Chapter 2-Literature review

Although skin is the largest organ of the body destruction of only 15% of the skin's total body surface area is sufficient to be life-threatening. Indeed, the rupture of the skin's barrier function induces massive water loss that will rapidly cause a deadly hypovolemic shock. In addition, burn is the most traumatic injury that the human body can bear and induces a complete disruption of the body's homeostasis, including immunodepression, massive hypermetabolism, and vascular hyperpermeability, which enhances edema formation[32-33].

In the United States of America, the Medicare dataset on all wound categories was estimated expenditures ranging from $28.1 to $96.8 billion on wound treatment[34]. Another report from Wales estimated a prevalence of 6% of chronic wounds at a cost of 5.5% for National Health Service (NHS)[35]. Therefore, the economic impact generated by wounds is a concern. There are approximately 15,000 –20,000 hospitalizations per year for acute burn injuries in the U.S. recorded by the American Burn Association[36].

Up to 70% of all burns occur in lower middle-income countries. Out of which two third occur in Africa and South- East Asia regions. About 200,000-300,000 people die from fire-related burns each year. The mortality rate is disproportionately greater in LMICs than the developed countries. Fire-related burns are the 6^{th} leading cause of death among females aged 15-29 years. The South-East Asia Region about 184,000 burn deaths per year, or 11.6 per 100,000 populations, accounting for a staggering 59% of the entire global mortality from burns due to fires[37]. In India the estimated annual burn incidence is approximately 6-7 million per year Nearly 10% of these are life threatening and require hospitalization and 50% of scrambles to their injuries [38].Since burn death rates is low in high income countries, therefore it is essential to develop efficient, robust and cost effective intervention to decrease

the motility rate and increase the quality of life using modern technique like tissue engineering.

2.1.Structure and function of skin

Skin is the largest organ of the body making 15 % of total weight of body and plays a crucial role in protecting against external insults, fluid homeostasis, sensory detection, and self-healing. Human skin composed of three main layers: the cellular epidermis, relatively acellular collagen rich dermis and hypodermis or subcutaneous fat[39-40].

2.1.1. Epidermis

The epidermis mainly consists of layers of keratinocytes separated from the dermis by the basement membrane. The epidermis having a high impermeability is the outermost barrier against microorganism. The epidermis can be divided in two layers – dead and the living epidermis. Dead epidermis is made of keratinocytes – with high lipid, protein and low water content. It provides protection against microorganism. Living epidermal consists of melanin producing of melanocytes. Melanin is protective pigment in skin against harmful UV radiation form damaging DNA[41].

2.1.2. Dermis

Dermis is located beneath the epidermis, consists of connective tissues, whose main components are collagen and elastin. Collagen is the most common protein in the human body (70% of the dry weight of the dermis) and its main function is to ensure tensile strength of the dermis[42]. Collagen in the skin is trihelical occurs as fibers, which are formed from fine microfibers. Elastin is present in relatively smaller proportion is highly branched structure responsible for physiological elasticity of skin. It also contains a range of immune

cells including macrophages and dermal dendritic cells, nerve fibers, blood vessels, lymphatic vessels, hair follicles, sebaceous and sweat glands are located in the dermis[43].

The dermis can be subdivided into the upper papillary dermis and the lower reticular dermis. papillary dermis contains papillae that integrated with the basal layer of the epidermis at the dermo-epidermal junction. The dermis contains hair follicles, sweat glands, sebaceous glands, sensory nerve endings, lymphatic vessels and blood capillaries which extend to the dermal side of the dermo-epidermal junction. This allows nutrient and oxygen delivery to, as well as waste removal from, the avascular epidermis to occur by diffusion across the dermo-epidermal junction[44].

2.1.3. Hypodermis

Hypodermis is innermost layer of skin chiefly composed of subcutaneous fat layer, which, in turn, is composed of fat cells. Fat cells are involved in thermal regulation and nutrient accumulation of reserves. Lipids occupy the majority volume of fat cells. Arteries, veins and larger lymphatic vessels are located in hypodermis[41].

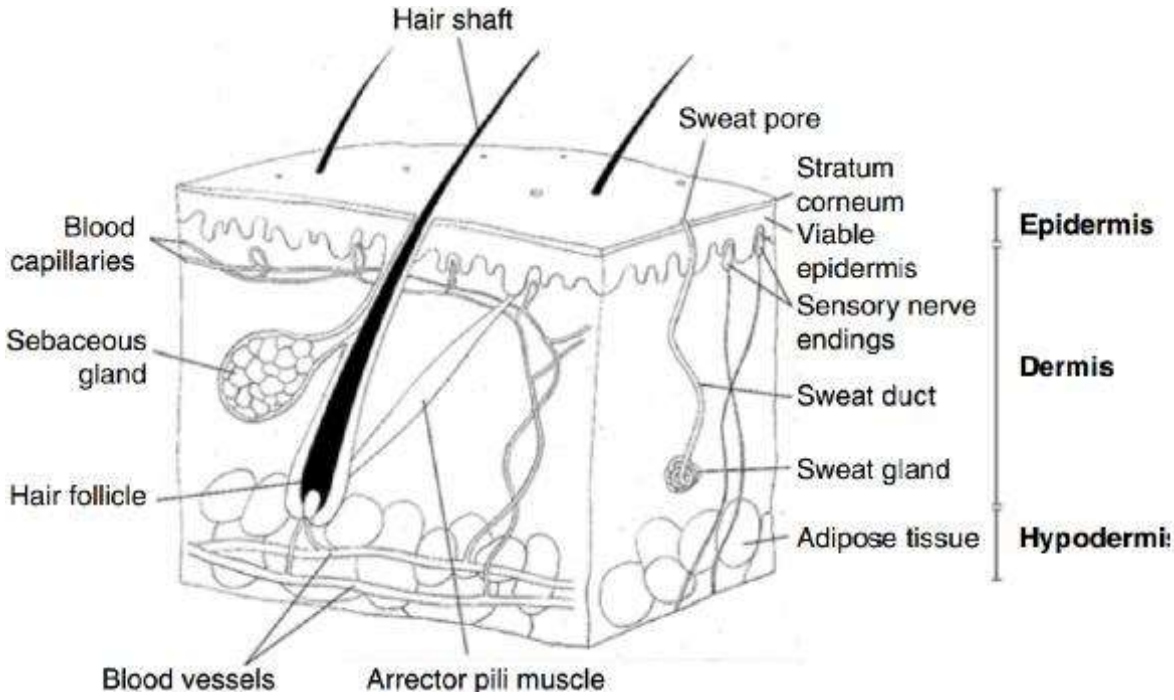

Figure 2.1. A diagrammatic representation of the structure of human skin in cross section[45].

2.1.4. Functions of Skin[46-47]

1. Skin provides protective barrier against mechanical, thermal and physical injuries. Skin acts as first line of defense against the microbes
2. It helps to maintain the homeostasis by regulation of excretion and temperature etc.
3. It acts as immune organ to detect and protect against microbial infection.
4. Skin converts precursor of vitamin D to active vitamin D
5. It regulates body temperature and salt concentration.
6. It senses touch, pain, pressure and temperature.
7. Protection against the UV radiation.

2.2.Wound & their types

Wound is the local disruption in the anatomy of skin. It occurs due to many reasons, which include acute trauma, genetic disorder, burns and scalds. There are several type of wound on the bases of the depth of the injury and disruption of tissue at the wound site. On the bases of depth wound can be dived into following types-

1. Epidermal
2. Superficial partial-thickness
3. Deep partial- thickness
4. Full thickness

Epidermal wound- They are typically of sunburns, light scalds or grazing. Characteristic of epidermal wound are erythema and minor pain. It does not require surgical treatment as only the epidermis is affected and this regenerates rapidly without scarring[48].

Superficial partial thickness wound-Epidermis and superficial parts of the dermis, with epidermal blistering and severe pain are the main characteristic of the superficial partial thickness wound. Such wounds are common in case of the first degree of burn. Such wound heals by the epithelization from the margin of the wound[49-50].

Deep partial-thickness wound it involve higher degree of dermal damage compare to superficial partial thickness wound which results in fewer skin appendages resulting into higher time taken to heal. In the depth of deep partial thickness wound scarring is more pronounced as remodeling of ECM occurs[51].

Full-thickness wound- It involves complete destruction of epidermis and most part of the dermis. Full- thickness wounds are more than one cm in diameter. They heal by contraction, with epithelialization form the edge of the wound which causes cosmetic and functional defects[52].

On the bases of source and its effect on the underlying tissue it can be divided into two types-

1. Closed wound
2. Open wound

In closed wound injury does not break the surface of the skin but causes damage to the underlying tissues i.e. it affect only the epidermal region of skin[53]. While in open wounds break the surface of the skin and may also damage underlying tissues i.e. it affects epidermal and dermal region of the skin[54-55].

2.3.Wound healing process

Since skin is very important for survival of the human as it has important role against the microbes, protects the body against any physical or chemical attack and maintains the homeostasis, therefore it must be healed quickly and efficiently in case of any wound**.** Wound has natural tendency of healing[56]. Although the process of wound healing is continuous process, it is arbitrarily divided into different phased in order to understand the physiological processes that are taking place in wound and its surrounding tissues. Wound healing involve the interaction between diverse immunological and biological system of various interdependent, interrelated and orchestrated steps which are following[57-58]-

1. Coagulation and homeostasis

2. Inflammation
3. Cell proliferation
4. Remodeling.

Coagulation and homeostasis-

After injury, coagulation and homeostasis takes place to prevent exsanguinations and protect the vascular system. It is marked with the blood vessel constriction which lead to reduction in blood loss and aggregation of platelets at the site of injury which lead to formation of blood clot to prevent or slow the bleeding[59-60].

Inflammation -

Homeostasis is followed by second step inflammation which is marked by the redness in the wound area. In this step humoral and cellular immune response occur with aim to establish an immune barrier against invading micro-organism. In this process white blood cell, macrophages migrate at wound site and bacteria, pathogens, debris are cleared away through the process of phagocytosis[61-62].

Cell proliferation-

When homeostasis has been achieved an immune response successfully taken place, then wound shifts toward tissue repair. Proliferative phase starts after three days and can last for 1-2 weeks. It is marked by cell proliferation phase which is characterized by epithelialization, angiogenesis (formation of new blood vessel), synthesis and deposition of collagen, fibronectin and elastin, formation of provisional extracellular matrix (ECM), formation of granulation tissue and wound contraction[63-64].

Remodeling-

It is the final stage of wound healing in which wound attempt to achieve normal tissue structure and function. This phase is responsible for the development of new epithelium and final scar tissue formation. The remodeling of acute wound is tightly regulated with aim to have a delicate balance between the degradation and synthesis of collagen bundles, hyaluronic acid and fibronectin. Initial deposition of collagen bundles is highly disorganized. After remodeling the collagen bundles become more organized and cross linked. Therefore, the strength of wound increases progressively. As wound heals the density of fibroblast and macrophages is reduced by apoptosis, the growth of capillaries stops, blood flow to the area declines[65-66].

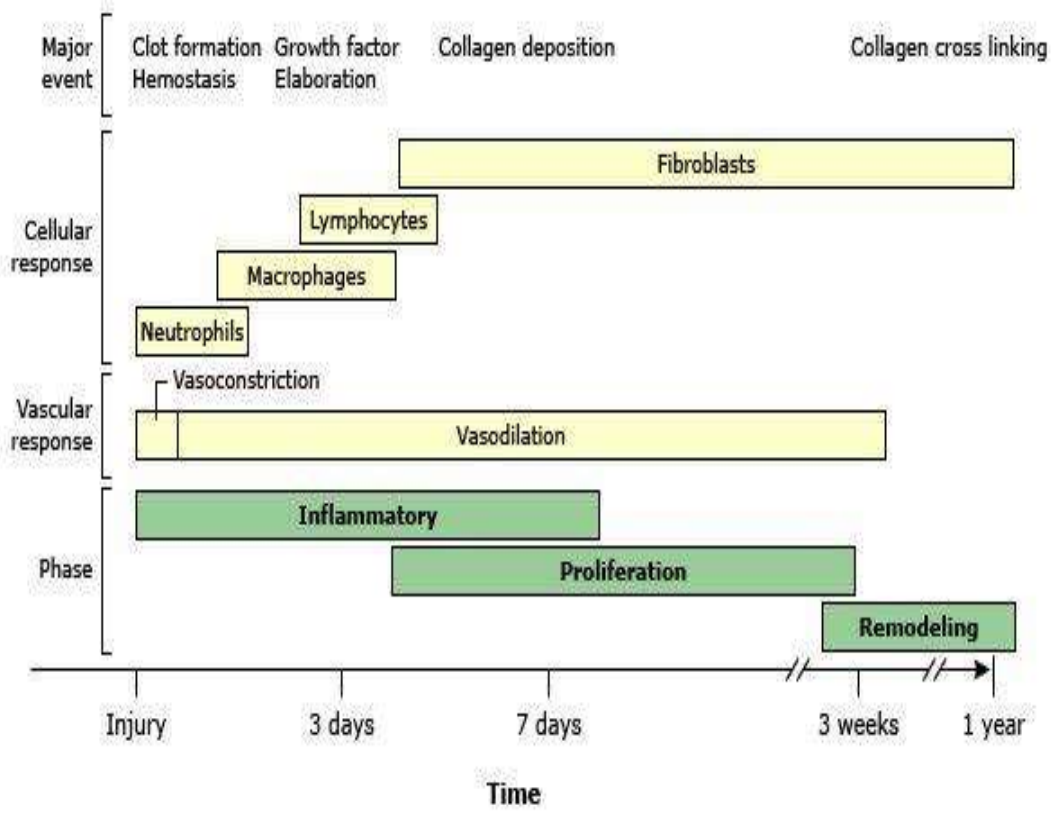

Figure 2.2. Various stages of wound healing [67]

2.4.Factors affecting wound healing process.

The wound healing process is not only complex but also fragile any disruption in any of the four process makes wound susceptible to failure in wound healing that leads to the non-healing wounds**.** Factors that contribute to non-healing chronic wounds are diabetes, oxygenation, sex hormones, medications, venous or arterial disease, infection, and metabolic deficiencies of old age, smoking, nutrition, inflammation, connective tissue disorders etc. [68-69].

Local factors	**Systemic factors**
Necrotic tissue burden	Diabetes mellitus
High bacteria burden or biofilm	Anemia
Excessive exudates	Malnutrition
High level of metalloproteinases	Immunodeficiency
Growth factors trapping or deficiency	Immunosuppressive medications
Corrupt extracellular matrix, cellular senescence	Age
Tissue hypoxia	Obesity
History of irradiation	Smoking

Table 2.1. factor affecting the wound healing process[70].

2.5.Biomaterial used in skin tissue engineering-

The field of tissue engineering has advanced dramatically and it offers the potential for regenerating damaged and diseased tissue or organ. Tissue engineering aims to restore, maintain, or improve functions of tissue that are defective or have been lost. Biomaterials play a decisive role in tissue engineering by providing synthetic frameworks called as scaffolds or constructs.

The art in biomaterials design has continuously evolved over the decades. In recent years, there has been increasing magnitude on materials that could be used in biomedical areas keeping the intention of biomedical applications target to develop artificial materials to renovate or restore function of diseased or traumatized tissues thus improving the quality of life[71-72].

Scaffold contains cellular and molecular modulators that stimulate wound healing are used for tissue engineering. Because of their open porous structure and good mechanical strength, they provide an optimal microenvironment for cell proliferation, migration, and differentiation. In tissue engineering applications, the criterion for selection of biomaterials is based on their material chemistry, molecular weight, solubility, structure, hydrophilicity and hydrophobicity, degradation pattern, nature of degradation product, water adsorption and swelling. Scaffolds is integral part of tissue engineering as it provides unique properties such as high surface to volume ratio, high porosity with small pore size, biodegradation, biocompatibility. All these vivid advantage leads to its use in biomedical application [72-74].

Scaffold materials can be synthetic or biological depending on the use. The properties of polymers depend on the composition, structure, and arrangement of their component

macromolecules. Naturally occurring polymers, synthetic biodegradable are the major types of polymers used as biomaterials[75].

Natural was first clinically used biodegradable biomaterials. Natural biomaterials are biocompatible due to similarity to macromolecule whichare recognized and used by human body that lead to enhance and better cell interaction between cell and its surrounding. Natural polymers can be classified as proteins (silk, collagen, gelatin, fibrinogen, keratin), polysaccharides (cellulose, amylose, chitosan). Natural macromolecules show a relatively low mechanical potency compared to synthetic polymers. By cross-linking or blending with synthetic polymers, the mechanical properties of natural polymers are improved. Natural polymers are widely used in the regenerative medicine due to its biocompatibility, biodegradability and similarity to the ECM[76].

2.5.1. Collagen

Collagen is the most abundant natural protein present in the human, account for 30% of the total proteins. Collagen provides structural integrity for connective tissues, such as dermis, bone, cartilage, tendon, ligament, and an internal organ thereby provides tensile strength and structural integrity to tissue. Collagen forms complex family due to structural complexity involved with tissue-specific expression, organization into heteropolymeric or homopolymeric fibrils and interaction with proteoglycans, adhesion glycoproteins, cells, and other collagens. Since collagen has wide spread distribution in various tissue and organs, it make it most important macromolecule for different biomedical application such as drug delivery as drug carrier , scaffold and wound dressing material[77]. Previously, it was believed that collagen have only structural function but later it was proven that collagen and its derivative controls many cell functions such as cell differentiation, migration and

synthesis of number of proteins. Collagen acts as natural scaffold in tissue system. Collagen protein is a helical polypeptide with the repeating sequences of glycine, proline and hydroxyproline. Main feature of collagen molecule is its stiff which is triple stranded helical structure. Three types of collagen namely type-I, II & III is found in connective tissue. Several animal origin collagen dressings have been found effective in wound healing of wound with third-degree skin burns and allergic conditions[78].

2.5.2. Chitosan

Another homoglycan polysaccharide, chitosan, which is a derivative of arthropod exoskeleton and crustacean shells called chitin, is the most popular polysaccharide biomaterial. Chitosan is composed of β (1–4) linked d-glucosamine residues with randomly distributed N-acetyl-D-glucosamine group. Chitosan has a wide range of applications in burn and wound treatments due to its hemostatic, antimicrobial and antifungal properties. It occupies a distinct position amongst other biomaterials due to its abundance, versatility, and unique properties including biodegradability, biocompatibility, non-toxicity, hydrophilicity, anti-bacterial and anti-fungal properties, and wound-healing effect. Chitosan is positive charged polymer which has been widely used for promoting the wound healing due to its antibacterial property and good drug delivery characteristics[79-81].

Chitosan promotes faster wound healing in many ways.

1. It accelerates blood clotting by activating platelets, thus promotes faster wound healing.
2. It promotes proliferation of fibroblasts, cytokine production through macrophage activation and angiogenesis.

3. It promotes synthesis and deposition of collagen and hyaluronic acid at the wound site. Thus, chitosan not only provides a wound healing effect by itself but also, in turn, helps in faster and scar-free wound healing by producing other important biomaterials.

Chitosan as a wound-healing accelerator has influence every stage of wound healing. Chitosan accelerate healing by enhancing the functions of inflammatory cells, such as polymorph nuclear leukocytes macrophages and fibroblasts[82-83]. Chitosan also enhances the tensile strength of wounds[84]. Properties of chitosan such as molecular weight, deacetylation degree and state of chitosan affect the wound healing properties of chitosan [83, 85-87]. Chitosan can be modified by chemical reactions with excellent elasticity, flexibility features, and a lower inflammatory response. Moreover, the degradation of chitosan produces harmless amino sugars, which can be absorbed completely by human body. Extensive studies using chitosan in combination with natural and synthetic materials have been reported to produce scaffolds for tissue engineering applications. Therefore, all these criteria make chitosan a viable candidate for a broad range of biomedical applications such as drug delivery carriers, surgical thread, wound healing, and for tissue engineering[88-89]. .

2.5.3. Gelatin

Gelatin is acquired by denaturing the triple helix structure of collagen, typically derived from bovine or porcine skin. Gelatin also exhibits excellent biodegradability, non-antigenicity, and cost efficiency similar to collagen. It has major drawback of forming colloidal sol at temperatures at or above 37 °C, and gels near room temperature. Gelatin scaffolds have been used for various applications such as wound healing, nerve, dental, bone, dermal tissue engineering applications and vascular grafts. Due to its similarity with collagen, it is mixed

with various synthetic polymers which have increased its tissue engineering applications in recent times [90-93].

2.5.4. Elastin-

Elastin is chemically inert, insoluble polymer with structural functions found in the wall of arteries and veins, lungs, ligaments, skin and intestines. It is present in ECM of connective tissues where elasticity and recoil are critical parameters. Elastin is used as a biomaterial as it has shown the ability to modulate the cellular physiology, influencing signaling, chemotaxis, and proliferation of cells. Elastin is used in several forms as insoluble and soluble form. The use of soluble elastin is advantageous over its insoluble form due to the straightforwardness in the handling and analysis of the material [94-98].

2.5.5. Silk

Silk fibers has been used in textiles for centuries and recently it has been explored for its biomedical and tissue engineering applications due to its high elasticity, high biocompatibility, high strength and toughness, and resistance to failure in compression. biocompatibility[99]. SF consists of a heavy and light chain (350 kDa and 25 kDa), that are linked together by a disulfide bond with β-sheet formation, which is responsible for the protein's tensile strength. SF is used in tissue engineering because of its natural strength, biocompatibility, slow degradation rate, good water vapor and oxygen permeability, minimal inflammatory response and ability to be used in several forms[68-70]. SF can be used as an electrospun scaffold, hydrogel, or film. Silk-based biomaterials can be tailored for vascular, bone or ligament and skin tissue engineering applications[100-103].

2.5.6. Alginate-

Alginate is a naturally occurring anionic and hydrophilic polysaccharide, extracted from brown seaweed and bacteria. It is made of blocks of (1-4) linked β-D-mannuronic acid and α-L-guluronic acid monomers[104]. Alginates provide a moist environment at the wound bed, absorb exudates, reduce wound pain, and lower the infection burden and odor and helps in hemostasis. Due to its wonderful properties of biocompatibility, biodegradability, non-antigenicity and chelating ability, alginate has extensive use in biomedical engineering, tissue engineering and drug delivery[105]. Alginate has anionic nature therefore it can react with anionic drugs, bio-molecules through electrostatic interaction. Alginate is widely used skin tissue engineering due to its ability to form sponges, hydrogels, electrospun mats[106-108]. Alginate has other attractive wound dressing properties such as good water absorption, antiseptic properties coupled with no toxicity and biodegradability. Composite made up of cellulose, chitosan and alginate by freeze drying facilitated fibroblast and endothelial cell migration, and enhances the expression of EGF, bFGF, CD31 in wounds[109].

2.5.7. Cellulose

Cellulose is the most abundant biopolymer in earth, made up of repeating units of β-d-glucose joined by β-1, 4-glycosidic linkages[110-111].Cellulose is used in wound management and wound mainly due to its moisture retaining properties as moist wounds heal faster . It also helps in the absorption of exudates resulting in the intake of cell debris and its porous architecture mimics ECM of skin tissue[112].

Modification of cellulose by surface immobilization with drugs and other therapeutic molecules such as signal peptides and blending with other polymers (synthetic or natural) and electrospinning have shown improved functional physical and chemical properties[113].

Cellulose can readily mix with other antimicrobials (antibiotics) to prevent the infection of the wound site. Cellulose loaded with vaccarin, povidone iodide and minocycline have been studied for efficient drug release and wound healing[114-115]. Hydrogel formed by mixing of bacterial cellulose and acrylic acid loaded with human epidermal keratinocytes and fibroblasts showed enhanced wound healing. Scaffold made using bacterial cellulose and gelatin showed complete regeneration of epithelium[116]. Effect of pH in the wound healing ability was assessed and Acidic cellulose was found with improved healing properties in cutaneous wound model[114].

2.5.8. Hyaluronic acid-

Hyaluronic acid is a high molecular weight glycosaminoglycan, present in the mammalian skin. It is made up of repeating units of d-glucuronic acid and N-acetyl-d-glucosamine linked by alternating β-1,4 and β-1,3 glycosidic linkages[117]. Hyaluronic acid has an active role in skin tissue engineering as it plays a critical role in wound healing by stimulating fibroblast proliferation, remodeling of ECM and keratinocyte migration[118]. On degradation hyaluronic acid have pro-angiogenic effect by activating a series of cascades to facilitates keratinocytes differentiation[119-120].. Hyaluronic acid has shown to alter the healing pattern with reduced scar and decreased collagen synthesis[121-122]. Li et al. showed accelerated healing with hemostasic ability due to improved anti-enzymatic degradation by hyaluronic acid grafted scaffold[123].

In addition to these widely studied biopolymers, there are several similar biomolecules of therapeutic interest such as; fucoidan, carrageenan, and glucans that are less explored. Research is being carried out to explore the potential capabilities of these molecules in

facilitating healing process. Fucoidan is fucose rich, uronic acid containing, sulphated polysaccharide isolated from brown algae. Heparin-binding cytokines like FGF-1 and FGF-2 in the wound exudates are activated by fucoidan inducing angiogenesis and wound repair. Another notable biopolymer with therapeutic applications is pullulan. It is derived from fungi and made up of repeating units of maltotriose. Wong et al. studied the wound healing effects of pullulan–collagen scaffold. Beta-glucans, a polymer of glucose, is a component of cell walls of cereals, mushrooms, algae and some bacteria also produce branched chain β-glucans.

2.6.Role of antibiotics in wound healing-

Skin has an important role in protection against aggressive agents by forming barrier against pathogens[124]. When tissue injury occurs, the body initiates a series of complex events, aiming to reestablish the structure and functions of the damages skin tissue. Repair process can be dived into four stages: hemostasis, inflammation, proliferation and remodeling[124]. This coordinated and regulated cellular, humoral and molecular processes lead to perfect tissue regeneration. However there are many factors like presence of infections, metabolic disorders (diabetes), and patient's agecan lead to an imbalance wound healing process which ultimately results into the inadequate wound healing and wound closure[125-126].

Chronic wounds represent a silent epidemic that worsens the patient's quality life by causing a significant financial burden on patients since treatment for chronic wounds is expensive and time-consuming. In addition to expense involved in treatment, when wound are not treated effectively, wound does not close and can lead to the sepsis, amputation and in worse case can lead to the death[127].

The progression of wound from a infected stage to chronic stage depends upon the various factors as the immune response of host and the amount and type of pathogen come in contact with injured tissue[128]. Open wound provides warm, nutritious environment for the growth of microorganism and forms the colonies. To check and restrain the colony formation, various interventions is been used (immunity modulating agents, bacteriophages and their lysines, antimicrobial peptides, pro and pre-symbiotics, plant extracts, pathogenicity inhibitors). In these interventions used, uses of antibiotics are most frequently and widely used. Use of antibiotics are frequent in wound management due to their favoring cellular and vascular proliferation which accelerates the wound healing by effectively reducing the infections[128-129].

Nitrofurazone-loaded PVA/ sodium alginate and clindamycin-loaded PVA/sodium alginate hydrogels, Gentamycin loaded polyvinyl Alcohol/dextran hydrogel were used to evaluate the effect of gentamycin in wound healing[130-132]. Ciprofloxacin loaded polyurethane–dextran nanofiber mat, collagen constructs with ciprofloxacin[133], gelatin loaded with ciprofloxacin[134] were used for its wound healing potential[22]. Therefore, knowing that one of the major complications of wound healing is infection, the correct use of antibiotics can speed up wound healing and significantly reduce health care costs[135].

2.7.Collagen-Chitosan based scaffold for skin tissue engineering-

Collagen is a unique biomaterial with various superior characteristics such as biocompatibility, biodegradability, which offer suitable platform for cells adhesion, proliferation and migration. Collagen can be dissolved in aqueous medium and can be molded in to various structures such as film, fiber and porous architecture.

Chitosan is biomaterials used in a variety of biomedical fields such as drug delivery carriers, surgical thread, and wound healing materials having many advantages such as hemostasis, accelerating the tissue regeneration and the fibroblast synthesis of collagen. Chitosan provides excellent cross-linking property when treated with crosslinking agent such as glutaraldehyde. Lie Ma et al (2003) fabricated collagen chitosan scaffold using freeze- drying method and glutaraldehyde was used to improve the biostability by crosslinking in aqueous medium[136]. Liu et al(2012) fabricated glutaraldehyde cross-linked collagen- chitosan scaffold using facile fabrication technique for skin tissue engineering[137].

Mahmoud and salama (2016) fabricated series of antibiotic norfloxacin-loaded cholagen-chitosan scaffolds using freeze-drying technique for wound healing. The result suggested that the norfloxacin loaded collagen/chitosan scaffold is a potential candidate for skin regeneration application[138]. Li it al (2017) fabricated ciprofloxacin loaded Poly(N-isopropylacrylamide)/poly(L-lactic acid-co-ε-caprolactone) fiber as wound dressing materials[139]. Liu it al (2018) has demonstrated the wound healing ability of ciprofloxacin loaded sodium alginate/poly (lactic-co-glycolic acid) electrospun fibrous mats using organic solvent binary mixture of fluoroethanol and chloroform[107]. Rathore et al (2019) evaluated the optimization of ciprofloxacin loaded chitosan film and its characterization such as swelling, degradation and its wound healing ability[140]. Sinha et al (2013) developed

ciprofloxacin hydrochloride loaded poly(ethylene glycol)/chitosan scaffold and studies the effect of polyethylene glycol in the mixed scaffold[21]

Shah et al (2018) has reported that in case of scaffold made of natural polymer as chitosan, collagen and gelatin etc, cross-linking process is crucial and therefore dual crosslinking process was used to attain the morphologically and mechanically stable scaffold[141].Chang et al (2014) discussed the cross-liking strategies and mechanism of different natural cross-liking agent such as genipin,Tannin acid and chemical cross-linking glutaraldehyde, 1-Ethyl-3-(3-dimethyl aminopropyl)-carbodiimide(EDC) and *N* hydroxysuccinimide (NHS) etc[142]. Zheng et al (2010)discussed the fabrication of electrospun PLGA/chitosan nanofibrous scaffold and antibiotic release profile from the scaffold[143]. Ghosh et al (2018) has discussed the release profile ciprofloxacin and effect of particular size on release profile of ciprofloxacin. They also discussed fitting of drug release profile into the various models such as Kursmyer-Pappus model, Higuchi model, Hixson-crowell model etc. [144].

2.8.Technique used for the fabrication for scaffold

Tissue engineering is an interdisciplinary field which involves engineering principles and knowledge of life sciences for the development of biological substitutes to restore maintain and improve the functions of organ or tissue. A well-designed scaffold is fundamental to guide tissue formation.

Characteristics of scaffolds

1. It should biocompatible. It should not cause unwanted immune response to host body.
2. Interconnected pore with suitable size should be present in scaffold so it can promote the cell infiltration and migration form scaffold.
3. It should have excellent surface chemistry so that it promotes cell attachment, proliferation of cells.
4. Control biodegradability
5. Suitable mechanical strength to control the structure and formation of new tissue.
6. It should mimic the function of the tissue or organ.
7. It should promote the ECM formation and provides suitable cell signals to promotes the tissue or organ preparation.

   Many techniques have been developed to fabricate three-dimensional porous scaffolds. They have their advantages and disadvantages depending upon the nature and type of function the scaffold.

S. No	Method	Merit	Demerit	References
1	Solvent casting	Economical and cost effective, Control over, pore size, porosity and crystallinity.	Limited mechanical property, residual toxic solvents, denaturation of protein and time consuming	[145-146]
2	Particulate leaching technique	Less amount of polymer required,	Pore shape and inter pore connectivity not retained,	[76, 147-148]
3	Gas foaming	Does not require toxic organic solvent, control over pore and porosity.	Limited mechanical property and inadequate pore connectivity.	[149]
4	Self-assembly	Fabrication of nano fibers with thin fiber diameter. Low cytotoxicity.	Expensive material, complex design parameters, complicated and elaborated process.	[150-151]
5	Electrospinning	Control over porosity, pore size and high surface area and high aspect ratio.	Limited mechanical strength, pore size decrease with fiber thickness, cells are hard	[152-153]

			to migrate, solvent toxicity can occur	
6	Phase separation	Activity of the molecule does not decrease. It can be integrated with other fabrication technique.	Selection of solvent and temperature is critical and scaffold morphology is difficult to control.	[154-155]
7	Rapid prototyping	Excellent control over geometry, porosity, provide highly reproducible architecture no supporting material required	Limited polymer type, highly expensive equipment and low resolution.	[156-158]
8	Fiber mesh	Large surface area for cell attachment, rapid nutrient diffusion for cell growth	Lack the structural Stability and low structural orientation and crystallinity	[159-160]
9	Fiber bonding	High surface to volume ratio, high porosity allow tissue ingrowth and allow the production of	Poor mechanical property, limited applications to other polymers	[161]

		extracellular matrix.		
10	Melt molding	Control over the scaffold architecture, pore size and porosity.	Not possible for all the polymer and required high temperature for non-amorphous polymer	[162-164]
11	Membrane lamination	Provide 3D matrix with defined anatomical shape.	Lack required mechanical strength, inadequate pore interconnectivity, long processing time	[165]
12	Freeze drying	Use in a variety of purposes Capability of obviating high temperatures pore size is manageable to be controlled by changing the freezing method	Small pore size and irregular size pore, long processing time High energy consumption, use of cytotoxic solvents	[166-168]

Table 2.2. Different method of scaffold preparation: their advantages and limitations.

2.9.Commercially available skin tissue engineering product

Wound can occur for many reasons, including genetic disorders, acute trauma, thermal trauma chronic wounds or even surgical interventions. Sometimes burn or mechanical injuries of skin can result in extensive deep wound which could not be healed with common techniques. Therefore, new innovative intervention has been devised in form of skin tissue engineering products which facilitated the wound healing process and improve the quality of life by accelerating the healing process.

Wound treatment approaches differ according to the nature of wound[169]. Epidermal injuries such as sunburn, light scalds or grazing does not require any surgical treatment[170]. Superficial partial-thickness wounds affect the epidermis and superficial parts of the dermis. Such wounds heal by epithelialization from the margins of the wound such wound are capable of self-renewal. Deep partial-thickness wound has greater dermal damage resulted in fewer skin appendages remaining which has resulted in slow healing and degraded quality of wound healing as scarring in healed site is more profound and vivid. Complete destruction of epithelial-regenerative elements which are more than 1 cm in diameter leads to full thickness wound such wound heals by contraction and epithelialization takes place from the edge of the wound, leading to cosmetic and functional defects[171].

Therefore, alternative life- saving approaches in the treatment of full thickness wounds includes bioengineered skin substitutes. Significant progress has been made in the field of designing and fabrication the bioengineered skin substitutes. Some commercially available skin tissue engineering products are listed below-

Serial number	Brand name	Scaffold material	References
1	Karoskin	Native human cadaver skin with dermal and epidermal cells	[172]
2	OrCel	Bovine collagen sponge	[173]
3	MySkin	silicone support layer with a specially formulated surface coating	[174-175]
4	AlloDerm	human acellular lyophilized dermis	[176-177]
5	OASIS Wound Matrix	porcine acellular lyophilized small intestine submucosa	[178-179]
6	PolyActive	PEO/PBT	1[180-181]
7	Tissue tech autograft system Laserskin and Hyalograft 3D	HAM	[182]
8	Apligraf	Bovine collagen sponge	[183-184]
9	Allograft (cadaveric)	native human skin with dermal and epidermal cells	[185-186]
10	Lyphoderm	Fibrin substrate	[187]

11	TransDerm	Fibrin substrate	[188]
12	PermaDerm	Bovine collagen	[189-190]
13	Integra Dermal Regeneration Template	polysiloxane, bovine cross-linked tendon collagen, GAG	[191-192]
14	Terudermis	Silicone, bovine lyophilized crosslinked collagen sponge made of heat-denatured collagen	[193-194]
15	Biobrane/Biobrane	silicon film, nylon fabric, porcine collagen	[195-196]
16	TransCyte (DermagraftTC)	silicon film, nylon mesh, porcine dermal collagen	[197-198]
17	Dermagraft	PGA/PLA, ECM	[199-200]
18	Hyalomatrix PA	HYAFF layered on silicone membrane	[201-202]
19	Hyalograft 3D	HAM	[203-204]
20	Tegaderm	Poly (e -caprolactone)/gelatin nanofibrous scaffold electrospun on polyurethane dressing	[205-206]

Table 2.3. Commercially available skin tissue constructs.

2.10. Role of oxygen in wound healing and oxygen producing scaffold for skin tissue engineering.

The survival and functional maintenance of cells in the 3D matrix (i.e., scaffold) until the in growth and maturation of blood vessels is the most important challenge in tissue engineering for producing a clinically relevant volume of tissues/organs This is true for large cell-seeded&non cell-seeded implants. Cells within a bioengineered tissue are more than a few hundred microns away from a blood vessel will not survive due to diffusion limitations. Thus, problems associated with lack of oxygen and nutrient diffusion occur, and these ultimately lead to cell and tissue necrosis. This has been a critical limiting factor for developing functional tissues for human applications.Tissue engineered tissues/organs such as the skin, trachea, and bladder have been developed but their dimensions caused low vascularization therefore limit oxygen diffusion that leads to the hypoxia which negatively affect its clinical efficacy. The oxygen dissolved in the aqueous solution can be diffused limitedly (100-200 μm) to cells seeded on the scaffold; therefore, the cells should be located within the critical distance from oxygen sources to prevent hypoxia and necrosis. It is well known that the central region of the engineered tissues with a volume larger than 1 cm^3 is usually in a hypoxic environment, and thus imperfect tissues with a necrotic center and viable cells only at the periphery are produced.

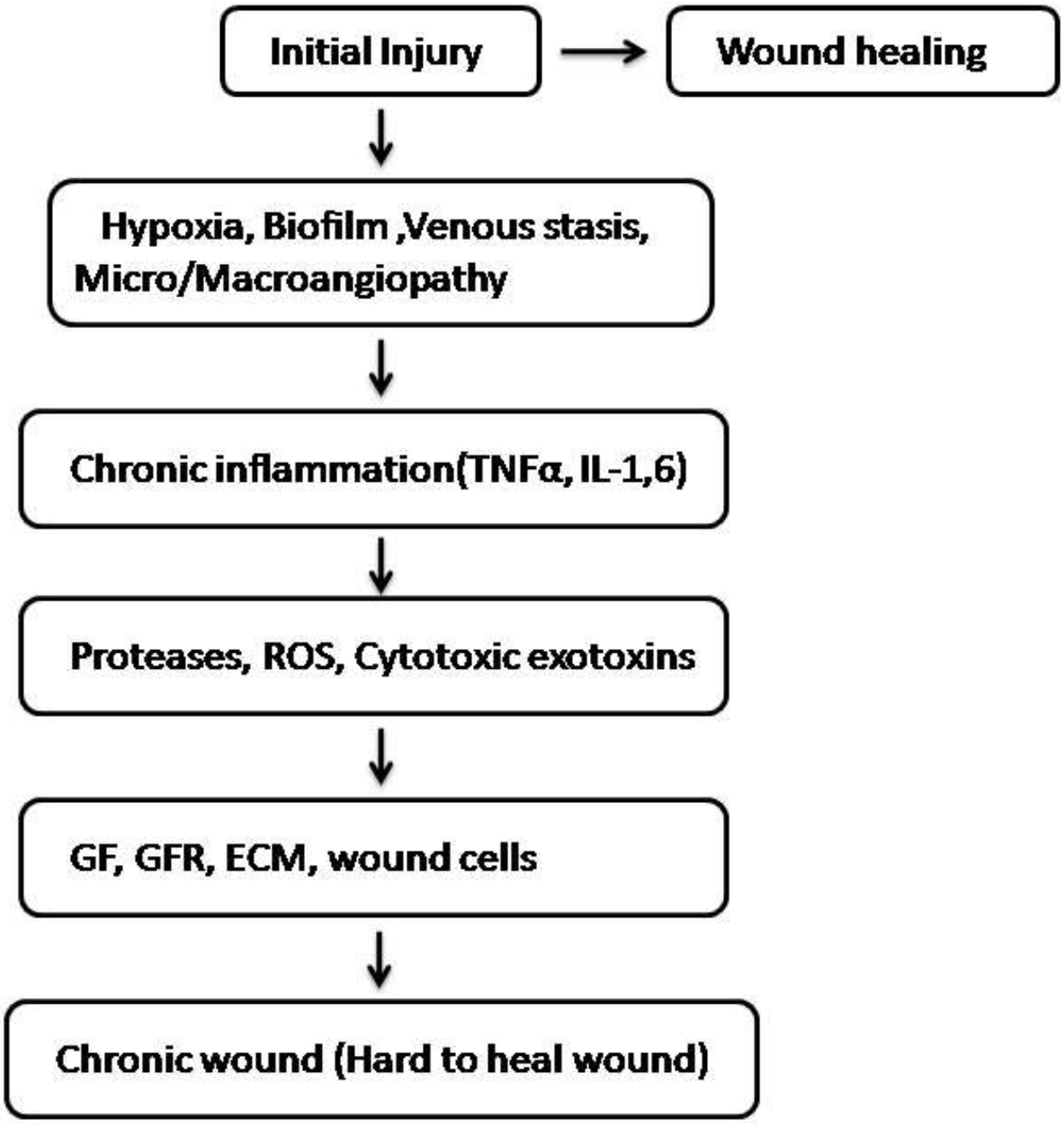

Figure 2.3. Schematic diagram of effect of hypoxia and infection in wound healing

2.10.1. Role of oxygen in wound healing -

Oxygen is a potent modulator of cell functions and wound repair in vivo. Oxygen concentration has important effects on some of the healing processes as aerobic metabolism of the cells, activity of many enzymes such as cyclooxygenases, collagen synthesis, expression of some angiogenic genes, and proliferation and differentiation capacity of stem cells[207]. The lack of oxygen (hypoxia) in one hand can potentially limit cellular respiration, cell growth and potentially cell necrosis. On the other hand it enhances the production of the specific extracellular matrix components and increase angiogenesis through the hypoxia-inducible factor-1 pathway. Therefore these divergent actions of hypoxia should be addressed[208]. To overcome the inherent limitation of tissue engineering, several strategies including the use of oxygen carriers (perfluorocarbons, fluorinatedzeolite and angiogenic factors (vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), endothelial cells, and VEGF are been used[209].More recently, oxygen-producing material loaded scaffold systems, which can produce oxygen by decomposition or chemical reaction of peroxides incorporated into the scaffolds, and thus can provide an appropriate oxygen environment. Using of oxygen generating biomaterials in tissue engineering construct may increase the oxygen availability to cell thereby increase the chances of the tissue survival at the site of implantation [30, 209-210].

2.10.2. Oxygen-generating materials

There has been continuous effort have been made to design and develop scaffold which provide sufficient nutrient especially oxygen, to overcome the effect of severe hypoxia at the site of wound healing. An emerging approach is using 3D scaffolds made from oxygen-generating biomaterials to tackle transport limitations deep within the engineered tissues. oxygen generally does not diffuse few millimeters beyond the open surface of skin. Therefore hypoxic condition generated must be deal This class of oxygen generating materials has opened a new window for overcoming the challenges associated with ischemia occurring within transplant[211].

Based on state, oxygen generating molecules are two types-

1. Solid oxygen generating materials
2. Liquid oxygen generating materials

It has been found that solid inorganic peroxides such as calcium peroxide (CaO_2), sodium percarbonate ($(Na_2\ CO_3)_2 \cdot 1.5H_2O_2$) and magnesium peroxide (MgO_2) have been proposed for generation of oxygen within liquid environments [27, 30, 212-213]. Liquid oxygenating materials are hydrogen peroxides and perflourocorbon. Because inorganic solid oxygen produces oxygen in more controlled and sustained manner than the liquid oxygen producing material. Prolong, controlled and sustained release of oxygen is required for tissue engineering. In addition to solid and liquid peroxides a new group of new group of oxygen producing chemicals such as perfluorocarbons (PFCs) are also used in tissue engineering application[214-215].

When inorganic peroxide mention above come in contact of water formation of hydrogen peroxide (H_2O_2) takes place in the first step, followed by decomposition of H_2O_2[216].

$$CaO_{2(s)} + 2H_2O \rightarrow Ca(OH)_{2(s)} + H_2O_2$$

$$MgO_{2(s)} + 2H_2O \rightarrow Mg(OH)_{2(s)} + H_2O_2$$

$$2H_2O_2 \rightarrow O_2 + 2H_2O$$

Calcium peroxide is more preferred than other inorganic peroxide because it found in purest form and cost efficient. Above mentioned inorganic peroxides have good cellular compatibility and generally do not show cytotoxicity[217].

The rate of oxygen release is of significant importance for tissue construct formation. When chemical reaction occurs following two conditions can occur-

1. If oxygen release takes place too quickly, the oxygen cannot be utilized due to super saturation. Which lead to formation of unwanted genesis of free radical and singlet oxygen. These free radical and singlet oxygen lead to increased inflammation, which ultimately cause tissue impairment.
2. If the oxygen is released too slowly, it does not provide a sufficient source to maintain healthy cellular function.

The rate of oxygen formation from peroxide compounds depends on a number of factors, including temperature, pH, ratio of solid peroxide to water, amount of catalyst and type of catalyst. In addition, the hydrophilicity of the surrounding biopolymer also influences the release rate of oxygen from the source. For example, if a hydrophobic material is used to

encapsulate solid peroxides, the rate of oxygen release reaction is slow due to the slow diffusion of water into the hydrophobic materials In this case; solid peroxide particles do not immediately come into contact with water, which leads to a slow release of oxygen. On the other hand, in the case of hydrophilic materials, reaction with water occurs quickly and burst release of oxygen and accumulation of ROS and singlet oxygen occur [210, 212, 218].

Oxygen generating materials forms hydrogen peroxide, which may be toxic in higher concentration, can lead to unwanted side reactions and cellular damage. Therefore, it is necessary to develop a strategy for efficient conversion of H_2O_2 in to water and oxygen. Therefore, use of Catalase enzyme system is a common strategy for removal of hydrogen peroxide. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. Catalase, an enzyme found in almost all living cells that is responsible for decomposing hydrogen peroxide to water and oxygen, can be encapsulated in biomaterial as an antioxidant to accelerate decomposition of H_2O_2 and O_2 [219]. Apart from Catalase, Vitamin C also acts as antioxidant and singlet oxygen quencher which has shown its efficacy to significantly improve the survival of fibroblast and muscles cell grown in hypoxic condition[220-221]. Without catalase, the formation of ROS, singlet oxygen and peroxide may lead to unwanted side reactions and cellular damage. Therefore, using a catalyst is a common strategy for the conversion of H_2O_2[222].

Various methods have been developed to incorporate oxygen-releasing material into biomaterials for *in situ* generation of oxygen. Two methods are most used

(1) Adsorption of oxygen-releasing molecules into fibers or scaffold

(2) Encapsulation of oxygen producing substance within 3D polymer networks.

Inorganic peroxide such as calcium peroxide (CPO), when come in contact with water, it immediately releases the oxygen, which will lead to production of various reactive oxygen species. Therefore, it is necessary to control the immediate and burst release oxygen and ROS. To achieve slow, constant and prolong release of oxygen particles of CPO is coated with the hydrophobic polymer PCL. PCL exhibits the limited bulk permeability and slower the diffusion rate of water. Therefore, CPO coated inside the PCL, releases the oxygen at slow, sustained and prolongs way. In one hand it promotes prolong and sustained release of oxygen and on other hand it efficiently limits the peroxide and free radicals' generation, thereby preventing the cell and tissue damage due to ROS and singlet oxygen.

Apart from coating and encapsulation with hydrophobic polymers other factors such as pH, temperature, purity, solubility, and presence of specific buffer or catalyst also affect the oxygen production in specific condition.

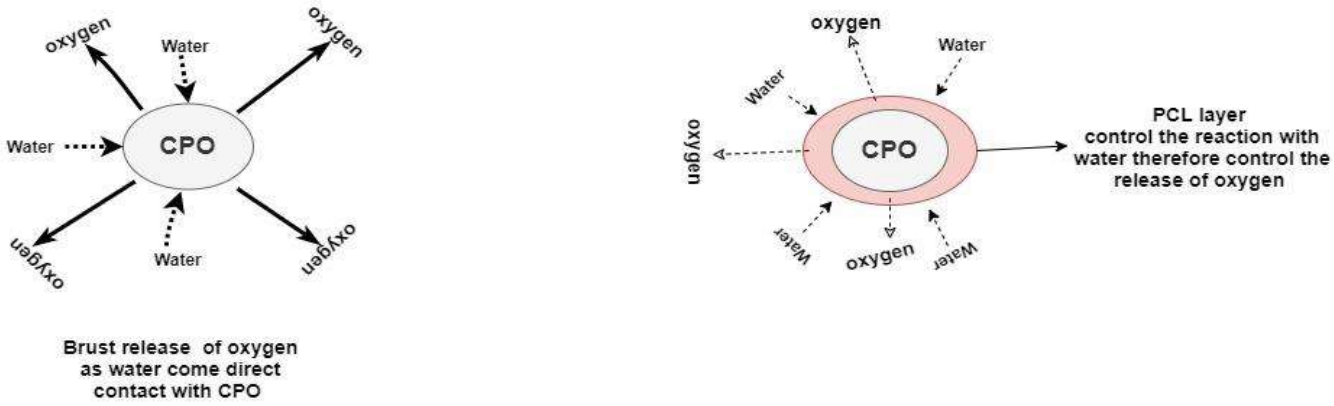

Figure 2.4. Schematic diagram shows effect of hydrophobic coating in the release of oxygen.

2.10.3. Notable work in field of oxygen releasing scaffold and hypoxia.

Mozafari et al (2018) fabricated oxygen releasing biphasic calcium phosphate (BCP) scaffolds were prepared using robocasting technique, has shown a great potential for bone tissue engineering. In the preparation of scaffold an oxygen releasing agent, calcium peroxide (CPO), was dip coated in PCL[28].Miao Tong et al (2017) has shown the role of oxygen in wound healing site using hyperbaric oxygen therapy (HBOT). Effect of oxygen in wound healing was shown in diabetic mice[223]. Though the mechanism of HBOT is still not clear. Se Heang Oh (2014) has used perfluorooctane emulsion was used to prepare scaffold. Effect of hypoxia in tissue necrosis and cell survival was studied on oxygen releasing hollow micro particle made up emulsion[27].

Stabler et al (2012) demonstrated that hypoxia induced death of cell was controlled by using PDMS-CaO_2 disk under hypoxic condition. They also find out that oxygen is very critical in the initial phase of implantion[224].Moztarzadeh et al (2018) has constructed the scaffold with oxygen releasing potential with controlled chemical synthesis of CaO_2 particles coated with polyethylene glycol and its characterization of crystallite size and oxygen release kinetics. Egaña et al(2014) developed innovative photosynthetic scaffold containing algae to treat broad spectrum of hypoxia. Since algae are capable of producing oxygen during photosynthesis hence it acts as oxygen producing scaffold[225]. Carsten Werner et al developed an oxygen producing gellan gum hydrogels for dual delivery of either oxygen or peroxide. It contains calcium peroxide and catalase for oxygen production[226].Xiaojun Yu et al (2010) have studied the antimicrobial properties of nano-fibrous PCL scaffold coated with CPO. Scaffold shows antibacterial property due to generation of the peroxides and free radicals[227]. Chao Feng (2016) fabricated silk- keratin oxygen producing scaffolds by

varying different proportion. CPO as oxygen generating chemical used without any hydrophobic polymer[26]. Ashok Kumar et al (2018) formulated antioxidant cryogel scaffold made up of polyurethane. Cryogel contains catalase enzyme that quench reactive oxygen species and help in sustained release of oxygen for Tissue Engineering Applications[228].Jeong Ok Lim et al (2018)fabricated an oxygen releasing scaffold using hydrogen peroxide instead of inorganic peroxide such as CPO. H_2O_2 is embedded inside the poly(lactic-co-glycolic acid) (PLGA) using double emulsion method[229].