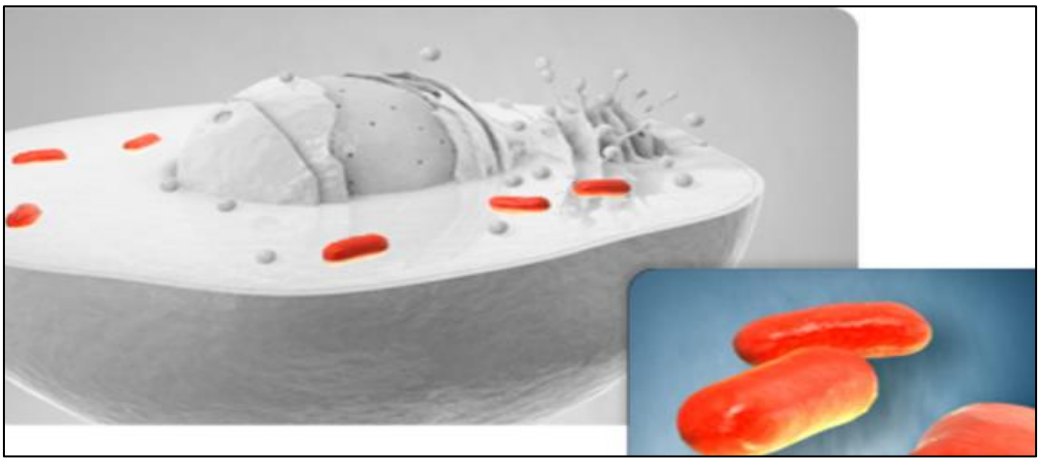


Figure 11: Cytoskeleton

Source: US National Library of Medicine

Endoplasmic Reticulum (ER)

Endoplasmic Reticulum (ER) is a continuous membrane system that forms a series of flattened sacs within the cytoplasm of eukaryotic cells and serves multiple functions, being important particularly in the synthesis, folding, modification, and transport of proteins. All eukaryotic cells contain an endoplasmic reticulum (ER); this organelle helps process molecules created by the cell. The endoplasmic reticulum also transports these molecules to their specific destinations either inside or outside the cell (Rogers, 2020).

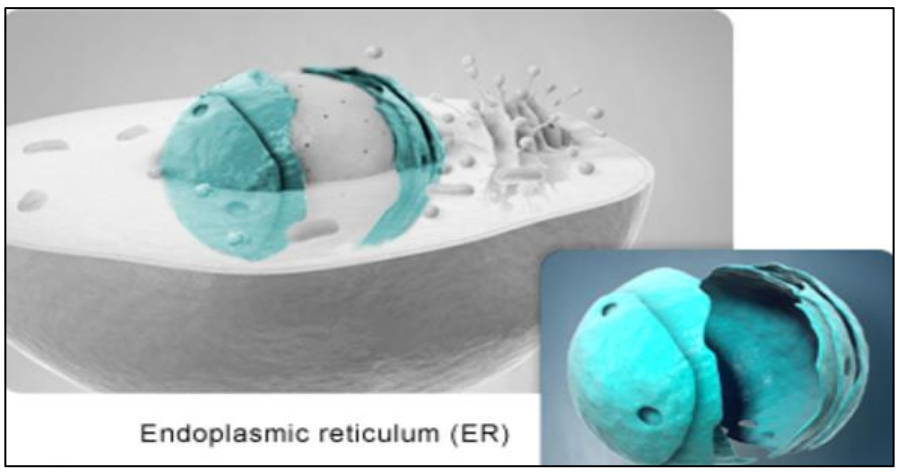


Figure 12: Endoplasmic reticulum

Source: US National Library of Medicine

Lysosomes and Peroxisomes

Lysosomes are small spherical organelles, enclosed by a single membrane. They measure about 0.5-1.0 µm across, and they contain digestive enzymes. Lysosomes fuse with membrane-bound endosomes (containing nutrients ingested by endocytosis), and the lysosomal enzymes digest large nutrient molecules. These organelles are the recycling center of the cell. They digest foreign bacteria that invade the cell, rid the cell of toxic substances, and recycle worn-out cell components. Cells can also degrade and recycle the components of their own organelles and structures when they are old or damaged, or if the cell is 'starving' in the absence of nutrients. This process, known as autophagy, usually involves formation of a membrane around the cell component and fusion of the resulting vesicle with lysosomes.

Peroxisomes are also small enzyme-containing organelles bound by a single membrane, which are very similar in size to lysosomes, measuring between 0.2 and 1.0 µm in diameter. They are thought to be present in all eukaryotic cells. Like lysosomes, peroxisomes also have a role in metabolism; they contain enzymes that break down fatty acids and amino acids, resulting in, among other things, the production of the toxic substance, hydrogen peroxide. Peroxisomes therefore also contain high levels of an enzyme known as catalase which breaks down the hydrogen peroxide into harmless products (water and oxygen).

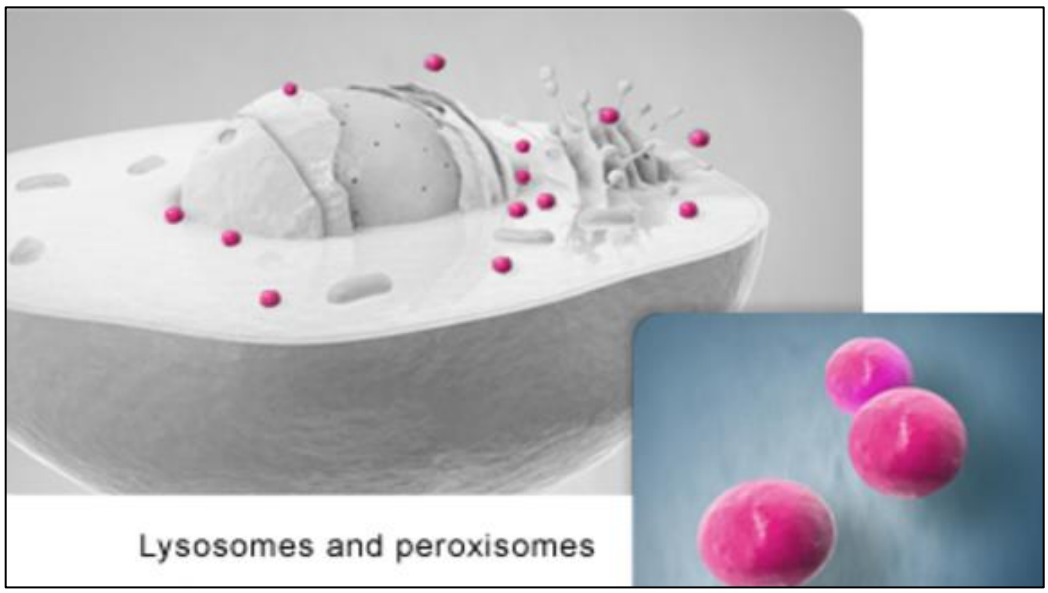


Figure 13: Lysosomes and Peroxisomes

Source: US National Library of Medicine

Golgi body

A Golgi body, also known as a Golgi apparatus, is a cell organelle that helps process and package proteins and lipid molecules, especially proteins destined to be exported from the cell. Named after its discoverer, Camillo Golgi, the Golgi body appears as a series of stacked membranes.

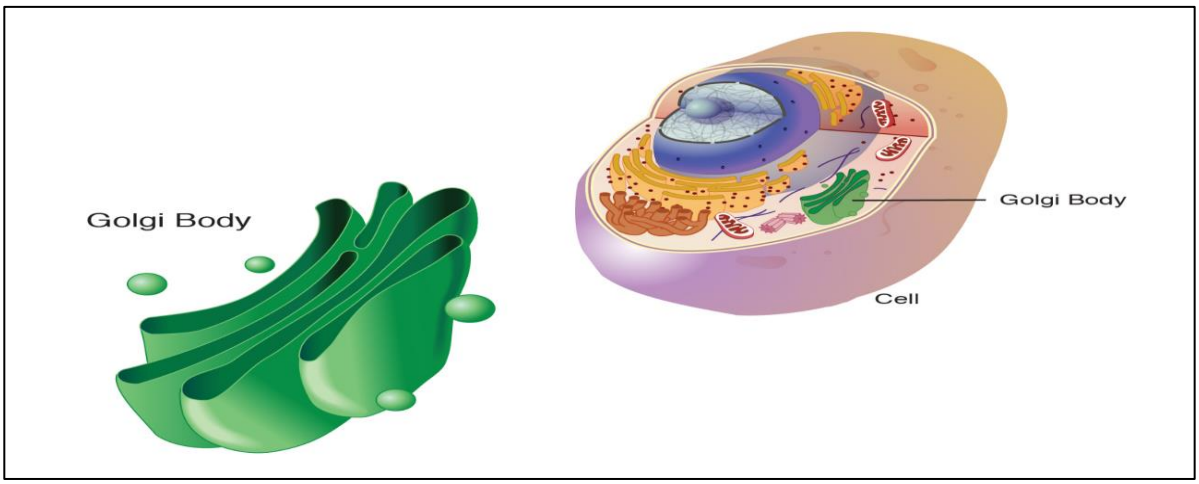


Figure 14: Golgi body

The Golgi body is a portion of the cell made up of different types of membranes. Some which are tubules, and some vesicles. The Golgi is located near the nucleus. It is called a perinuclear body, which is near the endoplasmic reticulum. Proteins move from the endoplasmic reticulum into the Golgi for further processing. For example, carbohydrates rest on some of the proteins, and then afterwards these glycoproteins – meaning they have carbohydrate as well as protein on them; these glycoproteins move out of the Golgi to the rest of the cell inside other vesicles. Those vesicles are made from the Golgi network. In fact, one of the functions of the Golgi is to form new vesicles out of the existing membrane of the Golgi and put into those vesicles the glycoproteins and other substances that are made in the Golgi network. Those vesicles, filled with the Golgi products, move to the rest of the cell, usually through the cell to the plasma membrane, which is their destination.

Cell Division

Cell division is the process by which a parent cell divides into two or more daughter cells. (Martin and Hine, 2020). Cell division usually occurs as part of a larger cell cycle. The cell cycle, or cell-division cycle, is the series of events that take place in a cell that cause it to divide into two daughter cells. In eukaryotes, there are two distinct types of cell division; a vegetative division, whereby each daughter cell is genetically identical to the parent cell (mitosis), and a reproductive cell division, whereby the number of chromosomes in the daughter cells is reduced by half to produce haploid gametes (meiosis) (Griffiths, 2012).

Cells are broadly classified into two main categories: simple non-nucleated prokaryotic cells and complex nucleated eukaryotic cells. Due to their structural differences, eukaryotic and prokaryotic cells do not divide in the same way. In cell biology, mitosis is a part of the cell cycle, in which, replicated chromosomes are separated into two new nuclei. Cell division gives rise to genetically identical cells in which the total number of chromosomes is maintained.

Eukaryotes have two major types of cell division: mitosis and meiosis. Mitosis is used to produce new body cells for growth and healing, while meiosis is used to produce sex cells (eggs and sperm). Mitosis will be discussed in the next subsection. The cell cycle is an ordered series of events involving cell growth and cell division that produces two new daughter cells via mitosis. The length of the cell cycle is highly variable even within the cells of an individual organism. In humans, the frequency of cell turnover ranges from a few hours in early embryonic development to an average of two to five days for epithelial cells, or to an entire human lifetime spent without dividing in specialized cells such as cortical neurons or cardiac muscle cells. There is also variation in the time that a cell spends in each phase of the cell cycle. When fast-dividing mammalian cells are grown in culture (outside the body under optimal growing conditions), the length of the cycle is approximately 24 hours. The timing of events in the cell cycle is controlled by mechanisms that are both internal and external to the cell.

Cells on the path to division proceed through a series of precisely timed and carefully regulated stages of growth, DNA replication, and division that produce two genetically identical cells. The cell cycle has two major phases: interphase and the mitotic phase (Figure 6). During interphase, the cell grows, and DNA replicated. During the mitotic phase, the replicated DNA and cytoplasmic contents are separated and the cell divides.

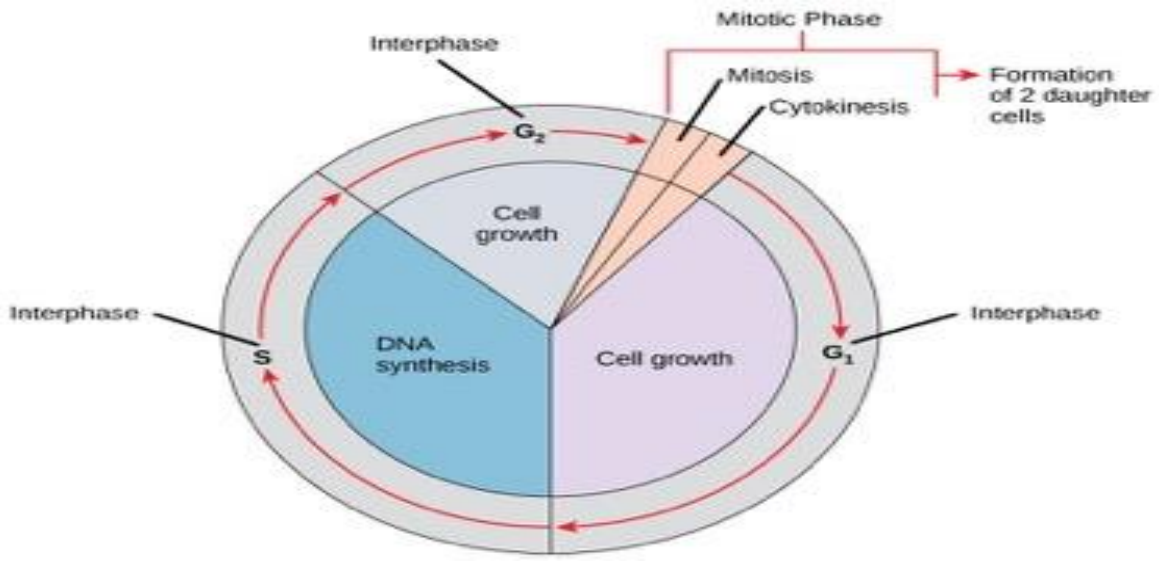


Figure 15: Phases of Cell Movement

Interphase

Before a cell starts dividing, it is in the "Interphase." It seems that cells must be constantly dividing, but each cell spends most of its time in the interphase. Interphase is the period when a cell is getting ready to divide and start the cell cycle. During this time, cells are gathering nutrients and energy. The parent cell also makes a copy of its DNA to share equally between the two daughter cells.

During interphase, the cell undergoes normal processes while also preparing for cell division. For a cell to move from interphase to the mitotic phase, many internal and external conditions must be met. Almost 80 percent of a cell's lifespan is spent in the interphase, which is the stage between mitotic cycles. During interphase, no division takes place, but the cell undergoes a period of growth and prepares itself for division. Cells contain many proteins and structures called organelles that must replicate in preparation for doubling. The DNA of the cell duplicates during this phase, creating two copies of each strand of DNA called a chromosome. A chromosome is a DNA molecule that carries all or part of the hereditary information of an organism.

Interphase itself is split into different phases: G1 phase, S phase and G2 phase. G1 phase is the period prior to the synthesis of DNA, during which the cell increases in size. During the G1 phases, cells grow and monitor their environment to determine whether they should initiate another round of cell division. During the narrow S phase, DNA is synthesized. This is followed by the G2 phase, when the cell synthesizes proteins and continues to get bigger. During the G2 phase, cells check to make sure DNA replication has successfully completed and make any necessary repairs. Not all scientists class interphase as a stage of mitosis because it is not an active stage. However, this preparatory stage is essential before any actual cell division takes place.

Mitosis Phase

Depending on the type of cell, there are two ways cells divide—mitosis and meiosis. Each of these methods of cell division has special characteristics. One of the key differences in mitosis is a single cell divides into two cells that are replicas of each other and have the same number of chromosomes. This type of cell division is good for basic growth, repair, and maintenance. In meiosis a cell divides into four cells that have half the number of chromosomes. Reducing the number of chromosomes by half is important for sexual reproduction and provides for genetic diversity.

Mitosis Cell Division

Mitosis is how somatic—or non-reproductive cells—divide. Somatic cells make up most of your body's tissues and organs, including skin, muscles, lungs, gut, and hair cells. Reproductive cells (like eggs) are not somatic cells. In mitosis, it is important to remember that the daughter cells each have the same chromosomes and DNA as the parent cell. The daughter cells from mitosis are called diploid cells. Diploid cells have two complete sets of chromosomes. Since the daughter cells have exact copies of their parent cell's DNA, no genetic diversity is created through mitosis in normal healthy cells.

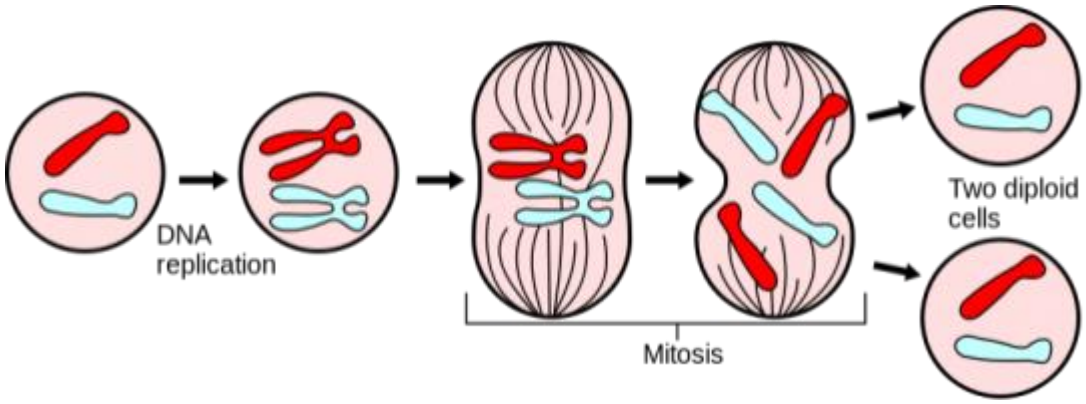


Figure 16: Mitosis Cell Division

It is important to note that when a cell divides during mitosis, some organelles are divided between the two daughter cells. For example, mitochondria can grow and divide during the interphase, so the daughter cells each have enough mitochondria. The Golgi apparatus, however, breaks down before mitosis and reassembles in each of the new daughter cells.

The Mitosis Cell Cycle

To make two daughter cells, the contents of the nucleus and the cytoplasm must be divided. The mitotic phase is a multistep process during which the duplicated chromosomes are aligned, separated, and moved to opposite poles of the cell, and then the cell is divided into two new identical daughter cells. The mitotic phase (mitosis) is composed of five stages, namely, Prophase, Metaphase, Anaphase, Telophase, and Cytokinesis, which accomplish nuclear division as shown in Figure 8. Each of these phases are discussed below:

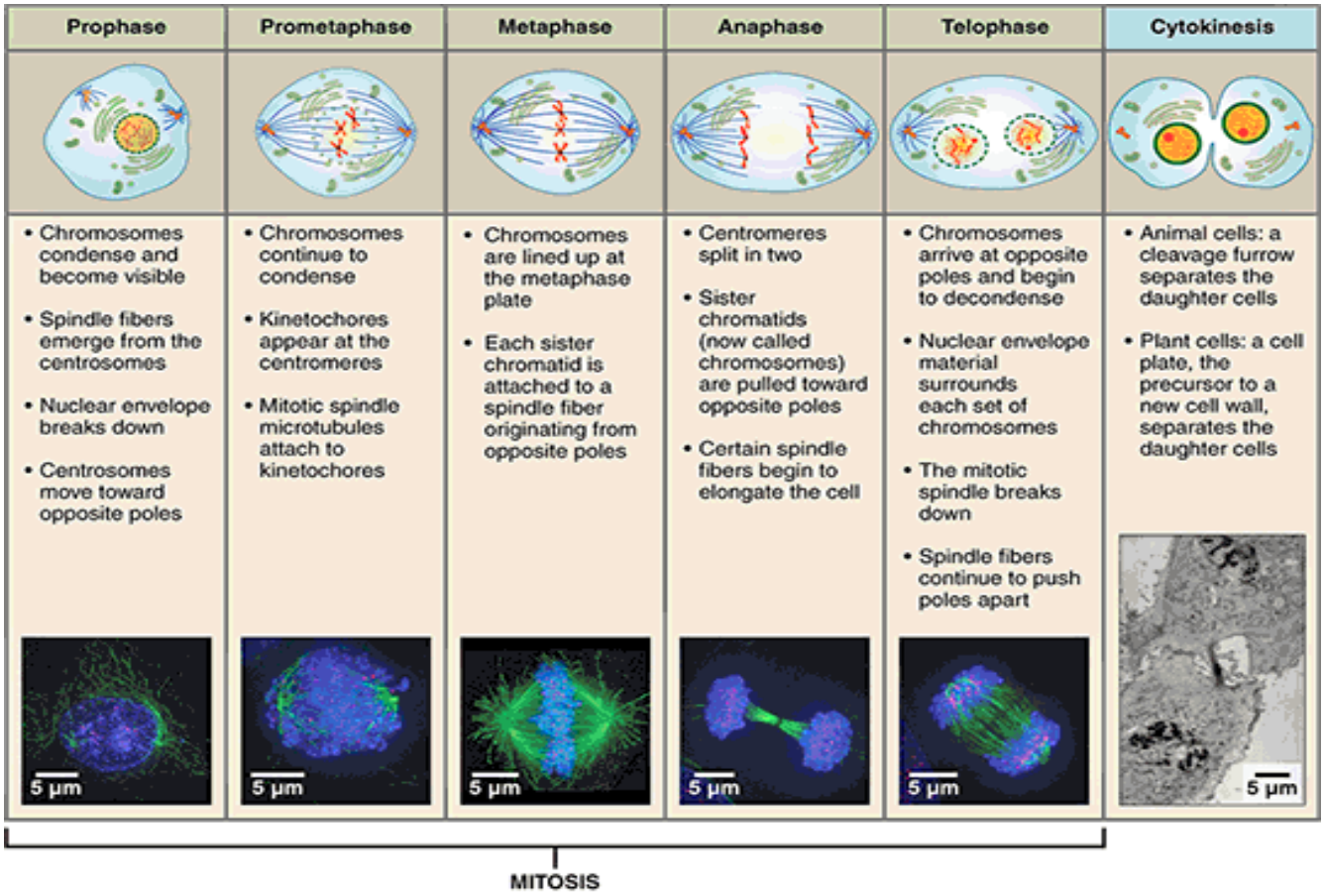


Figure 17: Phases in the Mitosis Cell Cycle

Prophase

Mitosis starts with prophase, which occurs after an initial preparatory stage, which occurs during interphase – a "rest" phase between cell divisions. During early prophase, the cell begins breaking down some structures and creating others, preparing for the division of chromosomes. The duplicated chromosomes from interphase condense, meaning they become compacted and tightly wound. The nuclear envelope breaks down, and an apparatus known as a mitotic spindle form on the edges of the dividing cell. The spindle is made up of strong proteins called microtubules, which are part of the cell's "skeleton" and drive the division of the cell through elongation. The spindle gradually lengthens during prophase. Its role is to organize the chromosomes and move them around during mitosis.

Toward the end of the prophase stage, the nuclear envelope breaks down, and the microtubules reach from each cell pole to the cell's equator. Kinetochores, specialized regions in the centromeres of chromosomes – regions of DNA where the sister chromatids are most tightly connected – attach to a type of microtubule called kinetochore fibers. These fibers interact with the spindle polar fibers connecting the kinetochores to the polar fibers, which encourages the chromosomes to migrate toward the center of the cell. This part of the process is sometimes called prometaphase because it occurs immediately before metaphase.

Metaphase

At the very start of the metaphase stage, the pairs of condensed chromosomes line up along the equator of the elongated cell. Since they are condensed, they can move more easily without becoming tangled. Some biologists separate metaphases into two phases: prometaphase, and true metaphase. During prometaphase, the nuclear membrane disappears completely. Then, true metaphase begins. In animal cells, the two pairs of centrioles align at opposite poles of the cell, and polar fibers continue to extend from the poles to the center of the cell. Chromosomes move in a random way until they attach, from both sides of their centromeres to polar fibers. Chromosomes align at the metaphase plate at right angles to the spindle poles and are held there by the equal forces of the polar fibers exerting pressure on the chromosomes' centromeres.

The metaphase plate is not a physical structure – this is simply a term for the plane where the chromosomes line up. Before moving on to the anaphase stage, the cell checks that all the chromosomes are at the metaphase plate with their kinetochores correctly attached to microtubules. This is known as the spindle checkpoint. This checkpoint ensures that the pairs of chromosomes, also called sister chromatids, split evenly between the two daughter cells in the anaphase stage. If a chromosome is not correctly aligned or attached, the cell will stop division until the problem is fixed. In rare cases, the cell does not stop division, and mistakes are made during mitosis. This can result in DNA changes, which can potentially lead to genetic disorders.

Anaphase

During anaphase, the sister chromatids are drawn to opposite poles (ends) of the elongated cell. The protein "glue" that holds them together breaks down to let them move apart. This means duplicate copies of the cell's DNA end up on either side of the cell and are ready to divide completely. Each sister chromatid is now its own "full" chromosome. They are now called daughter chromosomes. At this stage the microtubules get shorter, which lets the process of cell separation begin. The daughter chromosomes travel through the spindle mechanism to reach the cell's opposite poles. As the chromosomes approach a pole, they migrate centromere first and the kinetochore fibers shorten. To prepare for telophase, the two cell poles move further apart. Upon completion of anaphase, each pole contains a complete collection of chromosomes. At this point, cytokinesis begins. This is the division of the original cell's cytoplasm, and it continues through the telophase stage.

Telophase

In the telophase stage, cell division is almost complete. The nuclear envelope, which had previously broken down to allow the microtubules to access and recruit the chromosomes to the equator of the

dividing cell, reforms as two new nuclear envelopes around the separated sister chromatids. The polar fibers continue to lengthen, and nuclei start to form at opposite poles, creating nuclear envelopes from leftover parts of the parent cell's nuclear envelope, plus parts of the endomembrane system. The mitotic spindle is broken down into its building blocks, and two new nuclei form – one for each set of chromosomes. During this process, nuclear membrances and nucleoli reappear and chromatin fibers of chromosomes open out, returning to their previous string-like form. After telophase, mitosis is almost complete – the genetic contents of one cell have been divided equally into two cells. However, cell division is not complete until cytokinesis takes place.

Cytokinesis

Cytokinesis is the division of the cell's cytoplasm, starting before anaphase ends and completing shortly after the telophase stage of mitosis. During cytokinesis in animal cells, a ring of proteins called actin and myosin (the same proteins found in muscle) pinch the elongated cell into two brand new cells. A band of filaments made of a protein called actin is responsible for the pinching, creating a crease called the cleavage furrow. The process is different in plant cells because they have a cell wall and are too rigid to be divided in this way. In plant cells, a structure called the cell plate forms down the middle of the cell, splitting it into two daughter cells separated by a new wall. At this point, the cytoplasm, the fluid in which all cell components are bathed, is equally divided between the two new daughter cells. Each daughter cell is genetically identical, containing its own nucleus and a complete copy of the organism's DNA. The daughter cells now begin their own cellular process and may repeat the mitosis process themselves depending on what they become.

Meiosis Cell Division

Meiosis is the other main way cells divide. Meiosis is cell division that creates sex cells, like female egg cells or male sperm cells. What is important to remember about meiosis? In meiosis, each new cell contains a unique set of genetic information. After meiosis, the sperm and egg cells can join to create a new organism.

Meiosis is why we have genetic diversity in all sexually reproducing organisms. During meiosis, a small portion of each chromosome breaks off and reattaches to another chromosome. This process is called "crossing over" or "genetic recombination." Genetic recombination is the reason full siblings made from egg and sperm cells from the same two parents can look very different from one another.

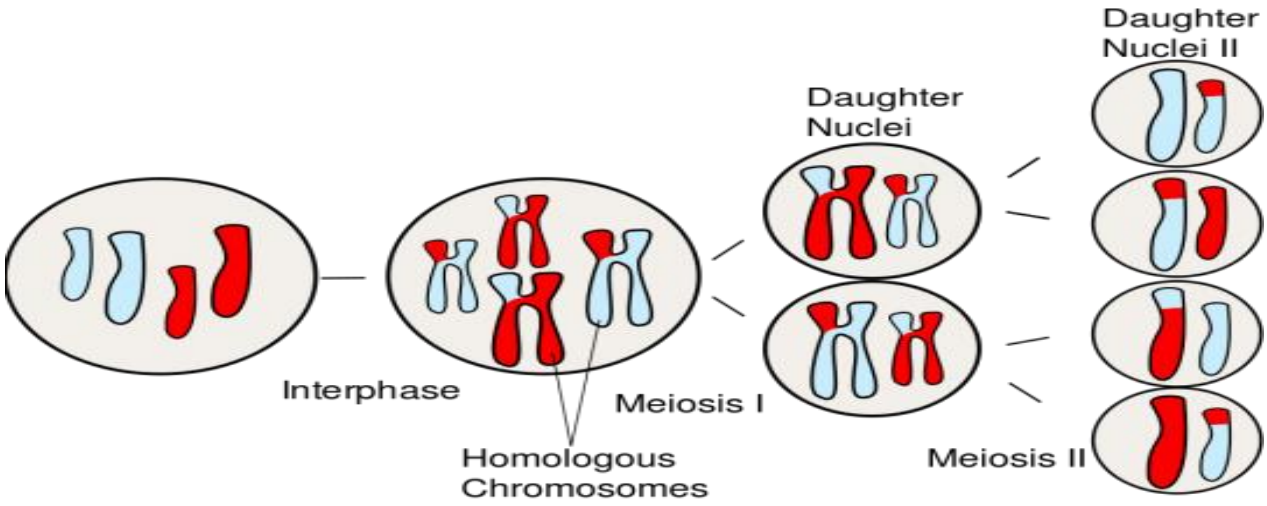


Figure17: Meiosis Cell Division

Cell Division and Aging

As mammalian cells age, they stop dividing. Cellular aging was first described by Hayflick and Moorhead in 1961. They showed that human cells in culture do not divide indefinitely but reach a limit (called the Hayflick limit) of replication and stop all further division (Mays, 2010).

Cristian et al. (2019) hypothesized that normal stem cell division rates might decrease as we age. To test this hypothesis, they evaluated cell division rates in the epithelium of human colonic, duodenal, esophageal, and posterior ethmoid sinonasal tissues. In all 4 tissues, there was a significant decrease in cell division rates with age.

Regenerative Medicine and Stem Cells

The regeneration of damaged tissues or organs implies the existence of cells able to proliferate, differentiate, and contribute functionally to the regenerative processes (Stoltz et al., 2015); this is done using regenerative medicines. Regenerative Medicine is a comprehensive term used to describe the current methods and research employed to revive and/or replace dead or damaged tissue (Carlson et al., 2009; Ullah et al., 2015). Typically, when the term 'Regenerative Medicine' arises, People automatically think about stem cells, particularly, embryonic stem cells. Being that embryonic stem cell research is currently a highly debated topic in both the scientific and political field, the assumption that Regenerative Medicine research only involves embryonic stem cell research can be narrowing to the field and does not allow one to understand its full potential.

A portion of Regenerative Medicine research revolves around the use of stem cells, including embryonic, adult, and induced pluripotent stem cells (iPS) (Ullah et al., 2015). However, many other resources are utilized to carry out the mission of Regenerative Medicine research. These include transplants,

biomaterials, scaffolds, machines and electronics, stimulation pathways, drug therapy, and many others. In regenerative medicine, four important issues must be considered including, the choice of the reparative cells that can form a functional tissue, if necessary, the choice of appropriate scaffolds for transplantation, the role of bio-reactive molecules, such as cytokines and growth factors that support the formation of the desired tissue, and grafting and safety studies (GMP compliance) (Ullah et al., 2015).

Stem cells have a very important role in Regenerative Medicine Research and have many potential applications. First, because of their role in development and their potential to develop into many different cell types, stem cells are vital to the field of developmental biology. Developmental biologists seek to uncover the genes and pathways involved in cell differentiation (how cells develop into specific cell types such as liver, skin, or muscle cells) and how these can be manipulated to create new healthy tissues. Second, stem cells can be applied to drug testing and development. New drugs that are developed in Pharma could be safely and effectively tested using differentiated stem cells (Jurgen, 2019). As scientists learn more about how stem cells develop to form new tissue, they will be able to apply their knowledge in maintaining differentiated cell types that can be used to test drugs.

Stem cells are the foundation cells for every organ and tissue in our bodies. The highly specialized cells that make up these tissues originally came from an initial pool of stem cells formed shortly after fertilization (Yanqiu, 2018). Throughout our lives, we continue to rely on stem cells to replace injured tissues and cells that are lost every day, such as those in our skin, hair, blood, and the lining of our gut. Stem cells are important for living organisms for many reasons. In the 3- to 5-day-old embryo, called a blastocyst, the inner cells give rise to the entire body of the organism, including all the many specialized cell types and organs such as the heart, lung, skin, sperm, eggs, and other tissues. In some adult tissues, such as bone marrow, muscle, and brain, discrete populations of adult stem cells generate replacements for cells that are lost through normal wear and tear, injury, or disease.

Given their unique regenerative abilities, stem cells offer new potentials for treating diseases such as diabetes, and heart disease. However, much work remains to be done in the laboratory and the clinic to understand how to use these cells for cell-based therapies to treat disease, which is also referred to as regenerative or reparative medicine. Laboratory studies of stem cells enable scientists to learn about the cells' essential properties and what makes them different from specialized cell types. Scientists are already using stem cells in the laboratory to screen new drugs and to develop model systems to study normal growth and identify the causes of birth defects.

The Skin

The skin is the largest organ in the body that covers the entire external surface. It protects the internal organs from germs and thus helps prevent infections. (Yousef et al., 2021).

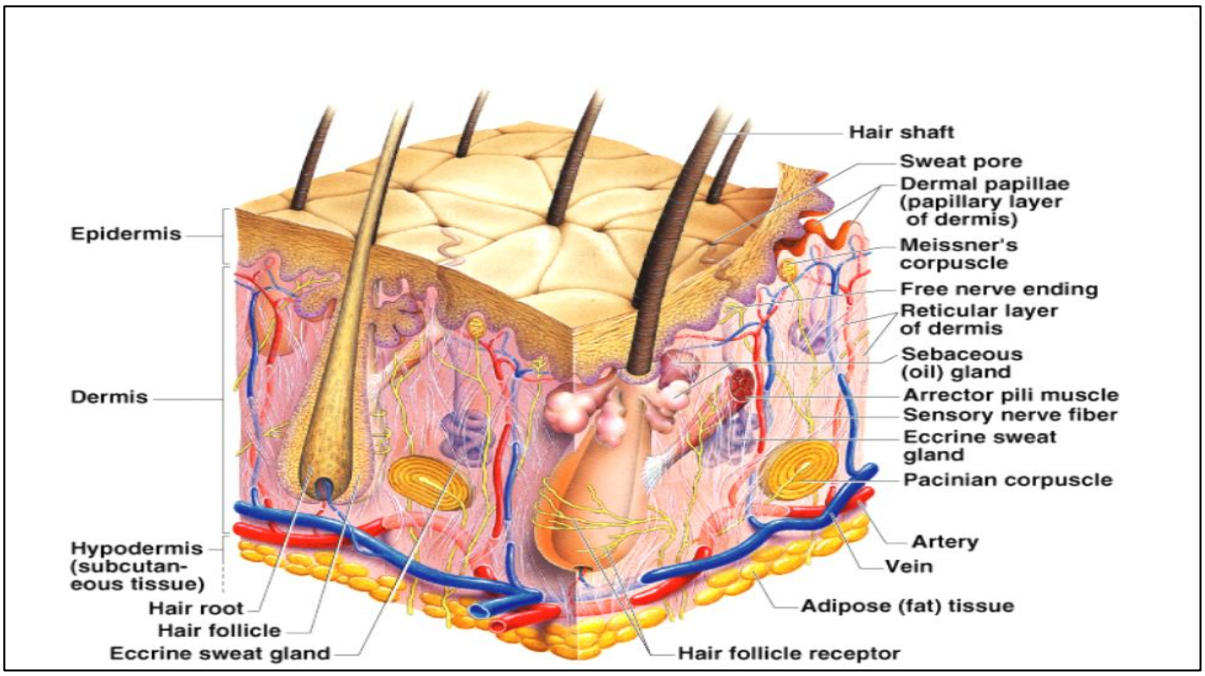


Figure 18: A cross-section of the Skin

Source: Pearson Education Inc.

The epidermis is composed mainly of keratinocytes. Beneath the epidermis is the basement membrane (also known as the dermo-epidermal junction); this narrow, multi-layered structure anchors the epidermis to the dermis. The layer below the dermis, the hypodermis, consists largely of fat. These structures are shown in Figure 18 and 19.

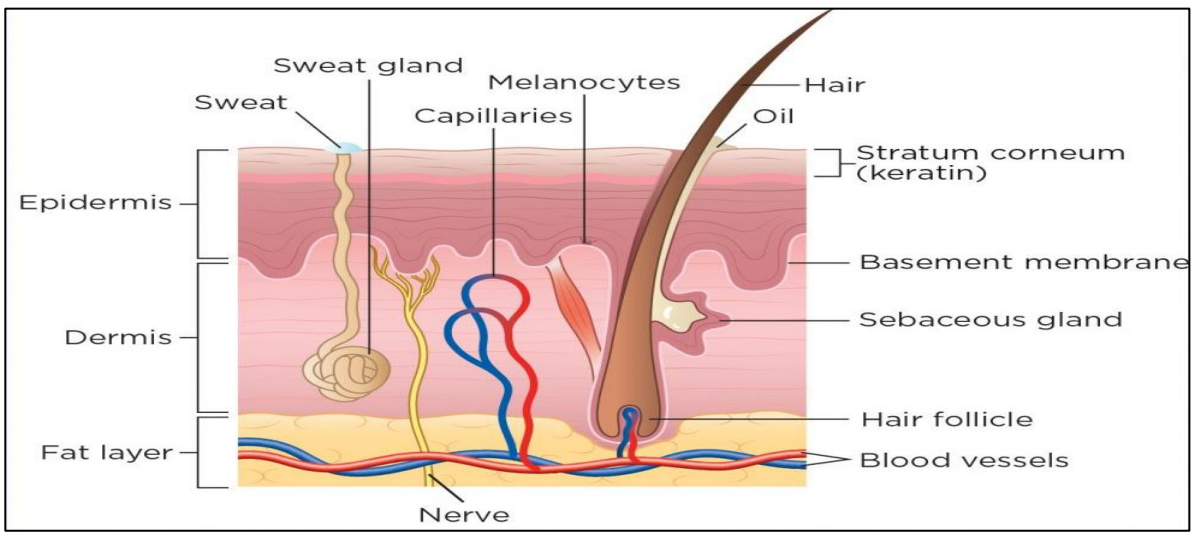


Figure 19: Cross-section through the skin

Epidermis

The epidermis is the outer layer of the skin, defined as a stratified squamous epithelium, primarily comprising keratinocytes in progressive stages of differentiation (Amirlak and Shahabi, 2017). Keratinocytes produce the protein keratin and are the major building blocks (cells) of the epidermis. As the epidermis is avascular (contains no blood vessels), it is entirely dependent on the underlying dermis for nutrient delivery and waste disposal through the basement membrane. The prime function of the epidermis is to act as a physical and biological barrier to the external environment, preventing penetration by irritants and allergens. At the same time, it prevents the loss of water and maintains internal homeostasis (Gawkrodger, 2007; Cork, 1997).

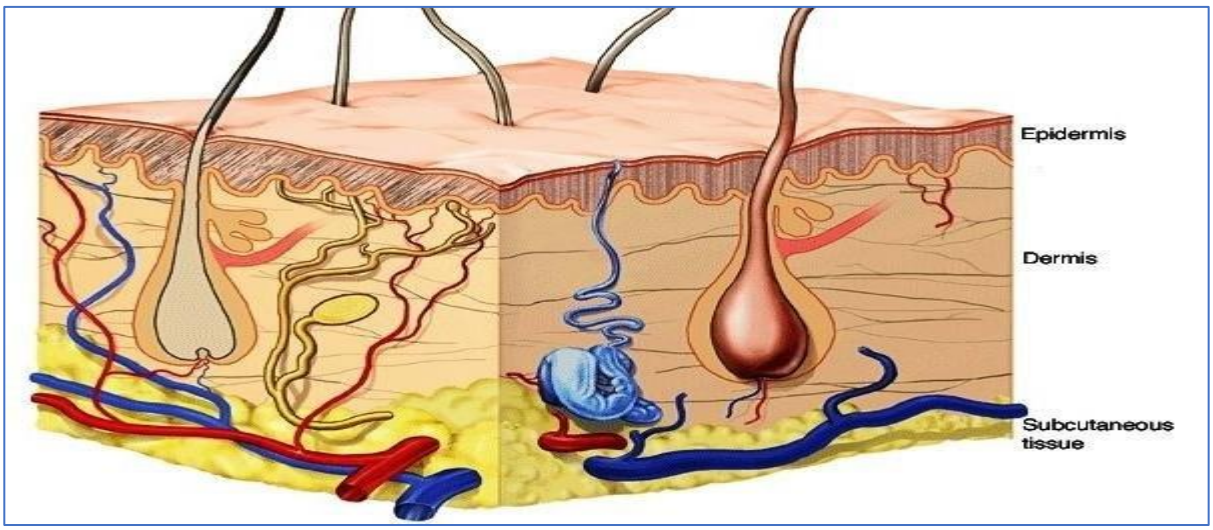


Figure 20: The Epidermis

The epidermis is a eukaryotic cell; it is responsible for skin rejuvenation. The epidermis is the outermost layer of the skin which protects the body from the environment. The thickness of the epidermis varies in different types of skin; it is only .05 mm thick on the eyelids and is 1.5 mm thick on the palms and the soles of the feet. The epidermis contains the melanocytes (the cells in which melanoma develops), the Langerhans' cells (involved in the immune system in the skin), Merkel cells and sensory nerves. The epidermis layer itself consists of five sublayers that work together to continually rebuild the surface of the skin

The epidermis is made from epithelial tissue and does not have a blood supply of its own; it is made up of five layers:

Stratum corneum – the outer layer of the skin, this is made up of scale like cells that are continuously shed (corn flakes).

Stratum lucidum - this is made up of small transparent cells through which light can pass. This layer is only present in the palms of the hands and soles of the feet.

Stratum granulosum – this layer is usually 1-3 layers thick. The cells have distinct granules and keratin is produced in this layer.

Stratum spinosum – this layer is 3-6 layers thick, and the cells are constantly dividing.

Stratum basale – a single basal layer of cells, which contain the melanocytes that produce the pigment melanin. The cells of the epidermis are produced in this layer, and each has a distinct nucleus. These cells divide continuously by a process known as mitosis.

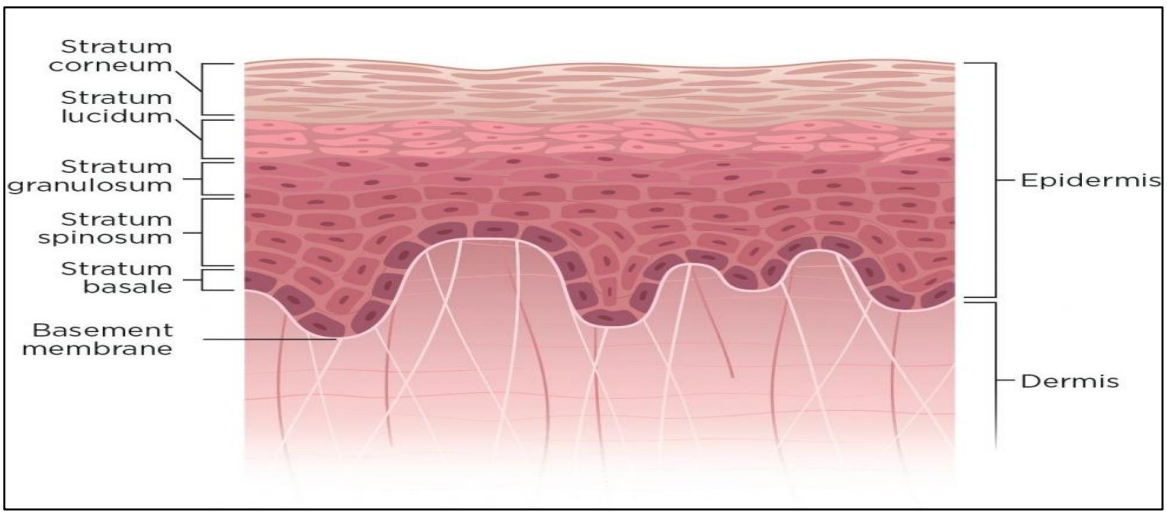


Figure 21: Layers of the Epidermis

One Square inch of skin contains:

- 9,500,000 Cells
- 65 Hairs
- 19-20 Yards of Blood Vessels
- 13 Sensory apparatuses for cold
- 19,500 Sensory cells at the ends of nerve fibres
- 1,300 nerve endings to record pain
- 650 Sweat glands
- 95-100 Sebaceous glands
- 78 sensory apparatuses for heat
- 78 yards of nerves
- 160-165 pressure apparatuses for the perception of tactile stimuli.

The development of the epidermis serves as a model to study how oriented cell divisions function to both shape a tissue and generate cellular diversity. The skin is one of the largest organs of the body and provides a protective outer covering in mammals. Primarily, the skin functions to control evaporation of water by creating a semi-impermeable barrier to water loss. Additionally, this tissue acts to protect us from external pathogens, regulate heat, and provide the sensation of touch. The epidermis, the outermost layer of our bodies, is essential for providing the waterproofing and barrier aspects of the skin. This protective function is established during development through the process of stratification in which the epidermis becomes a multi-layered squamous epithelium.

Dermis

This layer is often referred to as the true skin as it forms the bulk of the skin. The dermis has a good blood and lymph supply provided by lymph capillaries, arterioles, and venules. The dermis is made up of connective tissue and is divided into two layers:

- **Papillary Layer** – lies directly under the epidermis, it is quite thin and has cone like projections called papillae. It provides nutrients and oxygen to the germinating layer of the epidermis.
- **Reticular Layer** – this lies below the papillary layer and is the main section of the dermis. Within the reticular layer are collagen and elastin fibres. Collagen gives the skin a plump and youthful appearance and is a white fibrous tissue made up of proteins. Elastin gives the skin its elastic properties and is made up of yellow elastic tissue. These fibres are produced by the fibroblasts and are all held together in a ground substance. Whilst this network is strong, the skin will remain youthful and firm; however, as the fibres start to harden and split the network collapses and the ageing process starts to become visible.

Glycosaminoglycan

The glycosaminoglycan's (GAG's) make up a proportion of the extracellular fluid of the dermis are made by the fibroblast and of Hyaluronic acid, Heparan sulfate, Heparin and Dermatan sulfate.

The GAG's retain water and form a gel substance through which ions, hormones and nutrients can freely move.

A main component of this gel is hyaluronic acid, which is a large polysaccharide made of glucuronic acid and glucosamine that attract water and is increased in tissues under repair or growth.

Collagen

Collagen, is an abundant protein, it is the main component of connective tissue and is found not only in fibrous tissue like the skin, but also tendons, ligaments, cartilage, bones, corneas and blood vessels.

There are 18 collagen subtypes, 11 of which are in the dermis of the skin.

It will take approximately three months for type III collagen to mature into type I collagen.
As skin ages, reactive oxygen species, associated with many aspects of aging, lead to increased production of the enzyme collagenase, which breaks down collagen. Then fibroblasts, the critical players in firm, healthy skin, lose their normal stretched state. They collapse, and more breakdown enzymes are produced. People in their 80s have four times more broken collagen than people in their 20s.

Elastin

The same as collagen, elastin is present in many structures in the body, not just in the skin. Elastin makes up only around 3% of the skin, whereas collagen makes up 70% of the dry mass of skin. Degradation of elastic fibers is associated with UV exposure, and elastosis is one of the key features of photo aged skin. The fact that new elastin fibers are not produced is a challenge in the aesthetic industry.

Subcutaneous Layer

This is located under the dermis and is mainly made up of fat cells (adipose tissue). This fatty layer provides the plump contours of the body, protection, insulation, support, and a food supply if needed. A certain amount of fat in the face is beneficial as it plumps out the facial contours making the face look more youthful. If a client loses a lot of weight quite rapidly, you will notice that they look as though they have aged.

To support its specialized functions, the skin has basic requirements that must be satisfied for every tissue. It needs mechanical strength, provided by a supporting framework of extracellular matrix, mainly secreted by fibroblasts. It needs a blood supply to bring nutrients and oxygen and remove waste products and carbon dioxide, and this requires a network of blood vessels, lined with endothelial cells. These vessels also provide access routes for cells of the immune system to provide defences against infection: macrophages and dendritic cells phagocytose invading pathogens and help activate lymphocytes, which mediate more sophisticated adaptive immune system responses.

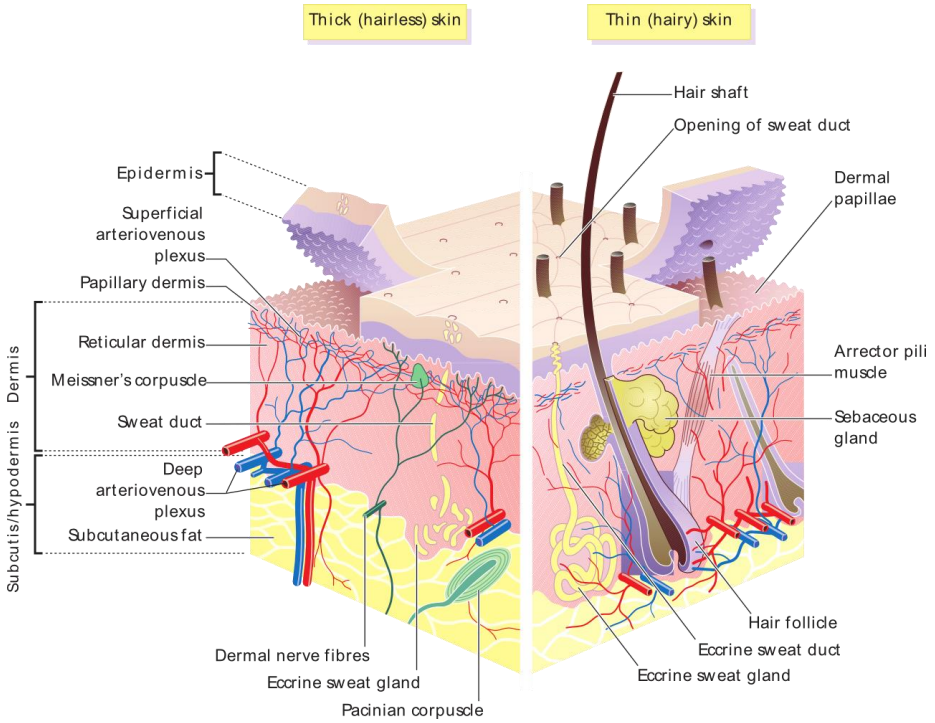


Figure 22: Thick and Thin skin

Chapter two

The Aging Process

Aging is the progressive accumulation of changes with time associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age (Harman, 1978). This process may be common to all living things since the phenomenon of aging and death is universal. If so, both aging and the rate of the aging process are under genetic control to some extent for the manifestations of aging, and life span varies by species. Further, like all chemicals and chemical reactions, the manifestations of aging which reflect chemical composition and the rate of the aging process are subject to environmental influences (Harman, 1981). Aging and death of single cells then can be viewed as being due to the aging process, the changes with time and their rates of production being under genetic control but subject to modification by the environment, with death ensuing when one or more activities vital to the cell are depressed below some critical level (Harman, 1978; Harman, 1981).

Several theories may explain the normal aging process, either alone or in combination with other theories (Table 1). These theories can be generally classified into evolutionary, involving historical and evolutionary aspects of aging, and physiologic or structural and functional changes. Processes that may explain these theories at a cellular level include intrinsic timing mechanisms and signals, accidental chance

events, programmed genetic signals making an organism more susceptible to accidental events, nuclear or mitochondrial DNA mutations or damage, damaged and abnormal proteins, cross-linkage, glycation, waste accumulation, general molecular wear and tear, free radical formation, and specific cellular components such as gene, chromosome, mitochondria, or telomeres. Physiologic processes that may explain aging include oxidative stress, immunologic, neuroendocrinologic, metabolic, insulin signaling, and caloric restriction (Pacala, 2010).

Figure 23: Major Cellular and functional changes of aging by prominent theories and major associated clinical disease outcomes

Table 1
Major cellular and functional changes of aging by prominent theories and major associated clinical disease outcomes

Organ System	Major Theories	Cell Level	Structural/Functional Changes of Aging	Disease Outcomes
Integumentary	Oxidative stress; free radical; genetic; autoimmune	Melanocytes, mast, and Langerhans cells	Thinning of stratum corneum and subcutaneous layer	Squamous and basal cell carcinoma; malignant melanoma
Oral	Oxidative Stress; free radical; genetic; autoimmune	Buccal	Increased thickness of tooth dentin, decreased dental pulp; thinning of oral mucosa and receding of gums; decreased sensitivity for smell and taste	Squamous cell carcinoma; tooth decay
Visual	Oxidative stress; free radical; genetic	Rods and cones	Reduced night vision, accommodative ability and increased glare	Macular degeneration; cataracts; diabetic retinopathy
Hearing	Oxidative stress; free radical; genetic	Sensory and neural cells	Stiffening of the inner ear bones	Presbycusis; osteosclerosis
Musculoskeletal	Oxidative stress; genetic; autoimmune	Myocytes	Apoptosis, reduced size of myofibrils, decreased type 2 muscle fibers; decreased hand grip strength with more in the lower extremities	Falls; disuse atrophy; chronic musculoskeletal disorders
Skeletal	Oxidative stress; free radical; neuro endocrine	Osteoblasts and osteoclasts	Change in bone architecture and accumulation of microfractures, disparity in the concentration of deposited minerals, changes in the crystalline properties of mineral deposits and protein content of the matrix; decreased height and thinning of bone	Fractures
Cardiovascular	Oxidative stress; free radical; neuroendocrine; genetic	Myocyte; pacemaker cell	Increase in left ventricular stiffness and decrease in compliance; decreased left ventricular diastolic filling and relaxation, increased stroke volume, reduction in maximal cardiac output and vasodilator response to exercise	Congestive heart failure; cardiomyopathy; heart block
Pulmonary	Oxidative stress; free radical; genetic; autoimmune	Alveolar cells	Chest wall stiffness; decreased arterial oxygenation and impaired carbon dioxide elimination; decrease in vital capacity and forced expiratory volume, increased residual volume and functional residual capacity	Chronic lung disease; carcinoma
Gastrointestinal	Oxidative stress; free radical	Mucosal cell	Decreased elasticity of connective tissue; reduction in phase I metabolism	Carcinoma; increased risk of drug–drug and drug–disease interactions
Renal/urogenital	Oxidative stress; free radical; genetic; neuroendocrine; autoimmune	Renal cell	Diminished proliferative reserve; apoptosis; loss of glomerular and tubular mass; decline in GFR, loss of tubular volume and narrowed homeostatic control of water and electrolyte balance	Carcinoma; chronic renal failure

(*continued on next page*)

Neurologic	Oxidative stress; free radical; genetic; neuroendocrine	Neurons; glial cells	Decrease in size of hippocampus and frontal and temporal lobes; decreased number of receptors of all types in the brain with increased sensitivity; decrease in complex visuoconstructive skills and logical analysis skills; decrease in processing speed, decrease in reaction time and decrease ability to shift cognitive sets rapidly; memory distraction and decline in executive function; abnormal reflexes	Neuropathy; neurodegenerative disorders
Hematologic	Autoimmune; genetic; oxidative stress; free radical	Stem cells	Decreased marrow cellularity, increase in bone marrow fat and reduction in cancellous bone	Chronic anemia; myelofibrosis; leukemia
Neuroendocrine	Neuroendocrine; oxidative stress; genetic	Neuroendocrine cells; mitochondria	Decrease or increase in hormone levels; inability to conserve or dissipate heat	Autonomic neuropathy; thyroid disease; adrenal insufficiency; male and female menopause

Changes in Skin during Aging

Skin changes are among the most visible signs of aging. Evidence of increasing age includes wrinkles and sagging skin. Whitening or graying of the hair is another obvious sign of aging. The skin contains nerve receptors that allow one to feel touch, pain, and pressure, helps control fluid and electrolyte balance, helps control the body temperature, and protects from the environment

Although the skin has many layers, it can generally be divided into three main parts: The outer part (epidermis) containing the skin cells, pigment, and proteins, the middle part (dermis) containing the skin cells, blood vessels, nerves, hair follicles, and oil glands, the dermis provides nutrients to the epidermis, and the inner layer under the dermis (the subcutaneous layer) contains sweat glands, some hair follicles, blood vessels, and fat. Each layer also contains connective tissue with collagen fibers to give support and elastin fibers to provide flexibility and strength.

With aging, the outer skin layer (epidermis) thins, even though the number of cell layers remains unchanged. The number of pigment-containing cells (melanocytes) decreases. The remaining melanocytes increase in size. Aging skin looks thinner, paler, and clear (translucent). Pigmented spots including age spots or "liver spots" may appear in sun-exposed areas. The medical term for these areas is lentigos. Changes in the connective tissue reduce the skin's strength and elasticity. This is known as elastosis. It is more noticeable in sun-exposed areas (solar elastosis). Elastosis produces the leathery, weather-beaten appearance common to farmers, sailors, and others who spend a large amount of time outdoors.

The blood vessels of the dermis become more fragile. This leads to bruising, bleeding under the skin (often called senile purpura), cherry angiomas, and similar conditions. Sebaceous glands produce less oil as one ages. Men experience a minimal decrease, most often after the age of 80. Women gradually produce less oil beginning after menopause. This can make it harder to keep the skin moist, resulting in dryness and

itchiness. The subcutaneous fat layer thins so it has less insulation and padding. This increases the risk of skin injury and reduces the ability to maintain body temperature.

Since humans have less natural insulation, hypothermia can occur in cold weather. Some medicines are absorbed by the fat layer; shrinkage of this layer may change the way that these medicines work, the sweat glands produce less sweat and this makes it harder to keep cool. The risk for overheating or developing heat stroke also increases.

Growths such as skin tags, warts, rough brown patches (seborrheic keratoses), and other blemishes are more common in older people. Also common are pinkish rough patches (actinic keratosis) which have a small chance of becoming a skin cancer.

Chronological ageing and wrinkles

As we age the epidermal cells become somewhat thinner and less sticky. This in turn makes the skin look thinner and more translucent. The decreased stickiness of the cells reduces the effectiveness of the barrier function allowing trans epidermal water loss to occur. This causes dehydration of the skin. The number of epidermal cells reduces by 10% every decade and start to divide less frequently. This slows down the skins natural ability to heal itself as quickly.

The dermal layers see a significant change during the ageing process. The dermal layer will start to thin, and less collagen will be produced. The elasticity in the elastin fibres also start to wear out. The skin will start to sag as the dermal papillae layer of skin starts to weaken between the epidermal and dermal layers. This also prevents the connection with essential nutrients and toxin removal that the skin requires in order to function normally.

Finally, fat cells start to shrink causing loss of volume to the face and further skin sagging and wrinkles.

Organ System Mechanisms of Aging

The various organ system mechanisms of aging can be viewed in the context of both the cellular and clinical characteristics of normal aging that occur. Cellular changes with normal aging include decreased proliferative capacity and potential of specific cells (lymphocytes and fibroblasts) associated with decreased secretion of interleukin-2 and diminished expression of T-cell populations that have an altered affinity for this cytokine. The clinical characteristics of normal aging include a change in the biochemical composition of tissues (lipofuscin and extracellular matrix cross-linking, protein oxidation, and altered rates of gene transcription), reduction of physiologic capacity, reduced ability to maintain homeostasis (adaptive processes under physiologic stress), and increased susceptibility and vulnerability to disease. The various organ system mechanisms of aging are discussed in terms of specific structural and functional changes of normal aging.

Body Structure and Composition

Normal aging is associated with a reduction in height related to a decrease in the height of the vertebral body, thinning of the intervertebral diStem Cells, a certain amount of flexing of the hips and knees, and flattening of the arch of the foot. Normal patterns of weight loss are different for males and females by decade, but generally weight gain is seen until the age of 55 to 60 years, when decline begins. Weight changes with normal aging are affected by dietary habits, activity levels, culture, and economics. Fat and water content change with normal aging with lean body mass decreasing by 1% per year after age 55, with a reduction of 40% by age 80; fat composition doubles to 30% of total body weight by the seventh decade, and there is a greater increase possible in females (Dharmarajan and Ugalino, 2003).

Integumentary System

Changes of aging relative to the integumentary system can be further divided into intrinsic (physiologic) versus extrinsic (environmental) changes. Physiologic changes include structural changes, clinical manifestations of these changes, and physiologic and immunologic changes. Normal structural changes of aging of the integumentary system include a thinning of the stratum corneum, reduction in the number of Langerhans cells, melanocytes, and mast cells, and a reduction in the depth and extent of the subcutaneous fat layer. With these normal change and exposure to ultraviolet rays of the sun, structural changes of the skin may include decreased DNR repair and increased DNA injury, lysosomal damage, and altered collagen structure (Cefalu and Nesbitt, 2006), resulting in an increased risk of skin cancer (basal cell, squamous cell, and melanoma). With normal aging, there is an increase in the proportion of hairs in the telogen or resting phase and shortening of the anagen or growth phase and a graying of hairs due to changes in the follicular melanocytes.

A result of these structural changes is varying degrees of thinning of the hair or actual balding; to some extent, this is related to genetic predisposition. Other clinical changes related to these structural changes include an increased frequency of benign and malignant epidermal neoplasms, irregular pigmentation, a propensity to blister formation, a reduction of dermal clearance of chemical agents leading to dermatitis and slower healing, superficial skin laxity, increased risk of skin tears, and thermoregulatory disturbances such as hypothermia and hyperthermia. Functional normal changes of the skin include beta cell dysfunction and increased levels of immunoglobulins A and G and a reduction in epidermal (Lajoie et al., 1996) dehydrocholesterol per unit area, resulting in a reduction in subsequent vitamin D production in the skin. This may result in an increased frequency of clinical disease including increased frequency of antigen–antibody reactions, increased risk of skin infection, and development of osteomalacia and fracture (Cefalu and Nesbitt, 2006).

There are some basic biological processes on which life depends on. For example, cells produce energy from break down of food and then from that energy cells make their new cellular components like proteins and DNA. Cells also need energy for the breakdown of waste products (Lopez-Otin, 2013).

Wound Healing

Wound healing is a complex and dynamic process of replacing devitalized and missing cellular structures and tissue layers. It refers to the replacement of living organism's destroyed or damaged tissues by newly produced ones (Nguyen et al., 2009). In undamaged skin, the epidermis (surface layer) and dermis (deeper layer) form a protective barrier against the external environment. When the barrier is broken, a regulated sequence of biochemical events is set into motion to repair the damage (Nguyen et al., 2009; Rieger et al., 2015). This process is divided into predictable phases. The wound healing process is not only complex but also fragile, and it is susceptible to interruption or failure leading to the formation of non-healing chronic wounds. Factors that contribute to non-healing chronic wounds are diabetes, venous or arterial disease, infection, and metabolic deficiencies of old age (Enoch and Price, 2004). When the skin is injured, our body sets into motion an automatic series of events, often referred to as the "cascade of healing," to repair the injured tissues. The cascade of healing is divided into these four overlapping phases: Hemostasis, Inflammatory, Proliferative, and Maturation.

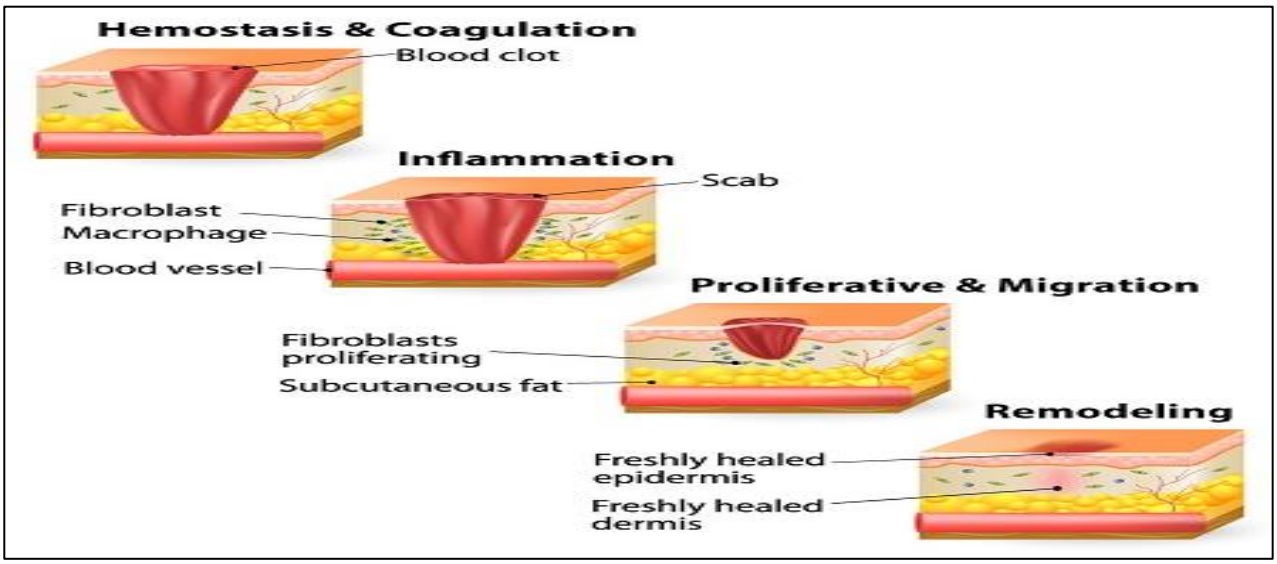


Figure 24: Phases of wound healing

Phase 1: Hemostasis Phase

Hemostasis, the first phase of healing, begins at the onset of injury, and the objective is to stop the bleeding. In this phase, the body activates its emergency repair system, the blood clotting system, and forms a dam to block the drainage. During this process, platelets meet collagen, resulting in activation and

aggregation. An enzyme called thrombin is at the center, and it initiates the formation of a fibrin mesh, which strengthens the platelet clumps into a stable clot.

Phase 2: Defensive/Inflammatory Phase

If Phase 1 is primarily about coagulation, the second phase, called the Defensive/Inflammatory Phase, focuses on destroying bacteria and removing debris—essentially preparing the wound bed for the growth of new tissue. During Phase 2, a type of white blood cells called neutrophils enter the wound to destroy bacteria and remove debris. These cells often reach their peak population between 24 and 48 hours after injury, reducing greatly in number after three days. As the white blood cells leave, specialized cells called macrophages arrive to continue clearing debris. These cells also secrete growth factors and proteins that attract immune system cells to the wound to facilitate tissue repair. This phase often lasts four to six days and is often associated with edema, erythema (reddening of the skin), heat and pain.

Phase 3: Proliferative Phase

Once the wound is cleaned out, the wound enters Phase 3, the Proliferative Phase, where the focus is to fill and cover the wound. The Proliferative phase features three distinct stages: 1) filling the wound; 2) contraction of the wound margins; and 3) covering the wound (epithelialization). During the first stage, shiny, deep red granulation tissue fills the wound bed with connective tissue, and new blood vessels are formed. During contraction, the wound margins contract and pull toward the center of the wound. In the third stage, epithelial cells arise from the wound bed or margins and begin to migrate across the wound bed in leapfrog fashion until the wound is covered with epithelium. The Proliferative phase often lasts anywhere from four to 24 days.

During wound healing, type III collagen appears in the wound about four days after the injury. Wound collagen or type III is immature collagen tissue and does not provide a great deal of tensile strength. It is initially deposited in the wound in a seemingly random fashion.

Phase 4: Maturation Phase

During the Maturation phase, the new tissue slowly gains strength and flexibility. Here, collagen fibers reorganize, the tissue remodels and matures and there is an overall increase in tensile strength (though maximum strength is limited to 80% of the pre-injured strength). The Maturation phase varies greatly from wound to wound, often lasting anywhere from 21 days to two years.

The healing process is remarkable and complex, and it is also susceptible to interruption due to local and systemic factors, including moisture, infection, and maceration (local); and age, nutritional status, body

type (systemic). When the right healing environment is established, the body works in wondrous ways to heal and replace devitalized tissue

Wounds that exhibit impaired healing, including delayed acute wounds and chronic wounds, generally have failed to progress through the normal stages of healing. Such wounds frequently enter a state of pathologic inflammation due to a postponed, incomplete, or uncoordinated healing process. Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or pressure. These wounds results in scars as the healing process is not complete or might get interrupted which result in scaring.

The Role of Stem Cells in Wound Healing

The role of stem cells (SC) in cutaneous wound healing and tissue regeneration is a topic of increasing research attention, with a focus on the role of adult stem cells such as epidermal stem cells and bone-marrow (BM)-derived cells (BMDCs).

Epidermal Stem Cells

Epidermal stem cells reside in the bulge area of hair follicles and in the basal layer of the epidermis and give rise to the keratinocytes that migrate andre-epithelialize wounds. Normal skin is also a target organ for BMDCs. Robust activation of Epithelial Stem Cells and efficient recruitment of their progeny toward an epidermal lineage are critical for re-epithelialization (Garcin and Ansell, 2017), a stage that is also called the re-establishment of an intact keratinocyte layer during wound healing (Gurtner et al., 2008).

Under homeostatic conditions, the major epidermal compartments are rejuvenated by differentiation of their own Stem Cells. IFE and SGs undergo constant self-renewal, whereas HFs undergo cycles of phases, including resting, growth, and involution. Each discrete Stem Cell niche behaves unipotently, replenishing its own compartment (Sada et al., 2016; Blanpain et al., 2004; Hirsch et al., 2017). However, during wound healing, Stem Cells have acquired the ability to repair neighboring compartments, and these compartments can repopulate one another (Adam et al., 2015). The way Stem Cells respond to injury varies drastically, does not only depend on the specific niches where these reside but also on how close they are to the wound (Wang et al., 2013).

During wound healing, Epithelial Stem Cells are activated and recruited from different skin regions when spatial confinement and lineage restriction of resident skin Stem Cells are transiently lost, allowing them to contribute to multiple Epithelial Stem Cells (Mascre et al., 2012). When HFStem Cells migrate toward the epidermis, they lose their specific markers and adopt a phenotype similar to that of IFEStem Cells. However, once in the epidermis, these cells are short-lived and disappear soon after the damaged tissue is

repaired (Mascre et al., 2012). Studies have shown that HFStem Cells temporarily contribute to wound re-epithelialization soon after damage but disappear several weeks later, suggesting that HFStem Cells serve as a transient bandage that allows other Stem Cells from the IFE and upper isthmus/infundibulum to maintain long-term repair (Quist et al., 2016). The role of HF Stem Cells was further defined by other researchers, who indicated a delay in the early stage of re-epithelialization when incisional wounds were created in HF-deficient mice, presumably through recruitment of IFE Stem Cells and indicating their capability for tissue regeneration (Kadaja et al., 2014). In addition, glabrous skin, such as the ventral part of the paw, heals properly with slower kinetics than human skin does, suggesting that HF Stem Cells are dispensable for wound healing (Ito, 2008). These studies suggest that the injury-induced vacant niches activate a broad range of Stem Cells to assume characteristics that differ from their homeostatic roles.

Bone-Marrow (BM)-Derived Cells (BMDCS)

Two main stem cell populations are present in the bone marrow: Hematopoietic Stem Cell (HSC) and Mesenchymal Stem Cell (MSC). BM-MStem Cells can differentiate into a variety of cell types, including adipocytes, osteoblasts, chondrocytes, fibroblasts, and keratinocytes (Cha and Falanga, 2007; Rea et al., 2009). Endothelial progenitor cells (EPCs) derived from the HSC lineage are key cells that contribute to neovascularization. Both BM-MStem Cells and EPCs are involved in the cutaneous wound-healing process. Wound-induced hypoxia triggers the mobilization of bone marrow EPCs to the circulation, playing a significant role in the process of neovascularization (Wu et al., 2007; Liu and Velazquez, 2008; Rea et al., 2009).

Several different cell types are involved in the wound-healing process, and as described above, the cellular activities of any cell type may also vary during different stages of repair. The complexity and coordination of the healing process are major hurdles to therapeutic approaches, since any therapeutic must effectively be sequenced to the appropriate stage.

Chapter Three

Aesthetic Medicines

Aesthetic Medicine comprises all medical procedures aimed at improving the physical appearance and satisfaction of the patient, using non-invasive to minimally invasive cosmetic procedures (AAAM, 2021). According to the Aesthetic Medical Journal (2021), aesthetic medicine is a broad term for specialties that focus on altering cosmetic appearance through the treatment of conditions including scars, skin laxity, wrinkles, moles, liver spots, excess fat, cellulite, unwanted hair, skin discoloration and spider veins through many non-surgical procedures like (needling, radio frequency skin tightening, non-surgical

liposuction, chemical peel, high intensity focused ultrasound, electromagnetic field, radio frequency fat removal), and many more . (Honigman et al., 2004).

Aesthetic medicine systematically deals with generally healthy individuals who are often dissatisfied with some minor deficiency, most often a physical one. It is one of the most innovative branches of medicine (HUEM, 2018). This medical specialty is aimed at improving the quality of life and general wellbeing and prevention of aging effects and as such presents the evolution of internal medicine. Prevention of body aging primarily refers to general aging and skin aging, but aesthetic medicine also deals with correcting physical and facial imperfections.

According to HUEM (2018), in a broader sense, aesthetic medicine deals with the construction and reconstruction of psychophysical balance of healthy individuals who may struggle with unease because of certain physical imperfection they have difficulty accepting. Aesthetic medicine also puts forward certain rules, suggestions and interventions referring to the way of life aimed at controlling the effects of general aging of the body as well as skin aging. Apart from making an important contribution to multidisciplinary approach to treatment of physical disadvantages as well as aesthetic and psychological consequences of disease, aesthetic medicine works to achieve and maintain individual's general health as recommended by the World Health Organization - "as a state of complete physical and mental well-being rather than merely the absence of disease" (HUEM, 2018).

Aesthetic medicine patients today, in addition to correcting an imperfection, often seek advice on improving the quality of life with the main goal of maintaining the optimal physical and mental health throughout the years. This branch of medicine makes way for the understanding of the role that various body parts and organs have in the general psychophysical balance; aesthetic medicine corrects potential imbalances and prevents the development of disease (HUEM, 2018).

Aesthetic Procedures and Effects

Over the last 10 to 20 years, there have been numerous advances seen in the world of aesthetic medicine. The benefits are now well recognized, and affordability makes this a constantly sought-after treatment. There are a variety of treatments offered in Aesthetic Medicine practices However, there only few are popular amongst patients.

Below are list of the aesthetic procedure that we are discussing in this research, with heir, mechanism of action, benefits and effect their effect on stem cells regeneration.

Chemical peel

Chemical peels are aesthetic treatments that can be applied to the face, hands, and neck. They are used to improve the appearance or feel of the skin. During this procedure, chemical solutions will be applied to the area being treated, which causes the skin to exfoliate and eventually peel off (Ana, 2018). Once this happens, the new skin underneath is often smoother, appears less wrinkled, and may have less damage. Chemical peels are a simple, non-invasive way of improving skin tone and texture. It is often offered as a treatment for hard-to-treat acne (and acne scars), liver spots, and freckles. The procedure is straightforward, and the results are good and depend on the type of skin peel. Deeper skin peels can take longer to heal, and patients can develop skin redness and irritation during that time. Despite this, it remains a sought-after treatment, with an increase of 15.5% between 2011 and 2012 in the number of patients seeking this treatment.

Mechanism of action:

To create specific skin depth injury, chemical peels are used which ultimately results in the formation of new skin and improved skin surface appearance and texture. The chemical peels exfoliative effect causes the formation of collagen and new epidermal growth with very evenly distributed melanin. In the aesthetic procedure of chemical peel, top layers of the skin are removed by applying chemical solutions on the skin surface. Chemical peels are of various strengths and depths. Outer layers of the skin are treated by light chemical peels while deeper skin layers are treated by strong chemical peels. Chemical peels contain acids in them which is very important component of chemical peels. When chemical peels are applied on the skin surface, the acids present in them causes an increase in the acidity of the skin. Normally, pH of the skin is around 5.5 while acids in the chemical peels bring pH of the skin to 3.8. This change in the pH of the skin effects those skin cells which are acting as glue between healthy and dead skin cells by chemically loosens them. When this bond becomes loosened, exfoliation of the skin surface occurs and hence healthy skin comes out by removing the dead skin present on the skin surface. Chemical peeling process enhances the growth of new healthy skin cells (Fischer et al., 2010).

Chemical peels induce keratocoagulation or keratolysis when they are applied on the skin surface and also causes protein denaturation present in the dermis and epidermis. It results in the release of chemokines and proinflammatory cytokines. These chemokines and proinflammatory cytokines causes inflammation which activates the natural healing signal cascade which includes structural scaffold proteins reorganization, the stimulation of new dermal elastin and collagen, their development and deposition, and new keratinocytes regeneration. This ultimately results in the increase in the volume of dermis as well as thickening and rejuvenation of epidermis (O'Connor et al., 2018).

The outcome of chemical peeling aesthetic procedure changes with the change in the chemical agent used for chemical peeling but the general purpose of using chemical peeling procedure is to enhance skin appearance by reducing quality and quantity of acne scars, decreasing noninflammatory and inflammatory acne lesions, dyspigmentation improvement, and forming more youthful skin appearance (Truchuelo et al., 2017).

Chemical peeling effect on stem cells regeneration:

Stem cells have the potential to naturally regenerate tissues and organs such as skin. It is safe to use secretome in the chemical peeling process. When a chemical peel acts on the epidermis, it loosened the lower skin layer and stimulates the basal skin layer which produces new skin cells by stem cell regeneration and skin cell division. They also produce collagen and elastin. These new skin cells replace the old, hyperpigmented and oxidized skin cells. While collagen and elastin tightens the skin layer. Hence the ultimate result is the formation of new skin cells layer in place of aged skin cells layer which is smooth and looks young and healthy (Stuzin, 2013).

Mesotherapy

Mesotherapy is a technique that uses transdermal injections of multivitamins, enzymes, hormones **medications, homoeopathic agents, and other bioactive substances and** natural plant extracts to rejuvenate and tighten skin, as well as remove excess fat. It is a non-surgical cosmetic solution aimed at diminishing problem areas in the body such as cellulite, excess weight through meso fat dissolve, body contouring, as well as face/neck rejuvenation by stimulating the biosynthetic ability of fibroblasts and facilitate interaction between cells; it is intended to increase collagen and elastin production (Aminetal.,2006; Lacarrubba et al.,2008).

In aesthetic medicine, there are several indications for which mesotherapy can be successfully used, for example, facial rejuvenation, Mesolift, Mesobotox, and mesotherapy

In general terms mesotherapy consists of three different techniques of injection which treat the skin at three different levels: First one is Epidermic level, Second is intradermic level known as ’nappage’ and third is dermoepidermic level known as ‘papula’, these three techniques serve different purposes as they reach different depths of the skin. Depending on the patient and on the quality of the skin.

Msotherapy can treat skin scars and wrinkles, stem cells can be administered into mesoderm either alone or in conjunction with other substances via the mesotherapy technique. Thymosin TB-4, a growth factor,

is injected into the mesoderm. TB-4 improves circulation and activates stem cells at the same time. The application of mesotherapy influences ageing, skin cell function, cell-to-cell interaction, and intermolecular transport and communication. Tired and dull skin on the face, neck, décolletage, and hands are the most typical problem areas can be treated with mesotherapy, they require intensive treatment to renew. Fat reduction component of mesotherapy can be used as well to target excess fat on the jowls/chin, cellulite on the thighs and buttocks, and sagging fat on the belly. Mesotherapy can be used to repair thinning hair, reduce the appearance of stretchmarks, and restore the skin's appearance after sun exposure, among other things. (Konda & Thappa, 2013).

Mechanism of action:

In mesotherapy, chemical injections are given to the targeted middle layer (mesoderm) of the skin with short, fine needles. A short, fine needle is injected into the mesoderm of targeted skin and then tiny drops of concentrated active substances are placed there. These concentrated active substances include amino acids, minerals, vitamins, biomimetic peptides, growth factors, traditional pharmaceuticals, homeopathic medications, or collegen. These concentrated active substances stay there for a longer time when they are injected intracutaneously as compared to their distribution by deeper injections. When skin absorbs these active substances, they stimulate the production of elastin and albuminoid, restore skin tone, remove skin wrinkles and reconstruct the skin’s inner structure as a result a new younger skin form. The active substances used in mesotherapy stimulate the fibroblast cells to synthesize more collagen to fight with the ageing symptoms. Some active substances used in mesotherapy help to decrease the skin’s unrequired pigments by controlling the pigment production rate of melanocyte in the skin (Mammucari et al., 2020).

Mesotherapy also removes the scars and marks of acne with the passage of time. For pitted skin surface, hyaluronic acid with the solution is used as an active substance in mesotherapy and it creates volume in skin and fill up the pitted surface. Amino acids and antioxidents in mesotherapy have an anti-inflammatory effect which causes healing of the skin. Enzymes are used as active substance in mesotherapy to treat the deposits of fat in localized areas (such as chin) and cellulite. These enzyme breaks down fat cells by disrupting the wall of fat cells which results in release of fat from cells which is reabsorbed by lymphatic system and at the end body excrete it out (Vedamurthy, 2007).

Mesotherapy effect on Stem cells regeneration:

Stem cells also called replacement cells are undifferentiated cells that can divide to form more stem cells and can differentiate into specialized cells. Dying, old or damaged cells are replaced by stem cells in the body. Stem cells can differentiate into any cel of the body according to need of the body. Using stem

cells extract as active substance in mesotherapy enhances the proliferation or multiplication of already existing skin cells which repairs the skin. Some mesotherapy active substances are antioxidant and anti inflammatory which provide active environment for stem cells to regenerate because stem cells are not able to regenerate properly under oxidative environment produced due to skin problem (Serra-Renom and Serra-Mestre, 2016)

Platelet Rich Plasma (PRP)

Platelet Rich Plasma (PRP), also called autologous conditioned plasma, is a concentrated platelet-rich plasma protein generated from whole blood that has been centrifuged to remove red blood cells. It is a form of cosmetic injectable treatment that uses the client's own platelet rich plasma. The plasma component contains stem cells and growth rich platelets. When injected back into the skin, it accelerates the body's natural production of collagen and elastin to provide overall skin rejuvenation.

Platelet-rich plasma (PRP) is a recent development in cosmetic medicine. PRP has been utilized to treat fine lines and wrinkles, acne, scarring, hair loss and. It can also be used to rejuvenate the skin and tighten the skin around the eyes. We can even use derma rollers that contain micro needles with different depth to stimulate the skin before injecting PRP to stimulate the collagen production and regeneration of stem cells as well as repair hair loss. The reasoning behind this is that it sends a signal to the hair follicles to begin the process of mending. PRP is then infused into the affected area to stimulate stem cells in the follicle even more. Multiple microscopic punctures under the dermis are used to inject platelet- rich plasma, which can be done with or without topical local anesthetic (Mehtaetal., 2008; Shinetal., 2012). If a sufficient topical anesthetic is used, the procedure is painless. When PRP is injected into the needed location, it stimulates the tissue and causes mild inflammation, which starts the healing process.
As a result, new collagen begins to develop. Collagen begins to increase which, tightening and strengthening the skin. Within three weeks to 28 days, you'll notice a difference in the texture and tone of the skin. Three months is required for complete collagen regeneration (Knightonetal.,1982; Kawazoeetal.,2012). All skin types and tones can benefit from PRP treatments. Swelling, bruising, and redness should be minimal during the first 12 to 24 hours.

Platelets stimulate growth factors, which aid in greater collagen stimulation, over the course of several weeks. Treatment outcomes vary for different skin types but it will always have an healthier looking skin after each treatment

The advantages of using PRP for aesthetic medicine include the following: tissue regeneration and rejuvenation, induction of cell differentiation, extra cellular matrix formation, recruitment of other cells

to the site of injury, and an increase in collagen production, which can increase skin thickness and over all skin health (Redaelli et al., 2010; Kim et al.,2011). In addition, PRP is non allergenic, is an autologous physiological product, eliminates donor transmissible infections, and is a biological glue for tissue adhesion, especially in skin flaps, bone grafts, and trauma.

Aging of the skin, dermal components, and cells means that the skin texture and appearance deteriorate and have been damaged (Cho et al., 2012). Aging affects the hands and soft tissue of the face, neck, and decollete. This is characterized by sagging jowls, thinning of the skin, puffiness, age spots, and wrinkling, PRP has been used to treat lots of skin ageing conditions, It is effective for skin rejuvenation and tightening around the eyes (for thin crepe-like skin and fine lines) as well as cheeks and mid face, thinning skin on the neck, jaw line and sub malar regions, back of hands, decollete, and others (eg; knees, elbows, and upper arms, as well as for post pregnancy skin laxity). Platelet-rich plasma is injected by multiple tiny punctures under the dermis, with or without topical local anesthesia. When PRP is injected into the damaged area, it stimulates the tissue, causing mild inflammation that triggers the healing cascade. As a result, new collagen begins to develop (Zenker,2010).

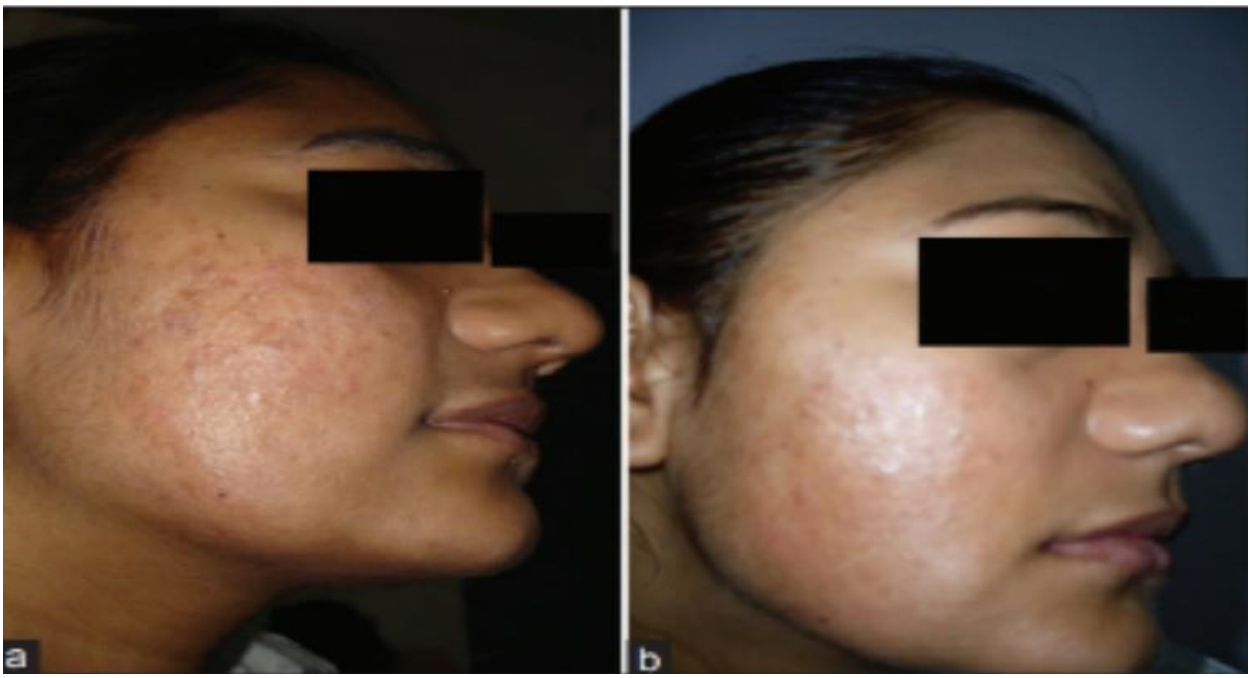


Figure 25: (a and b) Pre and post treatment of a 37 years old girl with acne scarring after three sessions of PRP

Mechanism of action:

Blood plasma is the liquid present in the blood. Plasma provides medium for white blood cell, red blood cell and platelet for circulation and mainly consists of proteins and water. Platelets present in the blood

are also called thrombocytes and are involved in the blood clotting and other necessary growth healing functions. Platelet-rich plasma (PRP) therapy includes injection of a person's own platelet for the purpose of healing. Hence PRP injections utilize a person's own healing system for the purpose of improving problems like skin problems. To prepare PRP injections, a person's blood is taken and then run through the centrifuge for concentrating platelets. These platelets are then introduced into the diseased or injured body tissue. This procedure took the time of 45 minutes to one hour. Platelet-rich plasma (PRP) therapy enhances the release of growth factors that increase the reparative cells number in the body (Banihashemi and Nakhaeizadeh, 2014).

PRP consist of seven main proteins which are adhesive proteins – vitronectin, fibronectin, and fibrin, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), transforming growth factor–β (TGF-β), and platelet derived growth factors (PDGF). At the injury site, PDGF glycoprotein emerges from the platelets degranulation. This protein activates receptors present on the cell membrane at the diseased site which develops phosphate bond in return, which initiate the specific activities there by activating signal proteins. These activities are macrophage activation, angiogenesis, and mitogenesis. Platelets and macrophages also secret TGF-β, which in the normal epithelial cells acts as an antiproliferative factor. Transforming growth factor–β (TGF-β) targets the stem cells and fibroblasts which cause regeneration, remodeling and long term healing. Vascular endothelial growth factor (VEGF) is a signal peptide that stimulates angiogenesis and vasculogenesis. Epidermal growth factor (EGF) stimulates differentiation, proliferation and cell growth (Jain and Gulati, 2016).

PRP enhances the natural healing process. PRP injections reduce the requirement for anti-inflammatories. The bioactive molecules and growth factors present in the platelet rich plasma promotes four main actions at the target site. These actions are proliferation, migration, cell differentiation, and angiogenesis. PRP has become a very effective non-surgical aesthetic procedure for skin treatment. PRP stimulates the collagen production and new cell growth by using a person's own blood platelets (Crutchfield III and Shah, 2018).

PRP effect on stem cell regeneration:

Platelet rich plasma helps in the restoration of lost facial volume, reduces skin acne and wrinkles, improves skin texture and revitalizes the skin. Platelet rich plasma increases stem cell numbers and guide the stem cells in healing process. PRP helps stem cells to find out in which cell they should differentiate either a skin cell or a collagen cell etc. Platelets also secrete many cytokines as well. Studies showed that PRP

increases the differentiation and proliferation of stem cells. In short, platelets guide the stem cells what to do (Lucarelli et al., 2003).

Skin boosters

Skin booster is a new aesthetic procedure which promotes skin improvement by injecting hyaluronic acid and amino acid in the dermis. Skin boosters are Bio skin revitalisers that continue to work weeks after each treatment. It restores radiance and firmness of dull skin, prevents and reduces sagging skin (face and body), reduces fine lines and signs of expression, prevents elastosis, improves the appearance of scars (including acne), keloids and red stretch marks. They stimulate key skin processes; fibroblast activity, cellular rejuvenation, free-range collagen activity, muscle mass and assists with inflammation whilst treating skin degradation, providing protection against free radical effects through the production of antioxidant activity.

Most of these formulas containing hyaluronic acid and amino acids helps to regenerate the Extra-Cellular Matrix (ECM) by stimulating the production of new collagen and elastin. This helps to restore the biological functions of the dermis and restore the natural youthful look of the skin.

Amino Acids are the building blocks that make up proteins and have a role in virtually all the biological processes that occur in our bodies. The sequence in which they combine together determines which particular protein they will form and how they will function. There are twenty amino acids, eleven of which are considered ‘nonessential’ and nine that are considered ‘essential,’ yet all are vital for the body’s survival. Nonessential means that the body can make them on its own. Essential amino acids must be obtained through the diet (the body cannot make them on its own.

Amino acids have many beneficial effects in skincare, including hydration, cellular repair and UV protection.

Hyaluronic Acid (also called hyaluronate, hyaluronan, HA) is a substance that is naturally produced by the human body and found in the skin, connective tissue and tears. This gel-like molecule has a special ability to hold 1000 times its weight in water which makes it an important skin structural element. HA works as a magnet for moisture, keeping cells well lubricated and moist; hence, the skin plumped, hydrated and healthy. HA also helps with the production of collagen, maintenance of skin elasticity, and cell protection against free radicals, thus, protects the skin against premature ageing. Moreover, it has antibacterial and anti-inflammatory properties that help with wound healing.

As we age, our body’s ability to produce HA slows down and the balance of water in cells become significantly impaired. By the age of 50, we lose almost half of all our naturally produced HA. As a result,

our skin becomes dehydrated, dull, saggy and wrinkled. When HA is injected into the skin, it replenishes the ‘missing HA’ spaces in the cells with moisture; hence, the skin becomes hydrated, plumped up, and fine lines and wrinkles are smoothed out.

This intradermal treatment corrects and protects the dermis from the signs of ageing. These skin boosters are a great option for people who wish to maintain a more natural look, those who do not want Botox or Dermal Fillers or as a special treatment skin booster ‘add-on’ to your treatments to improve the quality of your skin.

Mechanism of action:

Extracellular matrix and protein fibers are the components of dermis of the skin. Hyaluronic acid (HA) is naturally present in the dermal extracellular matrix and is an abundant glycosaminoglycan. This compound has high affinity to water and retain water in itself. This property is determinant of skin firmness, structure, hydration, and viscoelasticity. During the process of aging, production of elastin and collagen is decreased along with degradation and disorganization of dermic fibres. Glycosaminoglans concentration in the dermis is also reduced during aging. Due to these phenomena, the reduction of resistance, density, and elasticity of the skin occur(Landau and Fagien, 2015).

Skin booster is a new aesthetic procedure which promotes skin improvement by injecting boosters like hyaluronic acid and amino acids in the dermis. Hyaluronic acid stimulates the production of collagen and improves hydration of the skin dermis and amino acids are the building blocks for making the proteins, collagen and elastin. Eight specific amino acids are mostly used. This procedure uses small quantity of hyaluronic acid to reverse or delay the aging process, reduce the indicators of aging and maintain skin hydration. So, skin boosters increase the hyaluronic acid level and collagen procuction in the skin, which in turn produces firmer skin and improves wrinkles and fine lines. Some examples of skin booster products are Sunekos, Profhilo, Juvederm VOLITE, belotero REVIVE, Jalupro, Rejuran, and Restylane. Hyaluronic acid is the major ingredient of most of the skin boosters but some boosters contain other ingredients for example, Rejuran contain salmon DNA, Profhilo contain complex HA molecules (high molecular weight and low molecular weight HA) and sunekos contain many different amino acids. Skin boosters activate the fibroblast cells when they are injected into the papillary dermis. This stimulation of fibroblast enhances the formation of elastin and collagen restoring essential hydration, elasticity and volume to the skin. This makes the skin healthy looking, glowing and smooth. Many different areas of the skin can be treated by skin boosters. Among them the most common area of the skin which is treated by skin boosters is face followed by neck, decollate and hands (Guinan, 2022).

Skin boosters that contain HA are different from dermal fillers in the sense that Hyaluronic acid-based dermal fillers are clumpier and stiffer due to having cross-linking bonds and when these dermal fillers are injected into skin, they produce volumising and skin lifting effect. While on the other hand skin boosters are non-crosslinked hyaluronic acid which hydrate the skin and improves the skin quality instead of reshaping or volumising the face. Non-crosslinked hyaluronic acid is used by skin boosters which stimulate the production of elastin and collagen in the skin whose concentration was reduced due to environmental damage and aging. Youthful and firmer skin is obtained due to new elastin and collagen production (Kleine-Börger et al., 2022).

Hence skin boosters are a complete aesthetic procedure which rejuvenates the skin by reducing wrinkles and fine lines, hydrating the skin, improving skin tone and texture, and making the skin look fresh, smooth and glowing (Hall, 2019).

Skin boosters effect on stem cell regeneration:

Skin boosters make the skin youthful, hydrated and firmer by retention of water and production of elastin and collagen. They activate fibroblasts present in the skin. Skin booster promotes skin improvement by injecting booster substances like hyaluronic acid, and amino acids in the dermis. Hyaluronic acid hydrate the skin to make it smoother and brighter, activates stem cells regeneration to form fibroblasts cells which make more collagen and elastin. And amino acids are the building blocks for making the proteins collagen and elastin. Skin boosters activate the epidermal follicular stem cells which only activate during wound healing and remain dormant otherwise. Stem cell secretome can also be used as a skin booster (Xia et al., 2019).

Botulinum toxin & Mesobotox

Botulinum Toxin is a spore-forming bacterium that naturally occurs in soil. Seven distinct strains of C botulinum have been identified. Each strain is characterised by the type of botulinum neurotoxin that it is capable of producing and has been classified as type A-G. Although all of these neurotoxins inhibit the release of acetylcholine (ACh) at the neuromuscular junction, they all vary in their chemical structure and size as well as their mechanism of action within the nerve terminal itself. Only Five of these subtypes (A, B, E, F, G) will affect the human nervous system, whereas 2 subtypes (C and D) will not. Types A and B are the two most clinically relevant subtypes and, therefore, are commercially produced. Botulinum toxin type-A is thought to exert the most powerful neuromuscular blockade and is also capable of exerting its effect for the longest duration of time. In contrast, botulinum toxin type-E

and type-F are also capable of blocking myoneural transmission, however they have a shorter duration of action when compared to types A and B and, therefore, are not commercially made (Arnon, 2001)

Botulinum toxin type-A and type-B are composed of a 150 kD polypeptide, consisting of a disulfide bond linked light chain and heavy chain. These disulfide-linked molecules are associated with other nontoxic proteins during their synthesis to form a neurotoxin complex, which is approximately 500 kD in size. These non-neurotoxin accessory proteins may serve a beneficial role in stabilizing the fragile botulinum toxin molecule when it is reconstituted for use (Blitzer et al., 2000).

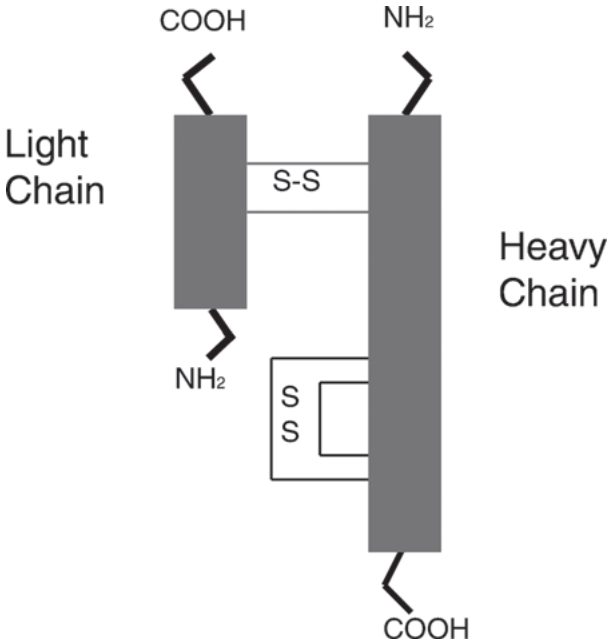


Figure 26: Botulinum toxin molecule

Botulinum toxin injections have both cosmetic and medical uses. They are noted primarily for the ability to reduce the appearance of facial wrinkles. The toxin functions by blocking off nerve endings to the skin and allowing the skin tone to increase, producing a younger look (Satriyasa, 2019). There are two categories of facial lines, dynamic and static. When the underlying muscle contracts, dynamic facial lines form, which can indicate an emotion such as anger or fear.

On the other hand, static facial wrinkles can occur through the frequent formation of dynamic facial wrinkles and develop involuntarily without muscular contraction. When dynamic and static lines occur "more frequently or more intensively than desired for expression, "the lines are called hyper-functional (Finnetal.,2003). These hyperfunctional lines are treated with Botox to reduce or eliminate them. Botox injections have both cosmetic and medical uses.

Mesobotox is the technique of injecting botulinum toxin matches to the criteria of mesotherapy injection when executing 'Mesobotox.' Intradermic injections must be made in the medium derma in order to create a dermic papule that can be spread. If the injection is made too shallow, the papule will become veside, the medication will remain at the level of the superficial dermis, and the underlying effect will be almost non-existent.

Mesobotox is an aesthetic procedure which makes younger looking and smoother skin by reducing wrinkles and fine lines. This procedure also reduces the oil production and pore size. It also reduces facial sweating by reducing glands functioning which are responsible for sweat production. This aesthetic procedure causes the skin tightening, skin smoothening and makes skin more youthful. Mesobotox procedure makes facial skin smoother by injecting mesotherapeutic solution along with high dilution of botulinum toxin into the skin. Its effect is different from botox injection because it contains highly diluted concentration of botulinum toxin as compared to concentration used in botox injections (Seo, 2005).

This aesthetic procedure is similar to botox injections in the sense that both receive injections of botulinum toxin. However, mesobotox uses high dilution of botulinum toxin that will speared widely through microneedles. Botox involves the injection of botulinum into the muscles of entire face while mesobotox involves the injection of botulinum into the surface skin layer. The diluted botulinum can be used in combination with mesotherapeutic solution composed of growth factors, peptides, amino acids, minerals, antioxidants, vitamins and non-cross-linked hyaluronic acid. Hence the skin is revitalized and hydrated. Mesobotox or botox injection causes improvement in the skin pigmentation and decrease in the skin scars (Wu, 2015).

Mechanism of action:

At the neuromuscular junction, the motor nerve terminal lies in close apposition with the adjacent muscle fibre and induces an excitation-coupling contraction. The motor neuron produces an action potential that travels down the axon to the nerve terminal. Upon the arrival of the action potential, voltage-dependent calcium channels open, causing an influx of calcium ions. This influx results in fusion of the presynaptic vesicles, containing Ach, with the nerve terminal. This fusion is mediated by the SNARE complex (soluble N-ethylmaleimide-sensitive factor attachment protein receptor). The SNARE complex is a neural exocytic complex that regulates the membrane docking and fusion of synaptic vesicles and the release of ACh. The proteins within the SNARE complex

include synaptic neural-associated protein (SNAP-25), syntaxin, and vesicle-associated membrane proteins (VAMP). Botulinum toxin targets these proteins (Blitzer et al., 2000).

When botulinum toxin is administered, the heavy chain (100 kDa) binds selectively to cell membrane receptors on the outer surface of the presynaptic nerve terminal. The entire neurotoxin complex (both light and heavy chains) is then internalized into the nerve terminal via receptor-mediated endocytosis. The vesicles containing the botulinum toxin then fuse with digestive vacuoles that cleave the botulinum toxin molecule into separate light and heavy chains. The light chain (50 kDa) exerts the paralytic effect of botulinum toxin by inactivating the SNARE complex, thereby blocking the release of ACh into the neuromuscular junction. Each serotype of botulinum toxin binds to a specific region of the presynaptic nerve terminal, and they each cleave unique proteins within the terminal itself. All botulinum toxin serotypes act upon the SNARE complex. Serotypes A, C, and E cleave the SNAP-25 molecule, whereas serotypes B, D, F, and G cleave synaptobrevin or VAMP, each at a distinct site. In each case, botulinum toxin enzymatically inactivates a specific protein that is required for the docking and fusion of vesicles containing ACh into the neuromuscular junction. The inhibition of ACh release results in localized muscle weakness (paralysis) that gradually reverses over time. The mechanism by which botulinum toxin-induced muscle weakness is reversed is unknown, but it may involve the intraneural turnover of the affected docking proteins (which are responsible for the release of ACh into the neuromuscular junction), the sprouting of new nerve terminals, or a combination of both of these mechanisms. At 2 months after administration of botulinum toxin, the axon begins to expand, and new nerve terminal sprouts emerge and extend toward the muscle surface. The motor nerve unit is re-established once a new sprout forms a physical synaptic connection with the previous neuromuscular junction. The new nerve sprouts that do not establish a connection to the motor endplate, however, subsequently regress and are spontaneously eliminated, whereas the parent, or former, nerve terminal is re-established.

An understanding of the mechanism of action of botulinum toxin allows one to understand the time required for the onset of paralysis as well as the duration of clinical effect. Botulinum toxin, once injected, takes approximately 3 to 4 days for its effect to become clinically apparent. This corresponds to the amount of time that is required for the botulinum toxin molecule to bind to the motor nerve terminal, undergo internalization via receptor-mediated endocytosis, and block Ach

release through inactivation of the SNAP-25 or VAMP SNARE complex proteins. In contrast, the clinical duration of effect, which is approximately 3 to 4 months in length,

corresponds to the time that is required for new unmyelinated nerve sprouts to grow from the nerve root to re-establish the motor endplate, beginning 28 days following injection. The completion of this process occurs approximately 90 days following injection. Therefore, the duration of effect is not dependent on the continued presence of botulinum toxin at the nerve terminal, but rather reflects the length of time that it takes for a particular individual's nerves to regenerate and develop a functional connection at the myoneural junction.

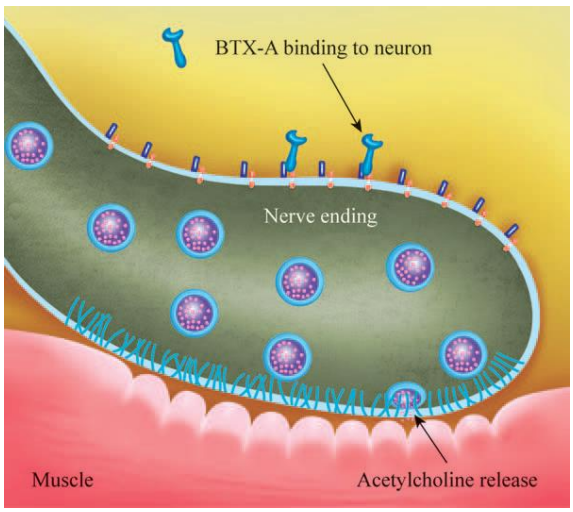


Figure 27(a): Botulinum toxin mechanism of action

The heavy chain of the botulinum toxin molecule binds selectively to cell membrane receptors via the heavy chain on the outer surface of the nerve terminal.

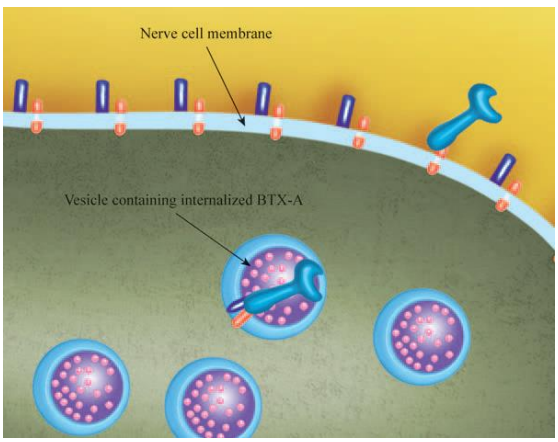


Figure 27(b): Botulinum toxin mechanism of action

The entire neurotoxin complex is then internalized into the motor nerve terminal through receptor-mediated endocytosis. The botulinum toxin type-A is then cleared into separate light and heavy chains. The light chain exerts the paralytic effect by inactivating the SNARE complex proteins, blocking the release of ACh.

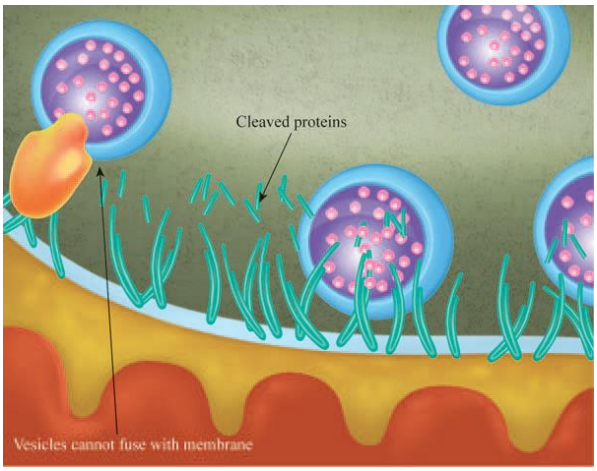


Figure 27(c): Botulinum toxin mechanism of action

The light chain of serotypes A, C, and E exerts its effect by cleaving the synaptic neural-associated protein (SNAP-25) that is responsible for fusion of vesicles containing ACh with the nerve terminal cell membrane.

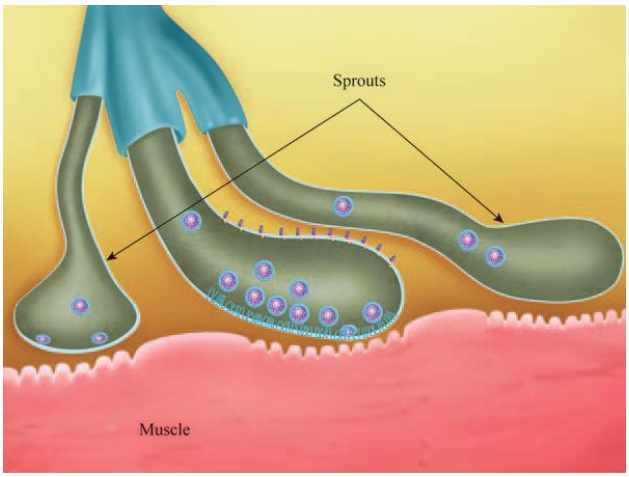


Figure 27(d): Botulinum toxin mechanism of action

Approximately 2 months after injection, the nerve terminal begins to expand, and new sprouts emerge and extend toward the muscle surface. Additional redundant nerve sprouts are also produced. The motor nerve unit is re-established once a new sprout forms a physical synaptic connection with the previous neuromuscular junction (Deineka and Dubinina, 2019).

Botulinum toxin & Mesobotox effect on stem cell regeneration:

Botulinum toxin used in mesobotox or botox injections is muscle relaxant which relaxes target muscles and provides powerful anti-wrinkle and anti-aging effect. Botulinum toxin did not put any direct effect on the regeneration of stem cells present in the body but mesobotox is used along with stem cells as a professional facial treatment. Mesobotox relaxes, weakens and reduces the wrinkles and fine lines (Gugerell et al., 2014).

Dermal fillers:

Dermal fillers are used by cosmetic facial surgeons and Aesthetic practitioners to reduce the signs of aging, minimize skin depressions and scars, and address fine lines and deep wrinkles or folds in the skin of the face and body. Dermal fillers help to diminish facial lines and restore volume and fullness in the face. As we age, our faces naturally lose subcutaneous fat. The facial muscles then work closer to the skin surface, smile lines and crow's feet become more apparent. The facial skin also stretches a bit, adding to this loss of facial volume. Other factors that affect the facial skin include sun exposure, heredity, and lifestyle. Fillers canal so be used on hollow areas below the eyes called tear troughs.

The following are the functions of fillers: plump thin lips, enhance shallow contours, soften facial creases and wrinkles, improve the appearance of recessed scars, reconstruct onto your deformities in the face, and lower lid shadows reduced or removed. Dermal fillers can be very helpful for individuals with early signs of aging, or as a value-added part of facial rejuvenation with or without surgery.

Types of Dermal Fillers

HyaluronicAcid:

This is a natural component of the skin’s connective tissue that gives the skin hydration and its pulpiness as the hyoloronic acid particals can hold thousand times its wait water .Its safeness and effectiveness have made it the most common filler used in the cosmetic facial procedures. Injections of hyaluronic acid can soften fine lines and restore fullness to the skin. The difference between hyaluronic acid products is the

size of the particles, which are designed to address different wrinkle depths: smaller particles for finelines and larger particles for deeper wrinkles and folds and the amount of particles in the 1 ml syringe.

CalciumHydroxylapatite:

Calciumhydroxylapatite treats deeper wrinkles and skin folds. It can help the tocontour the jawline and rest or evolume in and around the cheeks. These injections may last up to three years when used to fill wrinkles and upto one year when used for contouring.

Poly(methylMethacrylate) (PMMA):

This filler consists of microspheres suspended in a collagen-based gel. The gel provides immediate volume that lifts the skin to soften wrinkles and acne scars. The microspheres stay in place and provide structural support for smoother looking skin.

Plasma Bio Filler:

Plasma Bio fillers are the substances injected into the mesoderm to make the skin younger, more plumpted and fuller as of the normal HA fillers that we have explaned previously. Plasma Bio filling or plasma gel is a form of an autologous (made from patients own blood) method for medical/ facial rejuvenation. It is easy to obtain. It replaces Hyaluronic acid fillers and is far more beneficial than treatments such as Botulinum toxin. Plasma gel is a natural product that the body accepts and absorbs easily. Considering the low cost and plasma gel being an autologous product so there is no chance of hypersensitivity or allergic reaction.

Plasma gel is a filler used to treat wrinkles, ageing face, scar reduction. It has recently gained popularity in the medical field with excellent results in a single session for wrinkle reduction. The procedure involves the patient's own 10- 20 ml blood being centrifuged, filtered and processed to produce a platelet rich serum and then into a plasma gel or PRP gel.

Where the plasma which contains platelets (thrombocytes) which contain various proteins (growth factors) help in collagen formation and new blood vessel formation. Sonication process - In order to prepare the plasma gel and get maximal results, sonication process is an effective method used to break the cell membrane release maximum growth factors from platelets. This being a biostimulative treatment, the growth factors help the body to naturally produce collagen. This helps to tighten the skin and make it more radiant and glowing in one sitting.

Fillers that can modify the extracellular matrix to minimize scarring appear to be the most promising candidates for future targeted therapeutic development. Targeted stimulation of white adipose tissue stem cells might be a potential in filler usage, based on experience with midfacial soft-tissue augmentation.

Animal studies show that using a mix of fillers and stem cells improves and stabilises tissue augmentation outcomes. This might be a step forward in tissue regeneration that acne scar patients could benefit from in the future (Wollina & Goldman, 2015).

Human fat injections:

The doctor may harvest fat from the persons own body and re-inject it to enhance facial fullness, fill in deep creases and build up shallow contours. Using fat injections is more involved than other injectable fillers since it requires liposuction to extract the fat before injection. Fat injections are unpredictable in how long they last and vary from patient to patient and location of injection.

Facial aging is characterized by changes in skin quality, deflation of soft tissue volume, and descending facial muscles and fascia. The result is a change from a young, heart-shaped face to a rectangular or pear-shaped face (Donofrio and Weinkle, 2006). A major part of this volumetric change is due to the atrophy of facial fat deposits. Furthermore, the descent of soft tissues may be perceived as a forward drift from the malar region to the nasolabial region and jowl (Little,2000).

This movement of soft tissue is accompanied by the re-shaping of the underlying bone. These changes are reflected in the characteristics commonly associated with aging, including dynamic and static faciallines, temporal and cheek concavities, and facial musculature demarcation and protrusion.

Facial fillers can help restore a youthful appearance by enhancing soft tissue deflation and gravitational effects. The evolutionary paradigm for face rejuvenation by injection has been combination therapy. Chemodenervation with botulinumtoxin typeA (Botox and Dysport) helps shape the face through motion control, dermal.

Fillers soften wrinkles, and deep fillers regain and restore the volume of the face (Fagien, 2003; Coleman and Carruthers, 2006; Olson, 2007). A three-dimensional perspective of facial aging that focuses on volume loss has recently become the prevalent view point. (Donathetal., 2007). Even in the secular press, public perception today supports a concept of beauty that focuses on rejuvenating the multi-faceted face to restore anatural appearance of youth (VanMeter,2008).

In human Fat injections, Fat is harvested from the persons own body and then re-injected to enhance facial fullness, build up shallow contours and fill in deep creases. Human fat has adipose-derived mesenchymal stem cells, and treating the dermis with fat and stromal vascular fraction or expanded mesenchymal stem cells changes the collagen and elastic fibre pattern as well as stem cells also regenerate there. These methods appear to have a lot of promise for facial anti-aging surgery (Charles-de-Sá et al., 2015).

Mechanism of action:

Dermal fillers are injectable materials that restore skin volume either by collagen formation or by occupying physical space. Fillers actually correct various physiological changes that are produced in the skin as a result of scarring, aging, trauma, and sun damage. These physiological changes include reduced number of elastic fibers, fibroblasts, and collagen in the dermis and reduced number of fibroblasts and keratinocytes in the epidermis. The ideal fillers are predictable, biocompatible, and adjustable to the anatomy of skin to improve scars, wrinkles or atrophy. Facial fillers include: Hyaluronic acid (Restylane, Juvederm), Calcium hydroxylapatite (Radiesse), Fat grafting, Permanent soft tissue filler (Bellafill) and Poly-L-lactic acid (Sculptra) (Ahn and Rao, 2014).

Dermal fillers contain stabilized, non-animal hyaluronic acid. Once the viscoelastic characteristics of skin are lost, formation of wrinkles started. Dermal fillers boosts hyaluronic acid supply to the skin, add volume to the skin and revitalize the skin. As a result, wrinkles begin to disappear and skin begins to look younger. Furthermore, hyaluronic acid fillers also increase the production of collagen (Gilbert et al., 2012).

Within one week of injecting Perlane or Restylane into the skin, an acute inflammatory response started and around the dermal filler implant, a capsule is formed by single layer of fibroblasts then polymorphonuclear leukocytes came and attach to the implant. After one month of the implant, increased collagen production and deposition takes place around the implant. Between one to four months, foreign body reactions are minimized and macrophages started implant degradation. After four months, fibroblasts encapsulate the implant among dense collagen fibers and remain same over time. Hence it makes the skin youthful (Liu et al., 2019).

A more extensive procedure is required for human fat injections as compared to other soft tissues fillers. Firstly, a donor area is determined and then fat is extracted by liposuction. This fat is then used as filler and implanted into the skin of face. This implanted fat then required forming blood supply for its survival. Only fifty percent of the implanted fat survives but the survived fat lasts forever (Vedamurthy, 2008).

Dermal fillers effect on stem cell regeneration:

Dermal fillers did not put any prominent effect directly on stem cells regeneration in the body. But autologous stem cells can be used as the part of dermal fillers which will improve the results of this aesthetic procedure as described in a study in which dermal fillers are formulated with the combination of stem cells and hyaluronic acid and their effect was observed in rat models (Wollina, 2015).

Microneedling

Microneedling is a dermaroller procedure that uses small needles to prick the skin. Simply put, it is the insertion of very fine short, sterilized needles into the skin for rejuvenation. When these pinpricks penetrate the skin, the body naturally heals them, resulting in a plumped, more youthful appearance. The purpose of treatment is to generate new collagen and skin tissue for smoother, firmer, and more toned skin. Microneedling is mostly used on the face and may treat various scars, wrinkles, and large pores. A dermaroller is the most popular (and cost-effective) microneedling device, that contain micro-fine needles having the diameter of 0.2 to 3.00 millimeters.

The tiny injuries from a microneedling session boost collagen and elastin production to straighten lines and wrinkles. Collagen and elastin are compounds that add structure and strength to the skin, lending a thicker youthful skin. The wound-healing mechanisms also stimulate the body to produce new skin cells, making fine lines, crow's feet, and forehead wrinkles less apparent. Since microneedling stimulates collagen and elastin production, it is also super effective in addressing acne and other scars on the skin. The only type of scar not possible to treat is keloid, or raised, scars. In addition the buildup of collagen and elastin that occurs in response to these tiny wounds can improve skin structure and resolve sagginess. We can add the numerous benefits of microneedling process has the stimulation of dormant hair follicles; this stimulation implies growth. In a recent study by Dhurat et al. (2013), 100 test subjects were divided into two groups. One set was treated with minoxidil lotion, and the other received minoxidil lotion plus microneedling. After 12 weeks, 82 percent of the microneedling group reported a 50 percent improvement versus 4.5 percent of the minoxidil lotion-only group.

Mechanism of action:

In micro needling, needles of specific length are used for creating a controlled skin injury without damaging the epidermis. This controlled skin injury results in superficial bleeding which starts the healing cascade and causes the release of growth factors like transforming growth factor alpha and beta (TGF-α and TGF-β), platelet derived growth factor (PGF), connective tissue growth factor, connective tissue activating protein, and fibroblast growth factor (FGF), and heal up these micro wounds by synthesizing new elastin and collagen in papillary epidermis. Micro needling causes the endothelial cells migration, epithelial cells migration and release of growth factors. Micro needling results in activation of fibroblasts and the fibroblast's fixation, proliferation and migration to intercellular matrix starts neovascularization and neocolagenesis. This allows type III collagen deposition in matrix which is finally replaced by type I collagen. Old hardened scars also breakdown by micro needling which starts to revascularize. Hence, in real sense, micro needling do not produce wound but it makes the body fooled to believe that injury has

occurred. Micro needling also induced the expression of matrix metalloproteinases which causes reduction of hyperpigmentation of the skin. Furthermore, in acne patients, micro needling downregulate the high proliferation rate of keratinocytes (Alster and Graham, 2018).

Micro needling mechanism has three phases:

1) Inflammation
2) Proliferation
3) Remodeling

In the first inflammation phase which started just after the micro needling, neutriphils and platelets release takes place at the damaged collagen bundles and superficial blood vessels. In the second proliferative phase, monocytes replace the neutriphils and then these monocytes are transformed into macrophages. In this phase, reestablishment of basement membrane occur by keratinocytes and also formation of fibronectin matrix takes place which act as scaffold for the deposition of collagen. In the third remodeling phase, the neoformation of collagen I, collagen III, and collagen IV takes place. Unlike collagen fibers seen in scars which are irregularly spaced, these collagen fibers formed in third phase of microneedling are evenly spaced which removes the signs of scar from skin surface (Zduńska et al., 2018).

Microneedling effect on Stem cell regeneration:

Microneedling induces the growth factors activation by stimulating the stem cells. Stem cells possess growth factors which help in damaged tissue repairing, wound healing, starts skin growth, and aged skin regeneration. Two main components are present in stem cells. First one is growth factors which aided in cell division, cells growth, and elastin and collagen production while second component is proteins which regulate the division of stem cells. Micro needling activates immune system and stem cells to start the natural wound healing process (Hou et al., 2017).

Radiofrequency/ Thermage

Radiofrequency skin tightening procedures are non-invasive treatments with low risk, no scarring and no downtime. All radiofrequency devices work by delivering heat in the form of energy to the skin and underlying structures and creates mechanical and biochemical effects that lead to both immediate collagen contraction and delayed remodeling and neo-collagenesis due to the subsequent wound healing response.

Mechanism of action:

Nonablative radiofrequency is a technique that uses a rise in tissue temperature to treat skin issues. The purpose is to cause heat injury to the target area, which will encourage new collagen creation in the skin's

deep layers and subcutaneous tissue. This is a treatment method that uses a high frequency alternating current (0.3 to 10 MHz) to cause a specific and controlled rise in tissue temperature. The rate of temperature rise and the depth of heating are determined by the amount of energy utilized and the resistance of biological tissues. The ultimate objective is to cause heat injury in order to trigger changes in collagen conformation and the production of new collagen in the deep layers of skin and subcutaneous tissue (Ruiz-Esparza and Gomez, 2003a).

RF is one of the most often utilized non-invasive techniques for addressing skin wrinkles and sagging. RF for the treatment of skin sagging and wrinkles is based on the application of a heat source to cause collagen denaturation (which happens at temperatures ranging from 50^{o} C to 75^{o} C in the dermis) and subsequent connective tissue contraction. These mechanisms result in a tissue healing response and long-term dermal remodelling. The amount of collagen generated depends on the heating degree of the connective tissue, according to Ruiz-Esparza et al (2003). Collagen protein is made up of three polypeptide chains that form a triple helix shape. Thermal contraction of collagen begins with denaturing the triple helix, which breaks intramolecular cross-links and causes collagen to change from a highly ordered crystalline structure to a gel-like state (denaturation). The contraction of collagen is caused by the cumulative impact of the "unwinding" of the triple helix caused by the breakdown of intermolecular cross-links and the residual tension of such linkages. According to Ruiz-Esparza et al. (2003), the thermal effects of RF can affect the form, length, and diameter of collagen fibres, allowing for collagen rearrangement (Ruiz-Esparza and Gomez, 2003b).

RF Thermage is a revolutionary noninvasive skin tightening technique that uses volumetric heating of the whole dermis while protecting the epidermis from blistering with a cooled contact probe. In theory, this method results in controlled uniform heating at far larger depths than achieved with lasers and light sources. This deeper volumetric heating is hypothesised to promote early collagen contraction, which is then followed by secondary collagen production and repair. This therapy is delivered to skin tissue in three stages: (1) The cryogen coolant is released within the treatment tip to precool the surface of the skin during the precooling step; the coolant doesn't really make skin contact. (2) RF energy is given to the skin during the heating step, resulting in resistive heating; concurrent epidermal cooling occurs. (3) Finally, cooling continues even after the RF energy is turned off during the postcooling step (Fitzpatrick et al., 2003).

RF is colorblind; all afflicted tissues are heated. In brief, the combination of volumetric heating and contact cooling results in a reverse thermal gradient, with the deeper dermis having a greater tissue temperature (60^{o}C) than the epidermis and top dermis (40^{o}C). The triple helix uncoils as a result of heat-induced collagen contraction. The molecule collapses, increasing the fibril diameter and causing longitudinal contraction and tightness. Immediate collagen contraction and, subsequently, secondary collagen formation can be seen (Goldberg, 2004).

Radiofrequency/ Thermage effect on stem cells regeneration:

RF procedure is non ablative radiofrequency procedure which takes the target tissue into healing mood by non invasive delivery of radiofrequency to the deep skin. Radiofrequency pulse is given to the skin cause resistive heating with simultaneous epidermal cooling. Heat causes collagen contraction, with uncoiling of the triple helix resulting from hydrogen bond denaturation. This damage produced due to high temperature starts healing process by activating stem cells proliferation and fibroblast activation which produce new collagen in that area (Araújo et al., 2015).

Fractional Radiofrequency

Fractional Radiofrequency is new non-surgical aesthetic procedure that combined the use of microneedling and radio frequency togethher. This procedure lifts up the skin, tightens the skin, removes acne scars, reduces wrinkles and shrinks pores. The fractional radio frequency technology causes microneedles penetration into the target skin area without damaging the skin's surface (epidermis) extensively.

Mechanism of action:

Microneedles can penetrate to the depth of .5mm to 4.0mm. After reaching to the specific depth in the target skin area, these microneedles release laser energy which causes damage to the old skin cells. Tiny columns are generated in the skin due to the penetration of microneedles which stimulates production of new collagen and skin cells. Production of new collagen reduces stretch marks, wrinkles, scars, fine lines in the skin and tightens the skin. Hence it improves the appearance and texture of skin (Weiner, 2019).

There is no risk of burning or overheating because precisely controlled radio frequency is delivered into the skin. In microneedling radio frequency aesthetic procedure, underlying support system of skin is targeted and as a result, area damaged by stretch marks, acne scaring and aging is restructured. When the support system of skin is restructured, the skin became strong and smooth (Kim et al., 2014).

Radio frequency microneedling also causes tightening of the skin by contraction of collagen fibers. This contraction of collagen fibers occur due to breakage of intramolecular hydrogen bonds. Wound healing also promotes collagen tightening. Radio frequency also stimulates fibroblasts which reorganizes collagen along with the production of new elastin and collagen. Dermal layer of skin also become thick (Nilforoushzadeh et al., 2020).

Radiofrequency microneedling can be used along with other aesthetic procedures like prior to radiofrequency microneedling procedure, Botox may be administered. Similarly after radiofrequency microneedling procedure, fillers can be applied to reduce wrinkles and to provide volume to the face (Wootten and Rheins, 2022).

Fractional Radiofrequency Effect on stem cell regeneration:

Radiofrequency microneedling procedure delivers radiofrequency energy by puncturing the skin using ultrafine needles. This radiofrequency microneedling damage the skin resulting in the activation of fibroblasts and skin stem cells which enhances the collagen production. As a result, skin looks young and smooth. Radiofrequency microneedling is an effective and safe aesthetic procedure and better outcomes are expected when stem cell conditioned medium is used with radiofrequency microneedling (Seo et al., 2013).

High Intensity Focused Ultrasound (HIFU)

HIFU used as a one off (well every 1-2 years) treatment to firm and lift face and neck skin. It uses focused ultrasound energy to encourage stimulate natural collagen production, which results in firmer skin and will provide noticeable without making any cuts to the outer layer of the skin.

A high intensity focused ultrasound (HIFU) procedure is a non-invasive treatment for facial aging. This procedure is part of a growing trend for anti-aging treatments that provide some of the benefits of a facelift without the need for surgery (Danielle, 2020). According to the American Society for Aesthetic Plastic Surgery, nonsurgical procedures increased in popularity by 4.2% in 2017. These less invasive treatments have a shorter recovery period than surgical options, but the results they provide are not as dramatic and do not last as long. Due to this, dermatologists recommend HIFU only for mild-to-moderate or early signs of aging.

A HIFU facial uses focused ultrasound energy to create a deep and rapid heat with pin ball injoury in different layers of the skin. The cells are heated up to a particular temperature, where they are forced to cause cellular damage. This heat damages targeted skin cells, causing the body to try to repair them. As a result of cellular damage, there will be an enhanced production of collagen. Collagen is a protein that is essential for maintaining skin structure and elasticity.

With the enhanced production of collagen, the skin will become firmer and tighter. The number of wrinkles will also reduce. The ultrasound energy is usually focused on a particular site of the face to target the tissues. In this procedure, the patient will not experience any additional damage to the upper layer of the skin. According to the American Board of Cosmetic Surgery, nonsurgical ultrasound treatments such as HIFU can contract the skin on the neck, reduce the appearance of jowls, lift drooping eyelids or eyebrows, smoothen wrinkles on the face, and smoothen and tighten chest skin. HIFU is also meant for developing the elasticity of the facial skin after a few weeks or months of treatment.

Fatemi (2009) undertook a clinical studies to show exactly what occurs beneath the skin. They did a series of studies on gross pathology and histology; a total of 282 patients underwent a single HIFU treatment, which included areas of the anterior abdomen and flanks. The results of HIFU body sculpting achieved by the patients were summarized. Some examples of these results below:

Case 1 was a 27-year-old woman who received a single treatment to her abdomen and flanks. The HIFU parameters used were 2 passes of 74 and 52 J/cm2 at a focal depth of 1.3 cm. This patient experienced a 4.5-cm reduction in waist circumference after 8 weeks and 5.5 cm after 12 weeks.

Case 2 was a 32-year-old woman who received a single treatment to her outer thighs. The HIFU parameters used were 2 passes of 74 J/cm2 at a focal depth of 1.1 and 1.3 cm. This patient experienced a 3.0-cm reduction in circumference after 12 weeks.

Case 3 was a 38-year-old woman who received a single treatment to her outer and inner thighs. The HIFU parameters used were 2 passes of 74 J/cm2 at a focal depth of 1.3 and 1.6 cm, and a third pass of 52 J/cm2 at a focal depth of 1.3 cm on the areas where more reduction was desired. This patient experienced a 5.4-cm reduction in circumference after 12 weeks.

In HIFU procedure, the patient will not experience any additional damage to the upper layer of the skin. To get better results through HIFU, this procedure is linked with stem cells transplantation or activation of stem cells in the skin. When the targeted skin cells are heated up by HIFU, cellular damage is done. After this cellular damage, Stem cells already present in the skin become activated and starts regenerating and proliferating which repaired the damaged skin by making a new younger looking skin.

Mechanism of action:

The High Intensity Focused Ultrasound (HIFU) uses a focused ultrasonic energy that produces pin ball injury at the end of the focused point as well as heat. This combination of ultrasonic energy and heat delivers energy directly to the deep muscles and structural tissues of the skin. HIFU degrades subcutaneous adipose tissue and also cause rapid cell necrosis by increasing temperature of target tissue through induction of molecular vibrations. These degrading adipose cells release chemotactic factors. These chemotactic factors induce inflammations and attract macrophages that phagocytize cellular debris and also remove the extracellular lipids. This ultimately causes decrease in the subcutaneous fat layer without harming the nearby tissues (Ko et al., 2017). A high energy ultrasound transducer is used in HIFU. This high energy ultrasound transducer produces heat at a specific point inside the body by focusing sound waves without damaging the epidermis (skin's upper layer) and surrounding tissues. This specific sound wave focused tissues become very hot (150-200°F) in only 20 seconds. Once these specific sound wave focused tissues reach this high temperature, cellular damage takes place. This cellular damage results in

denaturation of collagen which increase the production of new collagen (it is a protein which provides structure to the skin). Increased collagen results in firmer and tighter skin with very few wrinkles. On the other hand HIFU can hit the SMAS layer under the skin which contract that layer resulting in immediate lift (Choi et al., 2016).

In HIFU, cellular damage is done by heating up the targeted skin tissue thrugh a small expotion when the focued ultrasout hits at the end targeted layer in the skin to a particular temperature and then allowing body to repair that damage by enhanced production of collagen after the denaturation of that collagen through heat, which causes the skin to become firmer and tighter. The number of wrinkles will also reduce.

HIFU and Stem cell regeneration

The High Intensity Focused Ultrasound (HIFU) causes cellular damage, breakdown adipocytes and produce micro lesions in the collagen. This takes the target tissue into healing mood and this healing process is speed up by fibroblast and stem cells activation to regenerate collagen. Stem cells direct collagen to the area of inflammation where collagen is required. Stem cells are like the manager at the site of construction guiding the workers about the place where they have to build. The secretions of stem cells activate the immune system to remove scarring which facilitates the natural rebuilding of collagen. Ultrasound when applied on stem cells enhances their ability of regenerative molecules secretion. And when applied on target tissue, it make that target tissue more favourable for stem cells homing. HIFU produces local gradient of adhesion molecules, growth factors and cytokines which helps in the regeneration of stem cells and stem cells homing (Yang et al., 2019).

Intense pulsed light (IPL)

IPL is a noninvasive skin rejuvenation procedure that uses high-powered pulses of light to improve skin damage and reduce the signs of aging as well as reducing the appearance of scars and treating/ lightening the darker patches on the skin.

IPL is an Aesthetic cosmetic skin treatment that works in a similar way to laser therapy, but it is not laser, it is a broad wavelength light that is pulsed onto the skin that produce heat which rejuvenates the skin

Mechanism of action:

IPL, also known as pulsed light and wide band light, is a non-laser light source used to treat active acne, photo damage, vascular and pigmented lesions, and undesired hair. Intense pulsed light (IPL) therapy is a non-surgical technique to enhance the texture and color of your skin. It can reverse some of the obvious solar damage known as photoaging. Bandpass filters and flashlamps are used in intense pulsed light

systems to generate polychromatic incoherent high-intensity pulsed light with a defined wavelength range, pulse duration, and intensity. Photorejuvenation is the therapeutic technology of noninvasive skin rejuvenation using IPL, and it has been widely utilized in cosmetic dermatology to address face photoaging. When your skin is heated, your body eliminates dead skin cells. Unlike lasers, an IPL device emits many wavelengths of pulsing light. It can cure many skin diseases at once. Because your skin tone is more even after IPL, you may seem younger. And because the light does not harm other tissues, you can recover rapidly (Babilas et al., 2010).

IPL (intense pulsed light) is a form of light treatment used to cure spots, wrinkles, and unwanted hair. It reduces or eliminates birthmarks, age spots, freckles, sun damage, damaged blood vessels on the face, varicose veins, rosacea, and hair on the face, or neck. IPL, like a photo flash, emits light of several distinct wavelengths. IPL light is more dispersed and less concentrated. IPL penetrates to dermis without affecting epidermis, causing less skin damage than laser. Skin pigment cells absorb light energy, which is then turned into heat. To remove spots and freckles, the heat eliminates the undesirable pigment. Alternatively, it kills the hair follicle to inhibit new hair growth. It uses light's skin-restoring capability to brighten the skin and to promote collagen production. This induces an increase in fibroblast activity, as well as hyperplasia and re-arrangement of elastin and collagen (Erol et al., 2008).

Intense pulsed light (IPL) treatment has been extensively acknowledged and proven to be successful in treating photodamage of the skin all over the world. This broadband light source's pulsed light operates on photodamaged skin's numerous target tissues using the principle of selective photothermolysis, which allows the light to target particular chromophores inside the skin while leaving structures that are not within the target chromophore alone. Through deeply penetrating wavelengths in the 800-1200 nm range, IPL treatments also stimulate fibroblast viability and the expression of collagenase, elastin, and procollagen I/III possibly through a combination of direct photothermal effects on the dermal matrix and chromophore-triggered cytokine and growth factor pathways (Raulin et al., 2003).

IPL Effect on stem cells regeneration

Intense-pulsed light (IPL) induces a healing state in the target tissue by delivering intense, broad-spectrum light to the deep skin in a non-invasive manner. IPL treatments are more broadly successful since they can treat a larger area of the skin. The strong, broad-spectrum light penetrates deep into the skin and delivers a substantial quantity of energy to the target region, contracting blood vessels and collagen fibres. Thus, damaged and photoaged skin is removed. The removal of damaged skin puts the target area in a healing state, which encourages stem cell regeneration and the formation of fibroblast cells. It also increases fibroblast viability and collagenase, elastin, and procollagen expression, perhaps by a combination of

growth factor pathways, chromophore-triggered cytokine, and direct photothermal effects on the dermal matrix (Gade et al., 2022, Wu et al., 2022).

Light Emitting Diode (LED light):

LED light therapy is a natural and completely non-invasive aesthetic treatment for all ages, skin types, and skin tones. It's fantastic for all-round skin health, delivering energy into the skin's cells to boost collagen, elastin and hyaluronic acid.

It hydrates and plumps the skin leaving your clients with a lovely glow and even skin tone, and is fantastic for those with more problematic or sensitive skin, correcting irregularities, such as eczema, psoriasis and acne.

Highly respected and much-admired by doctors, dermatologists and therapists, LED light therapy offers wide-ranging, scientifically-proven benefits.

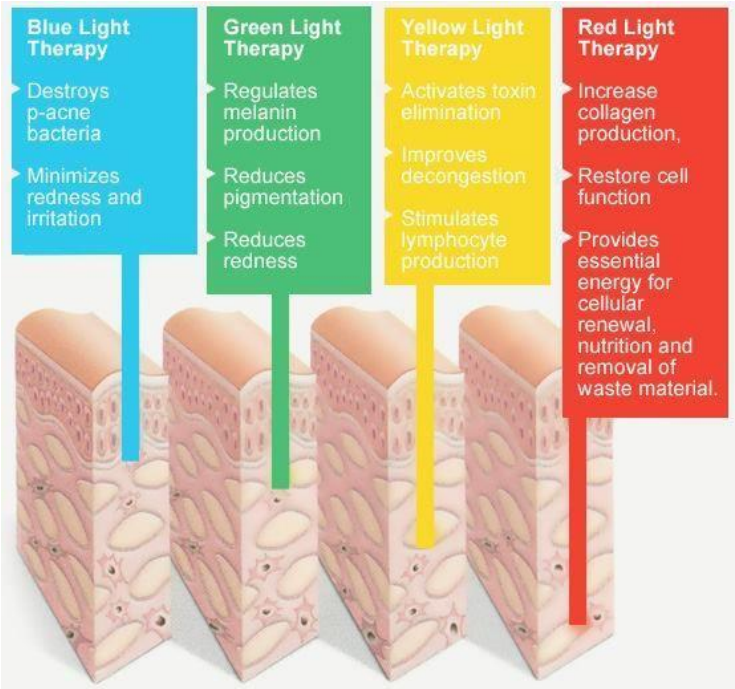


Figure 28: LED Light wavelength and depth in the skin

Low wattage light is directed through the skin's epidermis and aimed at fibroblast cells, which produce collagen and elastin. The light stimulates the cell's own energy transport system, therefore helping to stimulate and renew the skin.

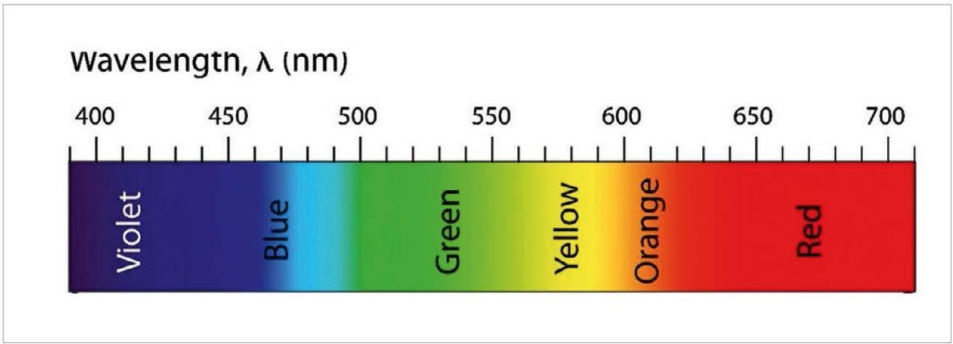


Figure 29: LED Light wavelength

RED: Anti-ageing, stimulates collagen, reduces fine lines, scar reduction and, rejuvenates skin.
YELLOW: Tightens and tones, detoxifies and stimulates the sensory motor nervous system.
BLUE: Treats acne, antibacterial, regulates oil glands
VIOLET: Treats psoriasis, dermatitis and Vitiligo
GREEN: Calms red skin, broken red capillaries, anti-inflammatory, Rosacea, traumatized skin (sunburn), aid in fading pigmentation and uneven skin color

Light doses ranging in colour have been proven to be most effective for skin rejuvenation treatments that relate to rehabilitation of the microcirculation and encouragement of fibroblast activity.

LED light therapy puts the skin into a state of repair, which leads to an increase in the production of collagen, elastin and hyaluronic acid. Hydration is boosted, improving plumpness, reducing fine lines and wrinkles, and brightening the complexion, allowing the skin to look fresher. The visible effects of ageing are lessened by the simple fact that the treatment boosts the levels of collagen, elastin and hyaluronic acid, which naturally diminish with age.

LED therapy also maintains youthful complexions and supports and repairs ageing skin, speeding up blood circulation that nourishes the treatment area, and further boosting regeneration. It switches on your immune system, allowing the body to efficiently ingest compromised and damaged cells, reproduce healthy cells and aid cell detoxification, which helps in the prevention and treatment of glycation, (a crepe-like texture to the skin).

Mechanism of action:

LED photo technology activates cellular metabolism and energy, assisting the production of collagen and elastin synthesis by increasing ATP synthesis and metabolising the fibroblast cells

Techniques that pulse the light at specific cycles or codes (on-time and off-time) have reportedly proved most effective and this technique is called LED Photo-modulation. LED Photo-modulation aesthetic procedure is the process in which cell activity is regulated or manipulated by using light source and without using any thermal effect. This process is performed by using portions of the visible light spectrum which is the part present between infra-red and ultra violet region. Hence a non-ablative, non-invasive, and non-thermal procedure of skin rejuvenation is achieved. LED light therapy causes the restoration of microcirculation and enhancement in the fibroblast activity. This aesthetic procedure causes skin rejuvenation by inhibiting or stimulating cell-signalling pathways. Studies show that LED Photo-modulation causes reduction of collagenase activity along with increase in the collagen deposition in the dermis. Hence it improves skin texture. This procedure causes both decrease in collagen breakdown and increase in the collagen production (Kim and Calderhead, 2011).

This aesthetic procedure metabolizes the fibroblast cells and causes increase in collagen and elastic production by enhancing the production of ATPs. This procedure also supports the synthesis of new protein in the skin cells by using cytochromes of skin cells. Red light therapy causes skin rejuvenation and scar reduction, reduction of fine lines, collagen stimulation and anti-aging. Yellow light therapy causes skin tightening and stimulation of sensory motor nervous system. Blue light therapy causes acne treatment, antibacterial effect and regulation of oil glands. Violet light therapy is used for the treatment of vitiligo, dermatitis and psoriasis while green light therapy treats uneven color of skin, fades pigmentation, treats sunburn, anti-inflammatory and calms red skin (Lee et al., 2007).

It is shown in various studies that the growth of skin cells is increased by 150 to 200 percent by LED light therapy. When a light reaches deep into the cell, a change is produced inside the cell which causes strengthening of blood capillaries of skin, improvement in the detoxification and oxygenation of skin. Red light therapy can be used as post treatment for microdermabrasion. LED light therapy helps to increase the process of skin healing and to reduce the inflammation and redness (Avci et al., 2013).

In short, LED light therapy repairs the skin by increasing hyaluronic acid, elastin and collagen production. It also brightens the skin, reduces wrinkles and fine lines, makes skin fresh looking, improves pulpiness and boosts hydration. With the passage of time, the level of elastin, collagen and hyaluronic acid is decreased naturally which is the main factor of aging. LED therapy boosts the level of elastin, collagen and hyaluronic acid and hence delays the aging process (Calderhead et al., 2008).

LED Light Effect on stem cell regeneration:

LED therapy is an aesthetic procedure in which skin problems are treated by light without using thermal effect. LED light therapy triggers the stem cells present in the body. As shown in various studies, light causes tissue regeneration by triggering the stem cells. Red light promotes stem cells migrations. Similarly, red and green light stimulates the wound healing and growth of fibroblasts (Lane et al., 2014).

Microdermabrasion

Microdermabrasion is a procedure where diamond tipped heads or fine Alum Oxide crystals, usually driven by airflow are used to abrade the skin. It offers a precise, non-invasive means of skin exfoliation that is progressive rather than aggressive and, it is also a very simple and speedy procedure that produces immediate results.

This painless abrasion gently removes dead skin cells, which, at the same time are gently vacuumed away into the crystal cylinders or filters thus eliminating cross contamination. Simultaneously, the procedure stimulates cell renewal and the synthesis of Collagen, Elastin and Fibroblast, normally resulting in an immediate, enhanced skin appearance.

Types of Microdermabrasion

Crystal Microdermabrasion: This is the original form of microdermabrasion. Crystal microdermabrasion uses sodium chloride or aluminum oxide crystals that works by directing these small stream of micro-particle crystals at the skin using a special device. This device simultaneously streams the crystals out against the skin and vacuums them back up along with the exfoliated skin cells. The friction caused by these tiny crystals on the surface of the skin at high speed that loosens the skin cells so that the suction can pull them away. This also promotes greater blood flow, further enhancing a healthy, glowing skin after treatment. This is an efficient method of removing the top layer of skin and the irritation is minimal. Although it does not hurt, it may feel a little rough and the skin may look slightly reddish and sensitive for a couple days afterward.

Diamond Microdermabrasion: This uses a real diamond-tipped tool to exfoliate the skin. Although this may be expensive, it may be more cost-effective than crystal microdermabrasion. This is because of the hardness of diamond—it is durable and long-lasting, unlike crystals that need to be consistently replaced. Another benefit of diamond microdermabrasion is that there is no risk of particles going where they are not needed. The diamond tip will not leave anything behind on the skin, unlike the crystals that could

potentially splash into the eyes or nose. Diamond microdermabrasion also includes the same vacuum suction as crystal, thus stimulating blood flow deep in the skin.

Hydro-Microdermabrasion: This is one of the newest forms of microdermabrasion. It is a great option for people with sensitive skins who do not want to undertake the risk of irritating their skin or sitting through an uncomfortable procedure. The main difference between the traditional forms of microdermabrasion and hydro-dermabrasion is that a stream of water is used to strip away the top layer of the skin. This is beneficial not only because water is gentler than crystals or diamond, but because water also soothes and hydrates the freshly abraded skin. Furthermore, hydro-dermabrasion can be customized for skin types by combining it with the dermal infusion technique. The of diluted Acid can be incorporate with hydro-dermabrasion to give a deeper peel and a better result which called Dermal Infusion.

Dermal Infusion: This is a double duty treatment that not only exfoliates the skin but infuses it with specialized skin care. This type of facial uses a unique device that combines a diamond-tip, suction, and the depositing of serums or peels. As the device moves over the skin, these three actions work in unison to unclog pores and clear away the stratum corneum so that serum immediately soaks deep into the skin, leaving it plump and hydrated. The diamond tip scratches away tough blackheads and flaky patches, allowing the vacuum to suck up anything that is underneath—dirt, bacteria, and congested pores. With the surface of the skin completely exfoliated, it is perfectly primed to receive skin-beneficial ingredients.

Microdermabrasion usually leaves the skin void of oil and moisture; the individual only has to apply moisturizer to restore some of what has been taken away. However, with dermal infusion, moisturizing serum is put right back into the newly clean pores so it can soak in immediately. Since the exfoliating diamond tip and vacuum clears off the top layer of the skin and unclogged pores, this serum can go deeper than it could during daily skincare routine.

Traditional stem cell treatment involves isolating stem cells from patients, propagating and differentiating them in vitro, and then injecting autologous cells back into the patient. Local stimulation and activation of endogenous stem cells to the site of damage for new skin regeneration is an alternate strategy that may be easier. This might happen as a result of some drugs that encourage stem cell growth and differentiation. Endogenous stem cell activation or autologous stem cell injection can be utilised in conjunction with dermabrasion and microdermabrasion techniques (Gozali & Zhou, 2015).

Mechanism of action:

Microdermabrasion is the peeling process which removes the dull, dead skin cells residing outer skin layer. In the aesthetic procedure of microdermabrasion, diamond head or tiny crystals are used which

breaks up the dull, dead skin cells residing in the outer skin layer to expose the softer and smoother skin underneath.

Microdermabrasion produce superficial trauma to the skin, enhances the amount of collagen and elastic fibres, collagen fibres become more consistently organized, more tightly packed, and thicker, elastic fibres gave more spring to the dermal layer by orienting vertically instead of horizontally (Spencer, 2005).

Microdermabrasion is applied for the rejuvenation of facial skin. Studies explained that gradual improvements in the damaged skin occur by repetitive intra-epidermal injury due to enhanced collagen production and fibroblast proliferation. This leads to the deposition of new collagen in the dermis. Microdermabrasion removes only the outer surface of epidermis and hence speeds up the natural process of resurfacing of skin. Microdermabrasion is a more shallow kind of dermabrasion that eliminates only the epidermis' outer surface, speeding up the natural resurfacing process (Shpall et al., 2004).

Microdermabrasion effect on stem cell regeneration:

During microdermabrasion, abrasive heads or crystals strike produce mechanical abrasion to the skin, which eventually removes the epidermis layer. This mechanical abrasion causes stimulation of the stem cells which results in the activation and release of growth factors. These stem cells released growth factors stimulate fibroblasts and other recovery cells after covering the abraded tissue to form new tissue. Stem cells are capable of making younger looking skin along with making the skin smother and toned (Gozali and Zhou, 2015).

Laser resurfacing

Laser resurfacing continues to be the most effective form of treatment for skin rejuvenation. Although many non-ablative devices have been developed over the years, none of them is able to deliver results equivalent to ablative devices. The effectiveness of ablative lasers is a direct result of their ability to completely vaporise the epidermis, thereby removing unwanted pigment and sun-damaged cells. Deeper penetration and diffusion of thermal energy heats dermal tissues, causes tissue contraction, and stimulates new collagen production. These processes culminate in the elimination of solar elastosis, yields a brighter and more luminous skin tone with a reduction in wrinkles and skin laxity(Borges et al., 2020).

Mechanism of action:

Laser is the abbreviation of Light Amplified by Stimulated Emission of Radiation. Laser resurfacing is an aesthetic procedure in which laser is used for improving skin appearance and for treating small facial

problems. Laser beam causes the destruction of skin's outer layer called epidermis along with heating the underlying skin called dermis. This stimulates the production of collagen for making better skin texture and tone. The laser beams of carbon dioxide reaches the dermis by penetrating epidermis. Due to these laser beams, small areas of thermal damage are produced which enhances the production of new collagen and causes replacement of damaged skin with new epidermal cells. For years, different skin issues like enlarged oil glands on the nose, warts, wrinkles, and scars has been treated by laser resurfacing (Ramsdell, 2012).

Laser resurfacing can be done by two types of lasers. First one is ablative laser which includes an erbium laser, a carbon dioxide (CO2) laser, and combination systems. This type of laser beam causes the destruction of skin's outer layer called epidermis along with heating the underlying skin called dermis which enhances the production of collagen for making better skin tighter and smoother. Second one is non-ablative laser or light source which includes intense pulsed light (IPL) therapy, erbium (Er:YAG) and pulsed-dye laser. This type of laser beam also enhances the production of collagen (Jordan et al., 2000).

Excessive skin cannot be removed by laser resurfacing. Surface imperfection and deep scaring can be improved by using carbon dioxide laser. Micro beams are used to treat severe scar which causes the production of new, healthier collagen after penetrating far under the skin (Borges et al., 2020).

Laser skin resurfacing vaporizes the skin in the form of layers and removes skin very precisely. This aesthetic procedure of laser resurfacing is also called laser vaporization, laser peel or laser-abrasion. Laser generated heat which would denature the collagen as a result collagen fibers contracts rapidly. This collagen shrinkage is behind the mechanism of tightening of skin along with other factors like ablation and intracellular water evaporation. After this, the phase of wound healing started in which collagenases level is increased which causes degradation of the fragmented collagenous matrix. After this process, new collagen and new epidermal cells begin to form and new ski surface is formed which is more youthful (Kaushik and Alexis, 2017).

Laser Resurfacing Effect on stem cell regeneration:

Laser resurfacing improves skin appearance and treats many facial problems, fine lines amd pigmentation by increasing collagen production and forming new epidermal cells. These epidermal cells are formed from the division of already existing cells and by promoting stem cells regeneration. Stem cell proliferation and regeneration is increased when skin is damaged by irradiating the cells with the laser energy. Laser irradiation also increases the secretion of growth factors at the target area.

Studies demonstrated that wound healing is enhanced after laser therapy by activation of fibroblasts. It enhances stem cell ability and increases stem cell proliferation (Xu et al., 2017).

IV Vitamins drips and Vitamins injections

Vitamin infusions therapy allows various types of vitamins and nutrients to be administered to the body intravenously. The most benefit in administering these vitamins and other essential nutrients directly into the blood stream, they bypass the digestive system, where many nutrients losses their vale through the digestive system and the stomach acids, so many of these vitamins and nutrients will not get absorbed well. With the IV infusion and vitamin injections, nutrients and minerals can go to work, instantly as they are injected directly IM into the muscles or intravenously into the blood replenishing and revitalizing your body and organs from the inside out, and its guaranteed that the body will receive the nutrients it needs as well as the skin, to be sure that theses Vitamins and nutrients can reach the skin which needs it to stay young and youthful. This method is especially beneficial for people experiencing digestive complications or irregularities, who may struggle with the normal natural absorption of certain nutrients and vitamins. Even though it's important to have foods that are rich in nutrients, but that does not always mean that those vitamins and minerals are reaching your body and skin to the fullest. This is when Vitamin infusions come into hand and allows larger quantities of vitamins to enter your system safely tolerated without the need to take them orally. The "concentration gradient" allows these vitamins and nutrients to be absorbed rapidly and efficiently and to transfer nutrients directly into your cells, yielding optimal results in helping the cells to get the best amount of nutrient needed to regenerate more healthy youthful stem cells and keep the skin healthy and younger for longer.

Below are some of the vitamins and minerals that can be administrated through IV treatments therapy and/or IM injections that helps with the skin ageing and the regeneration of healthy stem cells.

- **Vitamin C**

  Also known as Ascorbic Acid, Vitamin C is a water-soluble vitamin that plays many essential roles throughout the body, helping to maintain systems such as muscles, bones, immune support, and the circulatory system.

  Vitamin C promotes the biosynthesis of collagen, which is a fundamental part of connective tissues. In this capacity, Vitamin C reduces stretch marks and is an essential component of healing wounds. Vitamin C is an antioxidant that also helps regenerate other antioxidants within the body. This vitamin reduces the damaging effects of free radicals, which are unstable molecules that contribute to tissue damage and aging.

- **Glutamine**

  Glutamine is an important amino acid with many functions in the body. It is a building block of protein and a critical part of the immune system. Glutamine has a special role in intestinal health. One of the most important functions of glutamine is its role in the immune system. It is a critical fuel source for immune cells, including white blood cells and certain intestinal cells. Glutamine's immune system benefits are related to its role in intestinal health. Glutamine is an important energy source for intestinal and immune cells and it also helps maintain the barrier between the inside of your intestines and the rest of your body. This prevents harmful bacteria or toxins from moving from your intestines into the rest of your body.

  Due to the major role of the intestines in the immune system, glutamine may benefit your overall immune health by supporting the intestinal cells.

  Glutamine is also one of the building amino acids of Glutathione - an antioxidant that plays a crucial role in preventing cellular damage by fighting the free radicals that can damage our skin. This antioxidant binds with toxins in the body and facilitates their removal from the body. Glutathione is involved in many metabolic processes and biochemical reactions, as well as DNA repair and synthesis. Studies suggest that it also slows the speed of aging to regenerate healthy stem cells.

- **B-Complex**

  B complex vitamins refer to several different vitamins that are part of the same family. Together, these water-soluble vitamins perform a range of vital functions including cardiovascular support, helping the body convert food into energy, immune function, and more.

  In addition to these, B complex vitamins are most notable for their role in promoting healthy skin, hair, and nails. They reduce free radicals in the body that contribute to aging, resulting in an overall healthier and younger appearance.

  The B Complex Vitamins Are:

  B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin) and B12 (cyanocobalamin)

  Because the B vitamin complex is so versatile and performs so many functions throughout the body, it is featured in many IV drip formulas.

- **Vitamin B7 (Biotin)**

  Vitamin B7, also known as biotin, is part of the B-vitamin complex and is associated with improving the strength, appearance, and overall health of the skin, hair, and nails. To this end, biotin is a common addition to beauty supplements.

Biotin plays a role in converting food into energy by helping to metabolize carbohydrates and fats. B7 also helps maintain the nervous system and promotes healthy psychological function[12].

While rare, biotin deficiency is often marked by hair loss, a decrease in mental cognition, dermatitis, depression, hypotonia (decreased muscle tone), and ataxia (decreased muscle coordination). Biotin cannot be absorbed through the skin. IV therapy can quickly restore normal levels of biotin to prevent unwanted symptoms of deficiency.

- **Glutathione**

Glutathione is a molecule found naturally in your body. Produced by the liver, glutathione is made up of three amino acids: L-cysteine, glycine, and L-glutamate. This antioxidant primarily helps your body fight disease and injury by binding to free radicals (causing oxidative stress) and facilitating their removal from your body.

Glutathione and Free Radicals

Free radicals are atoms with an odd number of electrons and are a byproduct of cellular oxidation. Free radicals can be harmful to the body when they interact with certain types of molecules in your body.

Our bodies can tolerate a certain amount of free radicals; but, when these atoms accumulate faster than we can process them, our bodies start to accumulate damage. Exercise, stress, illness, medication, and exposure to environmental toxins such as heavy metals or pollution can all contribute to increased production of free radicals.

Free radicals can cause DNA damage, impairing cellular function or killing cells altogether. Damaged cells open the doorway to potential disease and illness. That’s where antioxidants come in. Antioxidants are stable molecules that bind to free radicals, stopping potential damage, and assisting in removing free radicals from your body.

Antioxidants occur naturally in many types of food, especially citrus fruits. Certain types of vitamins, such as vitamin E and vitamin C, are antioxidants that are easy to incorporate in your diet to help fight the damaging effect of free radicals.

Glutathione is another type of antioxidant that can easily be added to your wellness routine, helping prevent the damage that contributes to aging and illness.

Glutathione, Aging, and Illness

The accumulation of free radicals over the years can accelerate how quickly your body ages. A greater concentration of free radicals may lead to more cell damage, leading to the appearance of wrinkles, dull skin, and lower energy levels associated with aging. By getting rid of free radicals before they can build

up and cause cellular damage, glutathione can slow the aging process and help you keep a youthful and radiant appearance.

Glutathione for skin

Glutathione holds many anti-aging benefits. Since it is a natural detoxifier, it improves the health of the body's cells to reverse aging. Glutathione protects the skin against oxidative damage which leads to wrinkles - making it an excellent form of anti-aging skincare. It prevents or improves acne, wrinkles, and crows feet via detoxification of the skin and body. It also eliminates and gets rid of age spots, liver spots, brown spots, lentigines, and dark circles under the eyes.

- **NAD (Nicotinamide Adenine Dinucleotide)**

NAD is a coenzyme that is associated with metabolism. This coenzyme maintains regular levels of energy, regulates redox reactions, and maintains the health of mitochondria. NAD activates sirtuins, which are proteins that utilize NAD to improve metabolic efficiency, which in turn influences cell changes that contribute to aging, overall energy levels, and can improve neurodegenerative disorders.

In addition to these effects, NAD promotes anti-aging by lengthening telomeres, which are protective caps located at the ends of chromosomes that naturally get shorter as the body ages. The level of NAD in the body declines with age and reduces the effectiveness of the cellular function.

- **ALPHA LIPOIC ACID**

It is an antioxidant found in every cell of the human body. Antioxidants are compounds that inhibit cellular oxidation, a process that produces free radicals that can damage cells and undermine their function. ALA is sometimes referred to as the universal antioxidant because it supports other antioxidants like vitamins C and E, and glutathione. ALA is involved in multiple antioxidant functions in virtually all body tissues, protecting your body’s organs and systems from damage so they can keep you fit and healthy.

In addition to its role as a powerful antioxidant, ALA is critical to mitochondrial health. Mitochondria are tiny organelles inside human cells that help convert glucose to ATP, the energy molecule, through a series of chemical reactions. Lipoic acid is a highly essential cofactor in the mitochondrial energy production

cycle that enables cellular respiration and ATP production. Mitochondrial dysfunction has been linked to aging and disease in humans, and ALA supports mitochondrial health.

ALA is also fundamental to the breakdown of amino acids, the essential elements of proteins that are vital to new cell production. Your body's cells are continually turning over, replacing old dying cells with new ones. As we age, cellular turnover naturally slows down. Insufficient levels of lipoic acid can reduce your body's ability to replace old cells, which can speed up the aging process. Your body makes a small amount of ALA on its own, and a nutrient-rich diet of vegetables and humanely sourced red meat gives you the building blocks to produce sufficient amounts for optimal health. Nevertheless, some people need to supplement ALA to manage and overcome certain health conditions, through oral supplements or via IV infusion therapy.

Oxidative stress and free radicals are primary contributors to the aging process, damaging skin cells, vital organs and cellular mitochondria. ALA's powerful antioxidant properties fight oxidative stress and protect cells from damage, slowing the aging process. ALA also fights systemic inflammation which is a major contributor to metabolic disorders like heart disease, hypertension and diabetes. From the cellular level, ALA replenish, heal, and revive your body. Hair, skin, and nails can all be improved.

Vitamins injections improves the skin texture and reduces skin scars but to get better results, it is better to inject stem cells at the target site along with the vitamins injection intravenously. Vitamins and nutrients are needed to the skin to stay young but sometimes due to digestive problems, a person did not able to absorb vitamins and nutrients from digestive tract nd hence they did not reach to skin as a result skin become dull and a stressed environment is produced in the skin which is not favourable for stem cells regeneration. So, when vitamins and nutrients are injected intravenously or intramuscularly, they directly reach to the skin and make the skin younger as well as these vitamins and nutrients increase the stem cells regenerative ability in the stressed environment. Hence stem cells also start to regenerate which also helps in making the skin to look younger.

Mechanism of action:

IV Vitamins drips and Vitamins injections is a process in which essential vitamins, nutrients and minerals are administrated directly into the bloodstream through veins. A fraction of vitamins, nutrients and minerals are absorbed in the body through intestines and stomach when they are taken orally. So, the goal of IV Vitamins drips and Vitamins injections is to increase the concentration of absorbed vitamins, nutrients and minerals in the body by skipping stomach and intestine hence benefiting the body by making them readily available for the body. This procedure gives the skin a youthful appearance (Rimmer, 2019).

The combination of vitamins, essential fluids, electrolytes, and antioxidants is present in each drip. But most commonly, vitamin drips contain vitamin C, collagen, and/or glutathione. These components are very effective for prevention of skin aging as well as for skin improvement. Glutathione is composed of three amino acids and it is naturally present in the body. Glutathione helps body in the detoxification of substances. It also helps to decline melanin production in the skin. As a result, the whole body skin become light and bright. The collagen component commonly present in the drip is responsible for boosted radiance, improved skin firmness, and plumping, whereas vitamin C component makes skin more bright and youthful by supporting the production of new collagen and by acting as revitalizing agent (RIORDAN).

IV Vitamins drips and Vitamins injections effect on stem cells regeneration:

No any direct effect of IV Vitamins drips and Vitamins injections on stem cells regeneration is studied but in some intro studies it is seen that vitamin C induces telomerase activity in stem cells which leads to the increased production of cell matrix and higher expression of fibronectin, integrin and type I collagen. Vitamin D enhances the regeneration of muscle stem cells (Domingues-Faria et al., 2016).

Threads lift

Thread lift aesthetic procedure, threads are inserted into the skin. As a result, healing process of the body started for the purpose of healing the area of sutures and to remove the sutures. When the body senses any foreign object within it, body reacts against that object. Since the threads used for the purpose of thread lift are very small, person feels nothing of this happening. Even sometime patient will not feel any sutures after the healing process, because the skin has healed around them (Abraham et al., 2009).

Three types of thread are currently available;

- Polydioxanone (PDO)
- Poly L lactic acid (PLLA)
- Polycaprolactone (PCA)

PDO threads are the longest threads which are made up of synthetic biodegradable polymer. After six months of thread lift treatment, PDO threads are dissolved into the body due to hydrolysis. PDO thread lift stimulates the fibroblasts for the production of collagen at the targeted area.

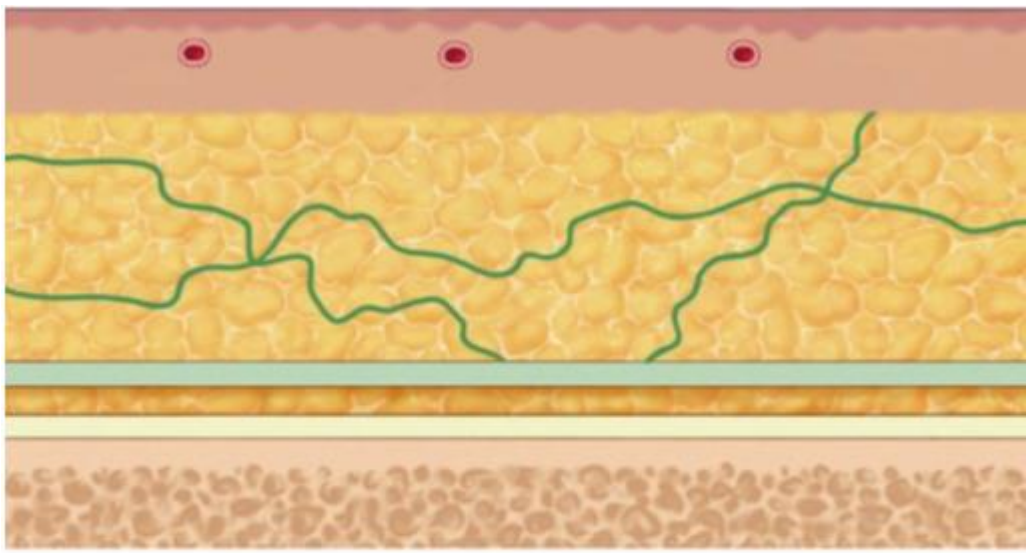
PDO threads used in this procedure are of three types;

- Mono threads
- Cog threads
- Screw threads

Mono threads provides small amount of lift and tightens the skin. They are smooth without barbs and are used on the face or the scalp. Cog threads have barbs or Cogs which lift and support the sagging skin by hooking onto the skin. Screw threads have interwinded one or two threads which helps in the restoration of volume at the target skin area.

PLLA threads are developed after PDO threads. PLLA threads are made up of lactic acid derived biocompatible polymer. PLLA threads produce collagen over a longer time as compared to PDO threads and are resorbable. PLLA threads restore facial shape by increasing saggy skin area volume along with providing a lift.

PCA threads are the newest type of threads which are bio-absorbable and are formed of caprolactone. These threads produce collagen over a longer time as compared to PLLA and PDO threads. They stimulate collagen production to support the skin and preventing the skin from sagging. As these threads stimulate fibroblasts so the skin lifting process will remain continue even after the resorbance of threads. When these threads are broken down in the skin then molecules having small molecular weight are produced which enhances the production of hyaluronic acid and collagen by the skin. hence the skin formed as a result is more vitalized, firm and moisturized (Wong et al., 2017).



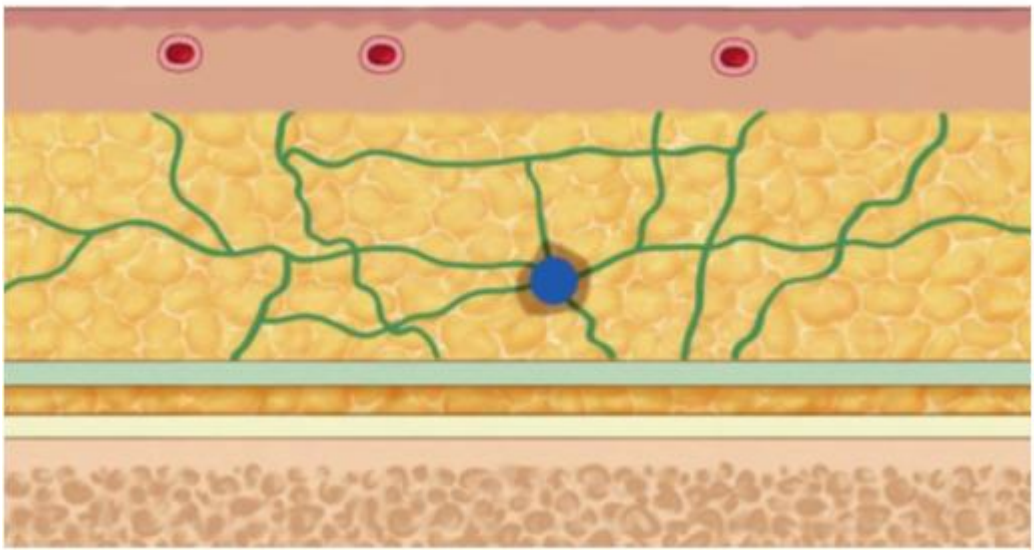


Figure 30: Change after thread insertion (five issue changes mentioned above)

Mechanism of action:

Thread lift is an aesthetic procedure in which temporary sutures are used to lift the skin. Thread lift stitches up the loose skin instead of surgically removal of loose facial skin. This causes slightly pulling the skin back and hence causes tightening and lifting of the face. In addition to skin lifting, threads also treat aging by stimulating the healing process of the body and enhancing the large production of collagen at the target site. This collagen production is important because collagen plays very vital role in aging process (Halepas et al., 2020).

Collagen supports growth factors which help in the rejuvenation of the skin. In addition to its role in wound healing, collagen makes skin vitalized, voluminous and strong. With the passage of age, the production of collagen in the body decreases which as a result reduces skin thickness. This reduced collagen production also causes decrease in the skin's strength as a result creation of excess skin and wrinkles takes place. As the skin become weaker and weaker with low level of collagen support beneath it, gravity pulls the skin downward and with the reduction of elastin the skin stretches down towards gravity(Savoia et al., 2014).

Thread lift Effect on stem cell regeneration:

Thread lift is an aesthetic procedure in which skin liftening is carried out by temporary sutures. In addition to skin lifting, threads also treat aging by stimulating the healing process of the body and enhancing the large production of collagen at the target site. The thread lift process enhances the production of fibroblasts and also support the growth and regeneration of stem cells present in the skin which revitalizes and smoothen the skin (Bertossi et al., 2019).

In general, Threads are known to cause changes to the dermis. As discovered in several studies, it has effects of improving pores or fine wrinkles. Especially, it is known to increase thickness of the papillary dermis, which is done through the method of fostering collagen formation in the dermal matrix.
There is an excellent study first published (29 July 2018) which can explain the changes to the skin and the subcutaneous layer after submitting the threads. The changes after inserting the Threads can be summarized as follows:
Many PMN cells, including eosinophile, are gathered making granulation tissues around the thread which is inserted. In the granulation tissues, newly made collagenous connective tissues are abundantly observed. The newly made collagenous connective tissues converge into the pre-existing fibrous connective tissues nearby (merging effect). Through merging effect, an inflammatory reaction is in progress to the surrounding area where the thread is inserted, and mechanotransduction cell signal delivery happens when granulation tissues are formed) starts and spreads as waves to surrounding tissues. Inside the granulation tissues which are newly made near the inserted thread, fibroblasts and myofibroblasts show. Myofibroblast is mostly clearly related to a wound contracture in the wound healing process, and it is a cell which serves a key role of causing elasticity in the area of the procedure and tight skin after the skin regeneration procedure. The cross-sectional area size of capillaries is larger in experimental group where the threads was inserted than control group. Also, many eosinophils were observed which shows effects for inducing fibrosis in the wound healing process. There was fat cell denaturation by granulation tissue only in the area where the thread was inserted, and there was no change to fat cells away from granulation tissues (Yoon et all 2018).

Sculptra

Sculptra is a collagen stimulator that aids in the healing of the skin's underlying structure. It is the same ingredient that is used for the PLLA threads but is used in a liquid form. I was not very sure where in this thesis I should explain this powerful product but thought after the threads it will be understood more.

Poly-L-lactic acid (PLLA):

It is a synthetic, biocompatible, biodegradable polymer. For its use in soft tissue augmentation, it is supplied as a lyophilized powder containing PLLA microparticles, the size and chemical attributes of which are tightly controlled. As a biocompatible material, PLLA generates a desired subclinical inflammatory tissue response that leads to encapsulation of the microparticles, stimulation of host collagen production, and fibroplasia. Over time, the PLLA degrades, the inflammatory response wanes, and host collagen production increases. This response leads to the generation of new volume and structural support that occurs in a gradual, progressive manner, and which can last for years. Coupled with consistent, optimized injection methodology, the use of PLLA in soft tissue augmentation can result in a predictable cosmetic effect that is completely controlled by the treating clinician.J Drugs Dermatol. 2014

Plasma fibroblast

Plasma fibroblast skin tightening is a new minimally invasive aesthetic skin procedure that is gaining popularity (Patel et al., 2020). Plasma treatments have gained a reputation as one of the best non-Surgical procedures in the medical aesthetic industry for skin tightening. The Plasma Pen Fibroblast treatment is a safe and effective non-surgical lifting and tightening procedure which offers similar results to traditional surgery but without the high risks, cost, and with a minor downtime.

The treatment involves a pen like device that holds a sterile single-use needle like probe that discharges a high-frequency electric current to small areas of the skin which will produce a small plasmic arc. This little arc, or flash causes microscopic points of evaporation in the tissue via a process called sublimation (turning a solid into gas). Consequently, shrinkage in the epidermal skin layer is caused, without any damage to the surrounding tissue. The plasma tip does not directly touch the skin, but instead releases a targeted current above the skin. The hot current creates small holes, or micro-injuries, in the skin's layer. A specific cluster or pattern of point sequences is placed in the treatment area, generating a reduction grid, and decreasing the amount of excess skin which results in immediate contraction and tightening in the area without cutting.

Plasma Fibroblast is successfully used to treat lines and wrinkles, loose skin on face and body, scarring, stretch marks as well as skin lesions, loose & saggy skin, lines & wrinkles, eyelid lift, brow lift, crow's feet, marionette lines, mouth and lip lines, frown lines, fore headlines, mini facelift, neck lift, scars and stretch marks reduction Histological studies performed on plasma resurfacing clients have confirmed continued collagen production, reduction of elastosis, and progressive skin rejuvenation even after the first year of treatment(Hernandez et al., 2022).

Plasma fibroblast therapy targets fibroblasts. These are collagens and protein producing cells in the dermis, the layer of skin just below the outer most skin layer. Fibroblasts play an important role in helping skin wounds heal as well as maintaining skin firmness and tightness.

According to a 2019 article published in the PMFA journal, the thermal disruption or heat damage from plasma fibroblast therapy breaks down proteins in the skin, encourages tissue regeneration, stimulates fibroblast activity, and causes tissue contraction (tightening). Plasma skin resurfacing is another term used by health professionals to describe this procedure.

Fibroblast therapy can improve skin texture, offer mild-to-moderate skin tightening effects, and result in some degree of skin facial contour change. According to a 2008 article published in the journal Clinical, Cosmetic and Investigational Dermatology, the effects are expected to stimulate fibroblast production for

upto 1 year after treatment. However, there is little amount of research on plasma fibroblast therapy as it is a relatively new aesthetic procedure. A 2007 study used plasma fibroblast therapies on eight participants. Each participant received one full-face treatment every 3 weeks. The patients reported a 37 percent reduction in facial wrinkling and a 68 percent overall improvement in facial appearance.

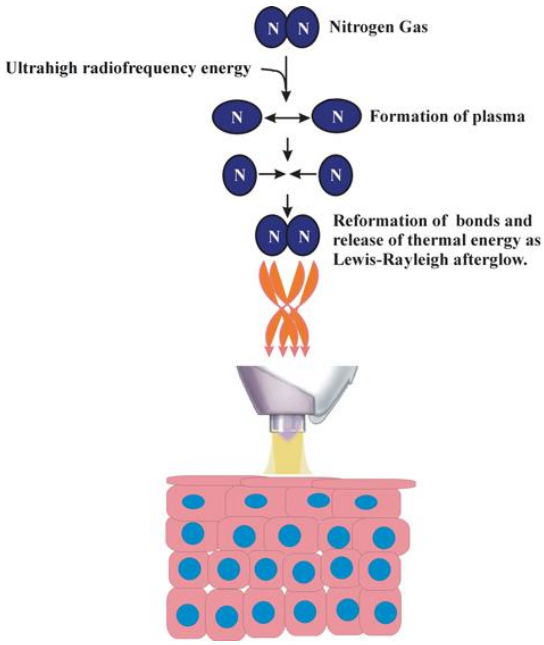


Figure 31: Plasma fibroblast Mechanism

Mechanism of action:

Plasma fibroblast, an aesthetic procedure which causes skin tightening, is a novel procedure. This procedure causes skin rejuvenation by improving skin pigmentation and facial lines, and reducing wrinkles. The skin is treated with topic creams for anaesthetic purpose prior to this procedure (Hernandez et al., 2022).

Plasma fibroblast therapy is different from microneedling. Microneedling smoothes the skin by using broad strokes while a pen like device used in plasma fibroblast therapy discharges electric current of high frequency to the small skin area. In plasma fibroblast therapy, small holes or microinjuries are created in the skin by the release of a targeted, hot electric current to the skin by using device. Fibroblasts activate in response to this microinjury and repair the skin. Gaseous diatomic molecular nitrogen is formed from electrical energy by plasma pen. Then device transmit this gaseous diatomic molecular nitrogen to the skin surface in a non contact way from above. This result in the formation of very small controlled burn

which can be seen as a small dot called plasma spot. Skin is contracted by each plasma spot and hence causes tightening of the skin. Plasma fibroblast therapy also causes heat damage or thermal disruption which results in the breaks down of proteins present in the skin, causes tissue contraction (tightening), stimulates fibroblast activity, and encourages tissue regeneration (Patel et al., 2020).

Plasma fibroblast therapy is an aesthetic procedure which improves skin appearance and helps in skin tightening. This procedure is also named as plasma lift, plasma needling, fibroblasting, plasma skin regeneration, and plasma skin resurfacing. Because plasma fibroblast therapy is a new procedure therefore its effectiveness is supported by very little evidences. This procedure uses plasma to improve skin appearance by stimulating fibroblast cells present in the deeper skin layer to repair the outer layer of the skin.

There are four states of matter and plasma is the fourth one. Plasma is a cloud of electrons, neutrons and protons and acting as a whole is the property of plasma rather than acting as bunch of atoms. Plasma has more similarity with the gas as compared to other two states of matter because in plasma atoms did not form a constant contact with each other. In plasma fibroblast therapy, plasma is the important component of plasma pen which generate plasma spots for the purpose of skin rejuvenation (Foster et al., 2008).

Plasma fibroblast Effect on stem cells regeneration

Plasma fibroblast therapy uses plasma to improve skin appearance by stimulating fibroblast cells present in the dermis. Within the body, dermal fibroblasts are derived from mesenchymal stem cells. The proliferation of dermal fibroblasts is stimulated by fibroblast growth factor. Fibroblasts did not become specialized cell or fully differentiated. Hence plasma fibroblast therapy stimulates fibroblast cells formed by stem cell regeneration in the body and repairs the skin.

The deep thermal effects of this procedure act in a unique fashion. First, immediate tissue contraction is accomplished via thermal denaturation of dermal collagen. Second, thermal disruption of solar elastosis and activation of the skin’s fibroblasts stimulating a wound healing cascade necessary for neocollagenesis and a reduction of solar elastosis.

Histologically, immediately after a high-energy treatment, an intact and nonablated epidermis with vacuolation of the basal cell layer is visible Four days after treatment, a line of cleavage can be noted, which demarcates shedding epidermal and dermal remnants from a newly formed stratum corneum and a regenerated epidermis and upper dermis. The overlying epidermal and dermal remnants serve as a biologic dressing, protecting the skin from infection. Ten days after treatment with, a fully regenerated epidermis is visible. Continued collagen production is observed for up to 1 year after treatment(Hernandez et al., 2022).

Fibroblast

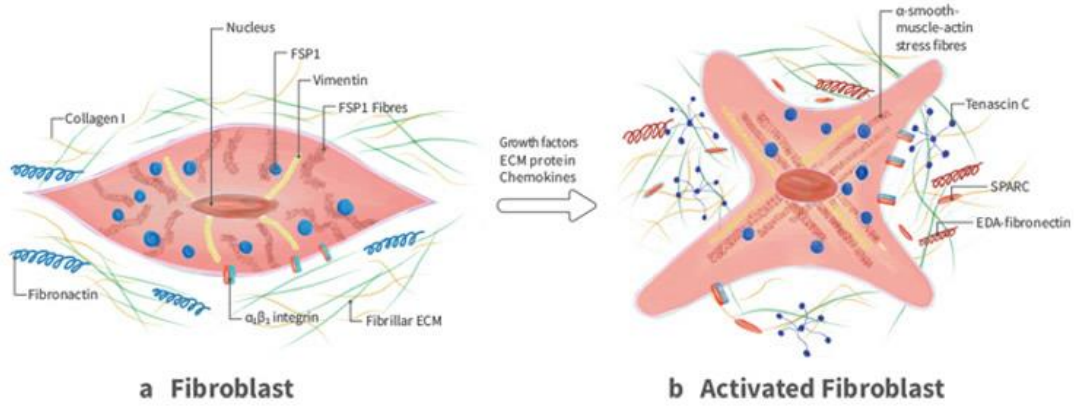


Figure 32: Fibroblast

The importance of the fibroblasts can't be overestimated. Fibroblast are involved in normal growth, healing, wound repair and the day to day physiological activities of every tissues and organ in the body.

Fibroblasts synthesize connective tissue fibers, including collagen and elastin fibers, as well as glycosaminoglycans and glycoproteins.

For the manufacture of collagen, the fibroblast first produces pro-collagen. This is produced in the rough endoplasmic reticulum, which is then in Golgi bodies from which arise secretory vesicles.

These will then discharge the pro-collagen into tropocollagen, which then combines with other tropocollagen to form the collagen fibrils. Trauma from Plasma Energy stimulates the fibroblast to produce collagen and elastin.

The Role of Fibroblast in Wound healing

Fibroblasts are critical in supporting normal wound healing, involved in key processes such as breaking down the fibrin clot, creating new extra-cellular matrix (ECM) and collagen structures to support the other cells associated with effective wound healing, as well as contracting the wound (Bainbridge, 2013). The ability to heal cutaneous injuries without scarring has aroused researchers' interest.

However, there is evidence that not all wounds heal without scarring, and the biological causes are still unknown (Fergusonetal,1996). Several factors influence the healing of specific wounds with hardly any scarring, and fibroblasts appear to play a key role. Fibroblasts are phenotypically unique, movefaster, and are better at organizing the ECM environment (Stephensetal, 1996).

When the injury is very small and minor the inflammation this will result in less infimation in the skin so the healing process will be less problematic and have a minimum possibility for scaring or post inflammatory pigmentation.

According to studies that is using dermal equivalents with epidermal keratinocyte/fibroblast co-cultures, fibroblasts influence keratinocyte proliferation and differentiation in the epidermal cells primarily through the release of soluble factors like GM-CSF, KGF, and IL-1 (El-Ghalbzouri et al., 2002; Russo et al., 2020; El-Ghalbzouri et al., 2002; El-Ghalbzouri and Ponec (Ghalbzouri and Ponec, 2004). Rognoni claims that a n early stage of vigorous dermal fibroblast proliferation and migration is required for effective wound healing, followed by a higher ECM production phase that slows fibroblast growth.

Papillary and reticular fibroblasts alter the composition of the relevant layer of the dermis. The reticular dermis has a variety of fibrillary collagen bundles that are well-organized, but the papillary dermis includes a lot of non-fibrillary collagens and proteoglycans like fibro modulin and decorin (Stunova and Vistejnova, 2018; Sorrell and Caplan, 2004). This suggests that these individuals have a considerable influence on the ECM components of the dermis and may respond to injury differently. Jiang and Rinkevich (Jiang and Rinkevich, 2020) investigated the fractions of dermal fibroblasts connected to fibrosis and found that no biomarkers or geographic position inside the dermis can separate non-fibrotic from fibrotic fibroblasts.

According to other research, papillary fibroblasts support lesion healing, but reticular fibroblasts are fibrotic due to their ability to rapidly generate collagenous ECM (Rippa et al., 2019; Philippeos, 2018). Keratinocytes cultured on ECM from the papillary dermis proliferated more than the others which are cultured on ECM from reticular fibroblasts (Janson et al., 2017).

Moreover, reticular and papillary fibroblasts have been demonstrated to react differently to ageing (Haydont et al.,2019; Mine et al.,2009). The proliferative and remodelling potential of old papillary fibroblasts is lower than that of aged reticular fibroblasts. The less specialized papillary fibroblasts may disappear or convert into reticular fibroblasts with age, according to the scientists. These results suggest that papillary fibroblasts are much more pro-regenerative versus reticular fibroblasts, and that activating them is essential for scar-free skin regeneration (Woodley, 2017).

Carboxytherapy

It is a non-surgical method which involves injecting CO2 at subcutaneous level with very fine needle by using an equipment designed exclusively for regulating the CO2 gas that exit with a low pressure.

Mechanism of Action:

Dark under-eye circles, stretch marks, Scars and even cellulite can all be treated using carboxytherapy. It was created in the 1930s in France. The eyelids, neck, cheeks, arms, buttocks, stomach, and legs can all benefit from this therapy. Carbon dioxide infusions, a naturally occurring gas in the body, are used in this therapy. Circulation, fine lines, skin flexibility, and wrinkles are all improved as well as those who have surgery. It also helps with collagen repair and fat layer destruction. It can also aid in the reduction of under-eye circles by improving blood flow to the lower eyelid. This procedure is usually recommended over more intrusive and high-risk procedures (like liposuction) for fat and cellulite removal (Soliman, 2018).

A flow regulator with plastic tubing is linked to a tank of carbon dioxide gas in this procedure. The gas is ejected into sterile tubing through a flow regulator with a filter at the other end. All pollutants are caught by the filter before they enter the body. The gas is subsequently passed through a very tiny needle on the filter's opposite side. The gas is injected beneath the skin using a needle. Almost little discomfort is experienced throughout the procedure. Before placing the needle (Pianez et al., 2016).

Dark under-eye circles, cellulite, and stretch marks are all caused by poor blood circulation. Carbon dioxide is emitted by the body's cells as a waste product. The oxygen which humans breathe is carried to tissues by red blood cells, which subsequently pick up carbon dioxide. The lungs eventually expel the carbon dioxide (Kroumpouzos et al., 2021).

By injecting carbon dioxide into a specific region, blood circulation is enhanced which encourages red blood cells to rush to the site. When blood cells arrive at their destination, they boost circulation. This improves skin elasticity and, changes pigment to a healthy glow in the case of dark circles beneath the eyes. Stretch marks, scars on the body are caused by poor healing to the tissue a dermal collagen rupture. Carboxytherapy stimulates the production of new collagen, thickening of the skin and improving its look (Nach et al., 2010). Carbon dioxide can also be infused into fat cells, causing them to rupture and be expelled from the body. Subcutaneous fat protrudes through the skin, causing cellulite. When it comes to treating cellulite, carboxytherapy is both effective and safe. Poor circulation causes vascular pooling, which results in dark circles beneath the eyes. When the gas is injected behind the eyelid, the bluish pooling is reduced and replaced with a blush tone (Koutná, 2012).

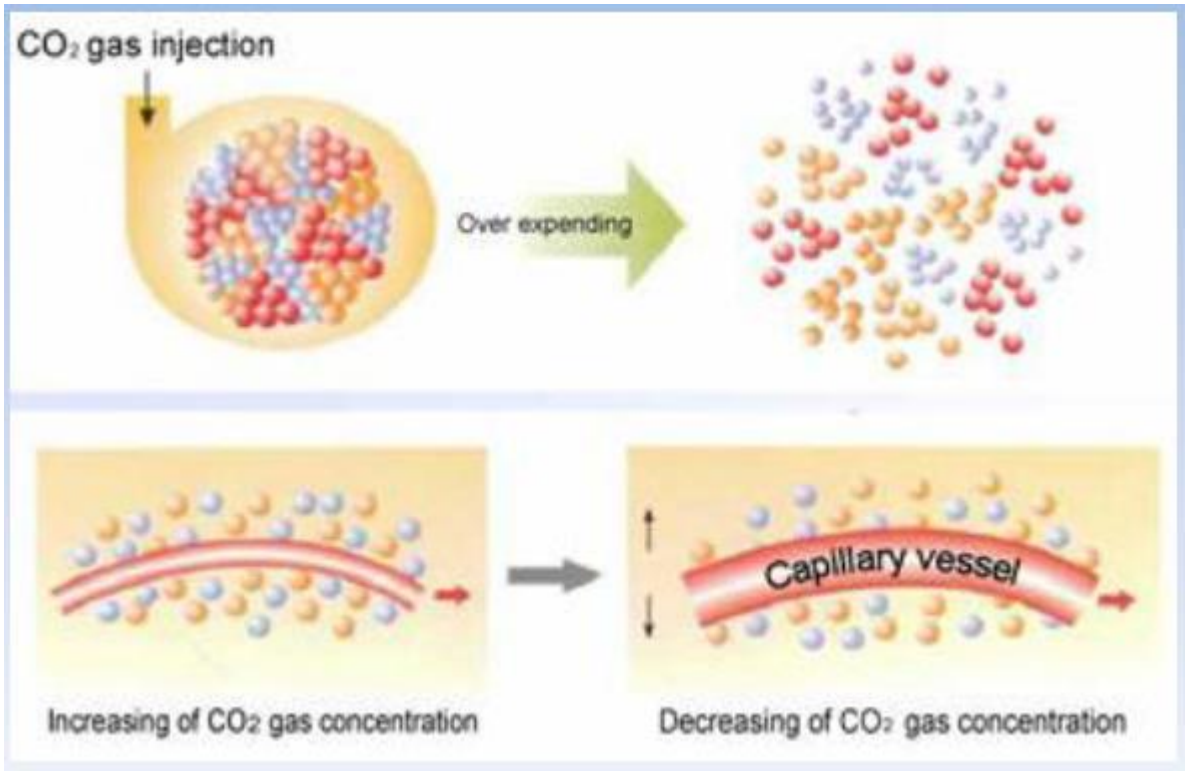


Figure 33: Carboxytherapy effect on stem cells

Carboxytherapy effect on stem cells

Carboxytherapy seems to have no significant impact on stem cell regeneration, and it induces new collagen to develop and thicken the skin, which helps to reduce the look of stretch marks, sacrs by repairing the collagen matrix. Carboxytherapy stimulates the production of new collagen by boosting blood flow (Kołodziejczak et al., 2018).

Chapter Four

Stem cell therapy

The use of stem cells to prevent or treat a condition or disease is stem cell therapy. They are rapidly dividing cells present in the body. They can divide to form new stem cells or they can differentiate into any kind of specialized cells. In some organs, stem cells replace or repair damaged tissue after differentiating into that organ or tissue cells. Similar function is also performed by plant stem cells (Wong et al., 2012).

Stem cell therapy is now applied in many conditions for therapeutic purpose. It has also applications in the field of dermatology. On the basis of their differentiation capacity and their source, stem cells can be

classified. In skin, stem cells are found in the adipose tissues, dermis, hair follicle, and inter-follicular epidermis where they assist in the regeneration and repair of the skin during injury and also assist in the maintenance of normal homeostasis of the skin. Due to unique characteristics of stem cells, they are applied for the treatment of several skin problems like alopecia, scleromyxedema, erythematosus, systemic lupus, systematic sclerosis, epidermolysis bullosa, wound healing, psoriasis, pemphigus, Merkel cell carcinoma and in aesthetic medicine with variable success rate. There is no doubt that discovery of stem cell therapy enables the treatment of those diseases which were previously thought as untreatable diseases (Jo et al., 2021).

What are stem cells?

Stem cells are cells with an essential role in tissue creation, regeneration, and healing. Their ability to create all other cells with specific functions is unique. It is the basis of stem cells 'immense medical potential. However, not all stem cells are the same. There are different types. Three main categories of stem cells are:

- Adult MSCs (Mesenchymal stem cells)
- Tissue specific stem cells
- Embryonic stem cells

Adult mesenchymal stem cells (MSC) are multipotent cells. They are present in various tissues, including bone marrow, fat tissue, and umbilical cord tissue. Mesenchymal stem cells have a formidable healing ability. They can differentiate into many tissue types, including skin, muscle, bone, neural tissue, etc.

Tissue-specific stem cells exist in all parts of the human body. Depending on their location, they can differentiate into specific tissues, such as fat, muscle, cartilage, bone, skin, etc. Human stem cells can have a therapeutic use. However, their products enjoy a wide spread application in aesthetic medicine. These are called secretome andexosomes. Secretome includes all proteins secreted by the cell into the extra cellular matrix. On the other hand, exosomes are microsize fluid-filled sacs released from cells.

More often than not, these two terms are synonyms in the medical literature. The reason is in the early definition of these cretome. It involved all substances from the extracellular matrix, including exosomes.

Emergence of Stem Cells

Stem cells offer tremendous potential for aesthetic procedures but we must be vigilant to avoid unscientific claims which may threaten this newly born field. With age stem cells were found to gain DNA damage, and depending on nature and extend of this damage, mutagenic lesions arising in stem cells have the potential to drive cells to transformation of tumor, apoptosis and senescence. As a result, because ageing

has been linked to a decrease in the regenerative ability of stem cells, claims of rejuvenation in the elderly people through cell transfer have become more doubtful.

Transplanted stem cells have recently emerged through as a promising area of research where stem cells where used in cosmetic facelift and volumetric rejuvenation, as biomaterial/molecule designed to improve facial volume For example, when autologous MSCs combined with hyaluronic acid were able to fill in deep skin folds in the face, showing progressive improvements of skin tone and decreasing lines of expression. Slowing or even reversing effect of aging thus volume filling alone.

How aging effects the stem cells

Another important consideration to be made within corporation of stem cells into aesthetic procedures is the effect of age on the cells themselves. Stem cells are not immune to the process of aging, and their function is tightly regulated by their surrounding environment known as the stem cell niche. The hematopoietic system provides the best evidence for the role local environment plays in guiding maintenance and differentiation of stem cell populations and the effects of aging on this balance. The functional decline of aged stem cells is due to a host of factors. These include both exogenous sources, such as genotoxic chemicals, UV irradiation, and ionizing radiation and endogenous sources such as a build-up of reactive oxygen species, shortening of telomere and stoppage of replication fork.

A series of experiments evaluating HSC reserves and functional capacity in young and old mice deficient in several different DNA-repair pathways demonstrated the impact of DNA damage on stem cell function.

Stem Cell Treatments

Stem cell treatment is a type of regenerative therapy that uses a special type of cell to repair injuries. Stem cell therapy is used to treat back pain and help regenerate tissues in the spinal discs and joints. Stem cells are special cells that do not have a specific purpose in the body that can develop into a type of cell that deserve a function.

When used in regenerative medicine, stem cells are collected from the patient’s own fat, blood, or bone marrow. They are then put into a medical machine called a centrifuge so the doctor can identify and separate the cells to find the right ones to use for the regenerative therapy treatment. The cells are then injected into the part of the body that needs to be repaired. For instance, to repair spinal discs, stem cells would be injected directly into the damaged disc. The cells will then develop into healthy spinal disc cells and repair the disc.

In the aesthetic medical field we can see the same technique is used to treat aging skin, it get injected around the eyes, inside the wrinkles and folds and even on scars. The cells will then develop and will repair the skin.

The development of regenerative medicine and aesthetics moves parallel forward. Already, some aesthetic procedures use the body's regenerative potential by provoking its potent healing response. Good examples of this are radio frequency, micro needling, chemical peels, and laser skin resurfacing. Still, stem cells offer much more.

Since the discovery of stem cells, regenerative medicine is spear heading medical research. The main reason, of course, is its therapeutic potential. As we learn how cells interact between themselves, our ability to utilize those interactions in medical treatments grows exponentially. One of the most promising uses of stem cells, and their products, is in aesthetics. So far, they've showing real potential in treating hair loss and all age-related skin changes, including scars.

The overall fashion in aesthetics nowadays is also helping. Patients look for more natural treatments and a more organic and unpretentious look. That's where regenerative aesthetics have plenty to offer.

Stem cell therapy can make anyone look the best version of themselves while avoiding the risk of an unnatural, frozen, or plastic appearance.

Mechanism of action:

Stem cells used for regenerative therapy are firstly isolated from adipose tissues or from person's bone marrow by stem cells isolation process. Then they are cultured and sub cultured under controlled and sterilized environment and then injected back to the person. Autologous stem cells are injected into the patient to avoid from any immune reaction. Firstly an adipose tissue is extracted from the person's body by liposuction and then this fat is washed with phosphate buffer saline and then treated with collagenase enzyme. After enzymatic digestion, fat is centrifuged and then stem cells are separated from fat are taken out which then either injected into the same person for treatment purpose or they are firstly cultured and their number is increased and then injected into the target area. At the target area, they release various growth factors which stimulate treatment process. They also start to differentiate into specialized cells like they form fibroblasts which are the cells that produce collagen. Proteins and amino acids are present in both animal and plant stem cells. These amino acids and proteins stimulate the cells of the body to repair the damaged part of the body. They rejuvenate the skin and make the skin youthful. Stem cells nourish the skin cells which results in the increase in the cell turnover and collagen production. Stem cells also have antioxidant properties (Ojeh et al., 2015).

Stem cells present in the basal layer of hair follicles and epidermis is important sources of stem cells for the injury repair, metabolism and development of skin. Stem cells transplantation enhances vascular formation, endothelial cell transformation, and skin healing. Stem cells already present in the skin can

also be transformed into sebaceous gland, keratinocytes and other tissues associated with the skin. However, stem cell's mechanism of action on skin regeneration and wound healing is not completely clear (Zarei and Abbaszadeh, 2019).

Skin aging is caused by functional disorder of stem cells or due to decreasing number of stem cells turnover with age. Therefore, injecting stem cells into the skin enhances anti-aging effect by affecting activity of humoral factors, maintaining skin homeostasis, and promoting skin regeneration. Hence they can be applied for anti-aging therapy such as for the treatment of photoaging, wrinkles and acne scars. Stem cells products such as exosomes and secretome are also applied for the treatment of various skin problems and give positive effect (Brown et al., 2019).

Remodeling of Collagen

Collagen remodeling represent as growth factor, can impact collagen remodeling through their effect on dermal fibroblast.

Give the availability cytokines such as vascular endothelial growth factors, platelets derive growth factor and transforming growth Factor-β to promote collagen synthesis and turnover stem cells capable of producing these factor may hold promise for anti-aging therapies.

Bovine Collagen

In the 1970s, there search into collagen production led to the use of bovine collagen for the purpose of clinical augmentation, and implicitly, for facial augmentations. The beginning of extracting bovine collagen started with Gross and Kirk who extracted collagen from fresh calf skin in 1958. They were able to produce a solid gel from the extracted collagen by gently warming the liquid collagen slowly to body temperature. The problem of antigenicity to bovine collagen molecules was not solved until the 1960s when the removal of helical amino and carboxy terminal telopeptides from the collagen gel allowed it to be injected in humans.

In 1977, the first successful injection of collagen was performed by Knupp, Luck and Daniels. They reported that the collagen grafts remained stable and were progressively infiltrated by amatrix of viable host connective tissue. Lateron, they injected the bovine collagen into the dermal and subcutaneous planes to correct depressed acne scars, subcutaneous atrophy, wrinkling and viral pock marks in 28 humans. The collagen fillers showed a 50–85% improvement that lasted for 3–18 months.

Porcine Collagen

Porcine collagen was only briefly used in the USA. It's resemblance to human collagen allowed for less risk for an allergic reaction and patients were not required to get skin testing prior to injection. Porcine collagen has been used outside USA since 2004. It gained popularity in the USA for about one year from 2008 to 2009. Currently, there are no available facial fillers on the American market based on porcine collagen.

Poly (methylmethacrylate) (PMMA)

PMMA is a synthetic material that is used in medicine and dentistry for bone cement, dentures and artificial eyelenses. When polymerized, the PMMA forms 30–40μm spheres that can then be suspended in collagen for injection into facial defects. The collagen only serves as a delivery mechanism for the PMMA beads, and it degrades after injection into the soft tissue, leaving just the PMMA beads to become the permanent implant.

The injectable compound is a suspension composed of 20% non-resorbable PMMA and 80% bovine collagen. The compound started being used in the 1980, but it wasn't until 1995 that a prospective study using 118 patients indicating a high patient satisfaction (90%) with results of treatment lasting a minimum of two years.

Stem cells as regenerative medicine

Regenerative medicine involves injecting stem cells into areas of the body effected by an illness. The stem cells multiply and work with the body existing resources to encourage the reaction of new, healthy cells to repair and replace damage tissue.

Risks of using Stem cells (Cell processing)

When considering clinical use of stem cells, one must be cognizant of the fact that cell and tissue processing pose a risk of contamination and or damage to cells. Regulation of stem cell therapy is thus paramount to ensure patient safety. For this reason, the growing number of "stem-cell based" cosmetic procedures is worrisome, due to the lack of oversight and dearth of scientific studies or trials to evaluate their efficacy and safety in-vivo. Potential contamination of and damage to cells becomes an issue when cell-based products involve more than minimal manipulation, including cell expansion in culture and differentiation. In theory, cells removed from a patient and replaced during the same surgical procedure

pose no greater risk of disease transmission than the surgery itself. However, cell culture typically involves the use of non-human serum, usually obtained from fetal calves, and therefore introduces a potential risk for prion infection.

In light of this, current FDA guidelines specify that fetal-calf serum must come from a country certified to be free of this disease. Additionally, cell expansion in vitro may also involve the use of xenogenic feeder cells, particularly in the case of human embryonic stem cells. This also poses a potential risk of infectious disease transmission. Another safety concern revolves around malignant stem cell transformation. Stem cells have clear similarities to cancer stem cells, and it has been demonstrated that mesenchymal stromal cells can undergo spontaneous malignant transformation following long-term in vitro culture. Despite the enormous potential for stem cells demonstrated in preclinical testing, it is thus essential to recognize and appreciate both the promise and limitations for use of stem cells in clinical therapeutics. As use of stem cells in aesthetic medicine continues to expand, it is important to note that FDA approval remains limited.

In June of 2011, after almost 10 years of review, the US Food and Drug Administration (FDA) approved azficel-T, a first-in-class personalized cell therapy for eliminating fine wrinkles or nasolabial folds around the nose and mouth.

Each azficel-T treatment involves harvesting a patient' sown fibroblasts from behind the ear, culturing them for 90 days, and then reinjecting expanded cells into the dermis during a series of treatments.
A search for "adipose derived stem cells" is on the clinical trials. Gov website yielded 109 results. However, only a small proportion of these clinical trials focus on aesthetic treatments.

Studies that do focus on aesthetic procedures include trials to establish the role of ASCs in volume retention of fat grafts, improving fat graft retention in the breast following breast cancer resection, reducing wrinkles when co-delivered with fat grafts, skin ulcers, diabetic wounds, and improving skin quality of irradiated breasts. In addition, one study is presently looking at the role of Adipose Derived Stem Cells (ADSCs) to improve osteogenesis in composite tissue grafts and another is looking at the role of ADSCs- enhanced fat grafts following cranio facial trauma.

Direct-to-consumer marketing of cosmetic stem cell therapies where mostly misleading as so many clinic website advertise stem cell therapies but they actually offer platelet-rich plasma (PRP) treatments which they marketed as stem cell treatment. Of note, PRP does not contain stem cells and is rather autologous plasma that is enriched with platelets. Indeed, platelets by their very nature are cell fragments, and technically do not even come under the umbrella term of cells as they lack a cell nucleus.

Nonetheless, PRP has found application in a range of clinical scenarios such as orthopedics,

ophthalmology, and wound healing, serving as a growth factor pool for improving tissue regeneration. However, to market PRP as a stem cell therapy is misleading.

Stem cells have captured the imagination of many due to deeper insights into the biology of cells they provide, as well as potential for treatment of many diseases. Stem cells are functionally defined by their ability to self-renew and to generate differentiated progeny cells with more restricted potential.

The role of stem cells changes significantly throughout life, as stem cells must alter their properties to match the changing growth and regeneration demands. Reflecting these changing roles, there are many different types of stem cells which have been defined. Pluripotent stem cells, such as embryonic or induced pluripotent stem cells, have the capacity to generate tissue from any of the three germ layers. Their clinical use, however, has been hampered by risks for teratoma formation and, in the case of embryonic stem cells, ethical concerns.

Multipotent stem cells, such as mesenchymal stromal cells (MSCs), lack these short comings, but have the capacity to differentiate into a more limited number of closely related cells. Finally, unipotent stem cells, while retaining the ability to self-renew, can only produce one cell type. This last class, while having limited utility in regenerative strategies, is intimately involved in normal tissue homeostasis at a wide variety of sites including skin, lung, liver, and intestinal lining.
Of these different types of stem cells, of particular clinical interest are the MSCs. MSCs have the capacity to differentiate into bone, cartilage, muscle, and fat. While the best-characterized MSC is the bone-marrow derived MSC, their clinical utility is limited as isolation of these cells is associated with considerable donor site morbidity and low yield.

Uses of Stem Cells in Aesthetic Treatments

We can sum up the crucial promise of regenerative aesthetics into one sentence. Your skin could be younger at 50 than it was at 40. It could be healthier and functioning better. That means younger, not only better looking. That is the potential of stem cells to naturally regenerate organs and tissues, such as skin and hair, and not only make them more physically attractive. Regardless of all that, many countries still limit live human stem cells in medical treatments. Therefore, regenerative aesthetics procedures use therapeutically active stem cell products.

There are many positive sides to this. Secretome is safer than live stem cells. There is no risk of injecting altered cells, and it's possible to make many doses from one stem cell sample. The Production and Application of Stem Cell Secretome Here’ show stem cell secretome

production works: First, a sample of adipose (fat) tissue is necessary. A simple procedure, such as mini liposuction, will do. Then, the mesenchymal stem cells are isolated from that sample in the laboratory.

Next comes cultivation. Mesenchymal stem cells multiply until they reach a sufficient number. The cells go through stimulation to produce the conditioned medium. A solution of secretome, exosomes, and other regenerative substances. After this, the cell products are ready for application. Usual methods are intravenous, topical, or through injections. There is no way to control where the therapy will have the best result, as it affects the whole body. However, in topical and injection applications of stem cell products, the best effect is local. They tend to respond to inflammation, and needle injection sites create just that (Xia et al., 2019).

That's why in aesthetic treatments for hair loss or age-related skin changes, it is best to inject regenerative substances in the affected areas. In and around hair follicles and wrinkles, for example. What to expect after a stem cell aesthetic treatment? Regenerative aesthetics treatments boost natural regeneration .The goal is to spark off the production of new cells of specific tissues. In that way, those tissues rejuvenate. Their physical appearance and function are improved.

The entire process starts immediately after treatment, and the results become gradually visible over time. The improvements are usually significant, long-lasting, and completely natural. Regenerative medicine encompasses innovative therapies that allow the body to repair or regenerate aging cells, tissues, and organs. The skin is a particularly attractive organ for the application of novel regenerative therapies due to its easy accessibility. Among these therapies, stem cells and platelet-rich plasma (PRP) have garnered interest based on their therapeutic potential in scar reduction, antiaging effects, and treatment of alopecia.

Stem cells possess the cardinal features of self-renewal and plasticity. Self-renewal refers to symmetric cell division generating daughter cells identical to the parents.

Discussion:

Skin ageing is a complicated biological process that is regulated by both exogenous (chemicals, radiations, toxins, chronic light exposure, pollution,) and endogenous variables (metabolic processes, hormones, cellular metabolism, and genetics). These elements combine to cause cumulative structural and functional changes, as well as gradual changes in each skin layer and changes in skin appearance, particularly in sun-exposed skin regions. There are several therapy procedures that can improve photo aging, make skin seem younger, and aid in skin rejuvenation (Zouboulis et al., 2019).

The plasma fibroblast aesthetic procedure has been shown to be the most successful in repairing wrinkles and depressed regions of skin on the face and body. Several investigations have demonstrated that plasma fibroblast is a novel therapeutic with little side effects. Plasma fibroblast treatment resulted in a lower Wrinkle Severity Rating Scale score. When patients were treated with the plasma fibroblast treatment, the level of patient satisfaction was 97.3 percent and the level of investigator satisfaction was 98.4 percent, according to the Global Aesthetic Improvement Scale results (Kamakura et al., 2015).

The technical advancement of the use of stem cells in skin rejuvenation is made possible by advances in cellular and skin ageing understanding and research. Adipose-derived stem cells therapy in skin rejuvenation is also an effective procedure but it is not largely applicable due to some ethical issues. Treatment with adipose tissue and PRP caused changes in the dermo epidermal junction, reticular dermis, and papillary dermis, most likely due to the presence of fat. (Rigotti et al., 2016).

Studies have shown that the treatment of periorbital dark circles (PODC) with PRP is more successful and comfortable than caboxytherapy. Periorbital dark circles are mostly a cosmetic problem that primarily affects women; few men are concerned about it. PRP outperformed caboxytherapy in terms of response, recovery time, and acceptable side effects. In one study, area percent of melanin is reduced by 46.6 percent following PRP injections but only 14.3 percent following carboxytherapy. This could be explained by the fact that the PRP includes growth factors such as epithelial growth factor, fibroblast growth factor, and keratinocyte growth factor in their alpha-granules. Melanogenesis is known to be reduced by TGF-β, which is produced in PRP. By the creation of hyaluronic acid, PRP has the capacity to improve skin tone, giving patients the appearance of more radiant skin that feels less pigmented and is pulled away from the underlying vessels. These components combine to create a complex that enables PRP to induce the proper degree of angiogenesis without leading to an excessive vessel creation. (El-Tahlawi et al., 2022).

Mesotherapy is the name given to a number of minimally invasive procedures that include injecting materials either intra- or subcutaneously. Amino acids, minerals, biomimetic peptides, growth factors,

conventional medicines, homoeopathic drugs, or collegen that can help with face skin restoration are some of the more popular chemicals that are injected in mesotherapy. Various studies have demonstrated that chemical peels, carboxytherapy, and mesotherapy procedures can be used for skin rejuvenation and reduction of fine lines. In contrast to the chemical peels and carboxytherapy, mesotherapy was shown to result in the greatest improvement and the highest degree of patient satisfaction; nevertheless, some patients considered it to be less comfortable due to the side effect of a burning feeling (Ahmed et al., 2019).

A recent method called carboxytherapy involves injecting CO2 subcutaneously and intra-dermally. While chemical peeling is a straightforward in-office procedure that has developed over the years, the injection of CO2 induces a relative state of hypercapnia that is compensated by vasodilatation and an increase in the capillary blood flow to the injected area, reduces cutaneous oxygen consumption, and stimulates the secretion of growth factors like vascular endothelial growth factor (VEGF), which results in the formation of new blood vessels. It is based on the notion that inducing chemical harm on the skin causes it to regenerate fresh, healthy layers. The skin may be rejuvenated with this easy, affordable procedure with minimal instruments. Hassan et al. contrasted the effectiveness of chemical peel treatment with carboxytherapy. Comparing chemical peel therapy to carboxytherapy, they discovered that with chemical peel therapy, the degree of improvement was superb in 46.7 percent of patients, great in 46.7 percent of cases, and average in 6.6 percent of cases, comparing the degree of improvement with carboxytherapy, where it was superb in 46.7 percent of patients, great in 40 percent of cases, and average in 13.3 percent of cases, with no statistically significant difference between these two procedures. These two treatments were successful. (Hassan et al., 2016).

Radiofrequency, carboxytherapy, and light emitting diode (LED) photomodulation are effective procedural techniques to treat skin ageing. In one research, the effectiveness and safety of LED, RF, and carboxytherapy in face rejuvenation were assessed and compared. 60 participants with facial wrinkles participated in that study. Three groups of patients were created. All patients underwent four sessions spaced three to four weeks apart, and they were all monitored three months following the course of treatment. Digital imaging and skin samples were used to evaluate patients both before and after therapy. In the LED group, the percent of participants who were satisfied varied from 50 to 80 percent, while the improvement percentage ranged from 40 to 70 percent. The percent of participants satisfied with the RF group was 30–50%, and the improvement percentage varied from 40 to 50%. While the percent of participants satisfied with carboxytherapy was 40–60%, and the improvement percentage varied from 40 to 60%. In terms of face rejuvenation, LED was the most effective, followed by carboxytherapy and, finally, RF. MMP1 immunohistochemistry expression confirmed these findings (Nassar et al., 2020).

Numerous high-level research studies support the safety, efficacy, and cost-effectiveness of hyaluronic acid fillers in facial rejuvenation. HIFU has strong evidence supporting its use, whereas Thread lifts lack high-powered data on their usefulness and safety. Hyaluronic acid fillers are the treatment of choice for facial rejuvenation, correction and adding volume safily, efficient, and cost-effective. When non-invasive therapies are chosen, HIFU is a viable option. Nonsurgical face rejuvenation is still the top of the line in aesthetic treatment. The shorter post-recovery period, along with the elimination of the need for surgical drains, has increased the appeal of non-surgical face rejuvenation for both patients and clinicians. The advancement of fillers used in cosmetic procedures is in its second decade, high intensity focused ultrasound in its first, and thread lifting procedures in its third (Chang, 2017).

The toolbox for minimally invasive face rejuvenation is rapidly growing, but because each individual is unique, no one method is appropriate for everyone. As a result, it is important not only to repair the wrinkles, but also to work with the patient to design an aesthetic overall idea. This implies that any of the procedures alternatives should be chosen based on the patient's health, needs, and objectives. Mesobotox, microneedling, and microdermabrasion therapy may be beneficial for wrinkles caused by mild and early degrees of photodamage in young patients with hyperkinetic muscles, whereas skin boosters, laser resurfacing, light sources, and RF techniques are more beneficial for more severe photodamage caused by collagen loss. Meanwhile, combined treatment is extremely beneficial for people who have moderate to severe photodamaged skin, hyperkinetic muscles, or volume loss. A good combination should be considered for appropriate reversal of indications of age on the face. A combination therapy is one that incorporates at least two distinct and unrelated therapies, such as a light or laser device mixed with non-laser technologies or other treatments or approaches. Appropriate patient selection and the combination of several procedures frequently allow for personalised therapy with excellent outcomes (El-Domyati and Medhat, 2013).

Vitamin reduces dark circles under the eyes and nourishes the skin by inhibiting the enzyme tyrosinase and acting as an antioxidant, reducing the creation of free radicals that might stimulate melanogenesis. It also stimulates collagen formation, reducing skin damage and promoting keratinocyte differentiation. Vitamin C promotes collagen synthesis and lowers hyperpigmentation by inhibiting melanin formation; vitamin E, an antioxidant, assists in skin anti-aging; and vitamin K influences clotting processes and so controls skin microcirculation (Farris et al., 2014).

Multiple sessions and integrated minimally invasive techniques, in addition to the utilization of future home devices, would bridge the gap between non-ablative and ablative techniques in alleviating skin ageing indicators and preserving clinical and histological improvement. Advanced understanding and

research in cellular and skin ageing pave the path for the technical progress of the use of stem cells in skin rejuvenation. Meanwhile, advancements in laser sources and procedures, fractional laser and other energy approaches, as well as new and safer filler choices and neurotoxin complexes, point to a hopeful future for minimally invasive skin rejuvenation and treatment of aged skin (El-Domyati and Medhat, 2013).

Conclusions

Regenerative Aesthetic medicine focuses on novel therapies that help the skin restore and regenerate old or damaged tissue, increasing overall skin quality and encouraging quicker healing while reducing repair time and adverse effects for patients. The results might be evident immediately after the Treatment or progressively growing over time (weeks or months), as we train the skin to help itself. Among the procedures discussed in this study, I will be addressing these procedures in groups depending on the technique that it's been introduced, administered and effect the patient's kin. Starting with Plasma fibroblast is the most efficient procedure which uses plasma to improve skin appearance by stimulating fibroblast cells present in the dermis. Plasma fibroblast therapy stimulates stem cell regeneration, which activates and forms the fibroblast cells and repairs the skin. The second aesthetic procedure in sense of efficiency will be stem cells therapy in which stem cells are injected at the targeted skin area where they release various growth factors which stimulate treatment process. They start to differentiate into specialized cells like fibroblasts which are the cells that produce collagen and helps in skin rejuvenation.

Laser resurfacing, chemical peeling and microdermabrasion therapies came after stem cell therapy as they all peel the skin indifferent ways and effectiveness. These procedures replace the full top layer of the skin by heating in laser therapy, by applying chemicals on skins top layer in chemical peeling and by applying tiny crystals or diamond heads which breaks up the dull, dead skin cells residing in the outer skin layer to expose the softer and smoother skin underneath in microdermabrasion. Hence go through the healing process. These procedures remove the top layer of the skin. Laser resurfacing goes much deep into the skin, chemical peeling less deep while microdermabrasion removes top skin layer containing dead skin cells. As a result, stem cells activated to heal this skin damage. Stem cell proliferation and regeneration is increased by irradiating the cells with the laser energy. Laser irradiation also increases the secretion of growth factors at the target area. Chemical peeling loosened the lower skin layer and stimulates the basal skin layer to produce new skin cells by stem cell regeneration and skin cell division. They also produce collagen and elastin. While microdermabrasion causes skin damage which causes stimulation of the stem cells regeneration which results in the activation and release of growth factors. These stem cells released

growth factors stimulate fibroblasts and other recovery cells after covering the abraded skin to form new skin cells. Laser enhances stem cell regeneration the most, chemical peeling enhances less while microdermabrasion enhances stem cell regeneration the least among them.

After these procedures, Skin booster, PRP and mesotherapy came which are the aesthetic procedures which also effect stem cell regeneration and makes skin youthful. These are the injectable treatments. Skin booster promotes skin improvement by injecting booster substances like hyaluronic acid, and amino acids in the dermis. Hyaluronic acid hydrate the skin to make it smoother and brighter, activates stem cells regeneration to form fibroblasts cells which make more collagen and elastin. And amino acids are the building blocks for making the proteins collagen and elastin. Eight specific amino acids are mostly used. Skin boosters activate the epidermal follicular stem cells which only activate during wound healing and remain dormant otherwise. Platelet-rich plasma (PRP) therapy improves skin texture by injection of a person's own platelet for the purpose of healing. PRP therapy increases stem cell regeneration and guide the stem cells in healing process. PRP helps stem cells to find out in which cell they should differentiate. Studies showed that PRP increases the differentiation and proliferation of stem cells. While in mesotherapy, concentrated active substances are injected to the targeted middle layer (mesoderm) of the skin with short, fine needles. These active substances are antioxidant and anti inflammatory which provide active environment for stem cells to regenerate because stem cells are not able to regenerate properly under oxidative environment produced due to skin problem. Among these injectable treatments, skin boosters enhances stem cell regeneration the most, PRP enhances less stem cell regeneration while mesotherapy enhances stem cell regeneration the least among them.

Next aesthetic procedures are radiofrequency microneedling and simple microneedling. Both these procedures involve injecting microneedles. Radiofrequency microneedling is found to be more effective procedure than microneedling alone. Radiofrequency microneedling procedure delivers radiofrequency energy by puncturing the skin using ultrafine needles. This radiofrequency microneedling damage the skin by needle insertion and causes collagen denaturation by heating due to radiofrequency delivery. This results in the activation of stem cells regeneration to heal this damage and fibroblasts activation which enhances the collagen production. As a result, skin looks young and smooth. Radiofrequency microneedling is an effective and safe aesthetic procedure and better outcomes are expected when stem cell conditioned medium is used with radiofrequency microneedling. Second procedure is microneedling which is less effective than radiofrequency microneedling. In this procedure, tiny needles are inserted into the skin to damage the skin. Microneedling induces the growth factors activation by stimulating the stem cells proliferation to heal the damaged skin. Stem cells possess growth factors which help in damaged

tissue repairing, wound healing, starts skin growth, and aged skin regeneration. Microneedling activates immune system and stem cells to start the natural wound healing process.

High intensity focused ultrasound (HIFU), non-ablative radiofrequency (RF)/ Thermage, intense-pulsed light (IPL), and light emitting diodes (LED) therapies came after radiofrequency microneedling and microneedling. These aesthetic procedures are noninvasive procedures and it does not have a downtime as they all penetrate deep into the skin. In sense of effectiveness, firstly comes high intensity focused ultrasound which takes the target tissue into healing mood by non invasive delivery of ultrasonic energy and heat energy to the deep structural tissues of the skin. This high energy produces heat at the target point inside the body without damaging the epidermis and surrounding tissues. As a result, cellular damage takes place and then healing process begins by fibroblast activation and stem cells regeneration and activation to regenerate collagen. Stem cells direct collagen to the area of inflammation where collagen is required. Ultrasound when applied on stem cells enhances their ability of regenerative molecules secretion. And when applied on target tissue, it make that target tissue more favorable for stem cells homing. HIFU produces local gradient of adhesion molecules, growth factors and cytokines which helps in the regeneration of stem cells and stem cells homing. Second procedure is non ablative radiofrequency an thermage, both which also takes the target tissue into healing mood by non invasive delivery of radiofrequency to the deep skin. Radiofrequency pulse is given to the skin cause resistive heating (with simultaneous epidermal cooling in the case of Thermage). RF heats he skin up to 41degrees for a period of 5 minutes continuously which result in cellular damage and collagen denaturation. Themage on the other hand use the combination of heating and contact cooling results in a reverse thermal gradient, with the deeper dermis having a greater tissue temperature (60^{o}C) than the epidermis and top dermis (40^{o}C). Heat causes collagen denaturation (contraction), with uncoiling of the triple helix resulting from hydrogen bond denaturation. This damage produced due to high temperature starts healing process by activating stem cells proliferation and fibroblast activation which produce new collagen in that area. In this method, all tissues (with and without pigment) are heated.

Third procedure is intense-pulsed light (IPL) which takes the target tissue into healing mood by non invasive delivery of intense, broad-spectrum light to the deep skin. IPL treatments are more widely effective as they have the ability to treat a broader region of the skin. The powerful, broad-spectrum light penetrates deep into the skin and provides a large amount of energy to the target area, causing blood vessels and collagen fibers to contract. Damaged and photo-aged skin is thus eliminated. This stimulates stem cells regeneration and activates fibroblast collagen production for the purpose of skin healing. Fourth procedure is light emitting diodes (LED) therapy which takes the target tissue into healing mood by non invasive delivery of different specific wavelengths of light. LED treatments are as relaxing as a massage.

LED light therapy is best for addressing superficial skincare issues like acne. LED light therapy uses a lower level of energy. In this procedure an array of light-emitting diodes delivers deeply penetrating, low-level light energy to the epidermis and deeper layers of skin which damage the target cells and stimulate the creation of Adenosine Triphosphate (ATP). As a result healing process starts by activation of stem cells regeneration and increased ATP rejuvenates damaged cells, increases collagen formation, and stimulates blood circulation for a more youthful appearance. Red light promotes stem cells migrations.

Next aesthetic procedure is carboxytherapy which is a minimally invasive aesthetic treatment. In this procedure, carbon dioxide gas is injected beneath the skin using a needle. By injecting carbon dioxide into a specific region, blood circulation is enhanced as carbon dioxide encourages red blood cells to rush to the site. Carboxytherapy stimulates the production of new collagen by stem cells and fibroblasts activation, thickening of the skin and improving its look. This procedure is very effective as well for treating cellulite. Next came thread lift procedure, Thread lift is an aesthetic procedure in which skin lifting is carried out by temporary sutures. In addition to skin lifting, threads also treat aging by stimulating the healing process of the body and enhancing the large production of collagen at the target site. The healing process enhances the production of fibroblasts and also supports the growth and regeneration of stem cells present in the skin which revitalizes and smoothen the skin.

Next aesthetic procedure is IV vitamins drips and vitamins injections. In this procedure, essential vitamins, nutrients and minerals are administrated directly into the bloodstream through veins. This procedure is effective for prevention of skin aging as well as for skin improvement. Vitamins induce telomerase activity in stem cells which leads to the increased production of cell matrix and higher expression of fibronectin, integrin and type I collagen. Dermal filler aesthetic procedure came after vitamin therapy. Dermal fillers are injectable materials that restore skin volume by occupying physical space. Fillers actually correct various physiological changes that are produced in the skin as a result of scarring, aging, trauma, and sun damage. Dermal fillers did not put any prominent effect directly on stem cells regeneration in the body. At the last is the botulinum toxin and mesobotox procedure. Botulinum toxin/ Mesobotox injection is aesthetic procedure which makes younger looking and smoother skin by injecting saline solution along to dilute botulinum toxin into the skin which reduces wrinkles and fine lines. Botolinum toxin is a neurotoxic protein which disrupts acetylcholine release which results in paralysis and hence resulted in decreased wrinkles at the target site. Botulinum toxin used in mesobotox or botox injections is muscle relaxant which relaxes target muscles and provides powerful anti-wrinkle and anti-aging effect. Mesobotox therapy did not affect stem cells regeneration.

Multiple sessions and integrated minimally invasive techniques, in addition to the utilization of future home devices and the use of good product ingredient, would bridge the gap between non-ablative and

ablative techniques in alleviating skin ageing indicators and preserving clinical and histological improvement. Advanced understanding and research in cellular and skin ageing pave the path for the technical progress of the use of stem cells in skin rejuvenation. Meanwhile, advancements in laser sources and procedures, fractional laser and other energy approaches, as well as new and safer filler choices and neurotoxin complexes, point to a hopeful future for minimally invasive skin rejuvenation and treatment of aged skin. The advancement of combination treatment to improve skin renewal is the future. Many of these combo studies are now observational. More study is needed to determine the sorts of combinations, sequence, timing, and number of sessions needed for best results. Clinical research with a longer follow-up period and a bigger sample size might aid in answering these concerns. More strong aesthetic procedures concentrating on the combination of various non-surgical therapies to provide high quality surgical results with low recovery time are hoped to be done in the future.

Future Perspective

The advancement of combination treatment to improve skin renewal is the future. Many of these combo studies are now observational. More study is needed to determine the sorts of combinations, sequence, timing, and number of sessions needed for best results. Clinical research with a longer follow-up period and a bigger sample size might aid in answering these concerns. More strong aesthetic procedures concentrating on the combination of various non-surgical therapies to provide high quality surgical results with low recovery time are hoped to be done in the future and replace lots of the surgical face lift that’s not needed.

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