INTRODUCTION

Burn is a type of injury to skin or flesh. Burns are classified into 3 different degrees, depending on how severe the damage is to the skin and its underlying tissues ***(Herndon, 2012).*** Burns that affect the epidermis only are known as superficial or first-degree burns. When damage penetrates into the layers of dermis, it is a partial-thickness or second-degree burn. In a full-thickness or third-degree burn, the injury extends to all layers of the skin. A fourth-degree burn involves injury to deeper tissues, such as muscle or bone ***(Pereira et al., 2012).***

Burn injury can be caused by heat, freezing, electricity, chemicals, radiation or friction ***(Singer et al., 2013).*** Thermal injury to the skin is generally characterized by variable necrosis and cell injury ***(Papp et al., 2004).*** Burn wounds evolve in depth and size in the first few days after injury, leading to loss of the integrity of the skin ***(Cleland, 2012).*** Burn sufferer may experience a number of complications including shock, infection, electrolyte imbalance and respiratory failure ***(Ha et al., 2010).***

Partial-thickness burn may heal within 2 weeks but inappropriate treatment may leave scars or may be complicated with infection that severely impairs the process of healing ***(Miyazaki et al., 2012)***. In case of full thickness burn injury, all regenerative elements have been destroyed, and healing only

occurs from the edges and is associated with considerable contraction and scar formation ***(Papini, 2004).***

A major gap in the treatment of burn is the lack of an effective therapy that reduces burn injury progression. Furthermore, during the healing process of deep partial or full-thickness burns treated by the known skin substitutes like autologous skin grafts or tissue engineered skin, wound contraction and scar formation are still unavoidable ***(Burd et al., 2007; Lu et al., 2009).***

The key step for burn therapy is to promote the wound healing as early as possible ***(Liu et al., 2014).***

The use of mesenchymal stem cells (MSCs) is becoming more realistic in burn treatment ***(Lu et al., 2009). Xue et al., (2013); Singer et al., (2013); Liu et al., (2014)*** reported that MSCs improved the quality of burn healing; reduced the formation of scars and re-established the normal function of the skin and its appendages.

AIM OF THE WORK

The aim of the work is to study the effect of bone marrow derived mesenchymal stem cells in improving the healing of burns.

REVIEW OF LITERATURE

Histology of Skin

Skin is known as the integument and is composed of epidermis, an epithelial layer of ectodermal origin and dermis, a layer of mesodermal connective tissue. At the junction between the dermis and epidermis, there are dermal papillae which are projections that interdigitate with invaginating epidermal ridges. Epidermal derivatives include hair, nails, sebaceous glands and sweat glands. Beneath the dermis lies the hypodermis which is a loose connective tissue layer usually containing pads of adipocytes ***(Mescher, 2013)***.

The epidermis of thin skin is a four layered stratified squamous epithelium. They are as follows: **Stratum basale** which is composed of a single layer of cubical to columnar cells. **Stratum spinosum** is composed of several layers of polygonal cells connected together by desmosomes**. Stratum granulosum** is composed of one to three layers of flattened cells. Keratinocytes of this layer accumulated basophilic membrane bounded keratohyalin granules. **Stratum corneum** is formed of 15 to 20 layers of flattened, non-nucleated keratinized cells whose cytoplasm is filled with cytokeratin filaments ***(Gartner and Hyatt, 2014).***

The dermis is formed of two layers, the papillary layer and the reticular layer**.** The papillary layer is the superficial

layer, consists of loose connective tissue containing collagen fibers type I, collagen fibers type III, elastic fibers, blood vessels and nerve processes. The reticular layer, lies deep to the papillary layer, is thicker and less cellular than the papillary layer. It is formed of thick, irregular bundles of type I collagen fibers and coarse elastic fibers. Thin skin contains hair follicles, their associated sebaceous glands and sweat glands. Each sebaceous gland opens into a hair follicle. Hair follicles, sebaceous glands and sweat glands extend through the whole dermis and may also be present in the hypodermis ***(Ross and Pawlina, 2016)***.

Skin Burn Injury

Skin burn represents a type of injury that can be caused by heat, electricity, chemicals, radiation or friction. Most burns are due to flame injuries. Burns due to scalds are the next most common ***(Allgower et al., 2008)***.

Jackson (1953) described three zones of tissue injury resulting from a burn. This description remained the foundation of understanding the pathophysiology of burn injury to the skin. The three zones of burn were **zone of coagulation** which was the central severely damaged zone where irreversible tissue loss occurred due to coagulation of the constituent proteins. **Zone of stasis** surrounded the zone of coagulation and was characterized by decreased tissue perfusion due to vasoconstriction and ischemia. The tissue in this zone was

initially viable and then converted to coagulation as a consequence of the development of edema and infection. **Zone of hyperemia** was the outermost zone where tissue perfusion was increased resulting from vasodilatation. The tissue in this zone remained viable unless there was severe sepsis or prolonged hypoperfusion (Fig. 1).

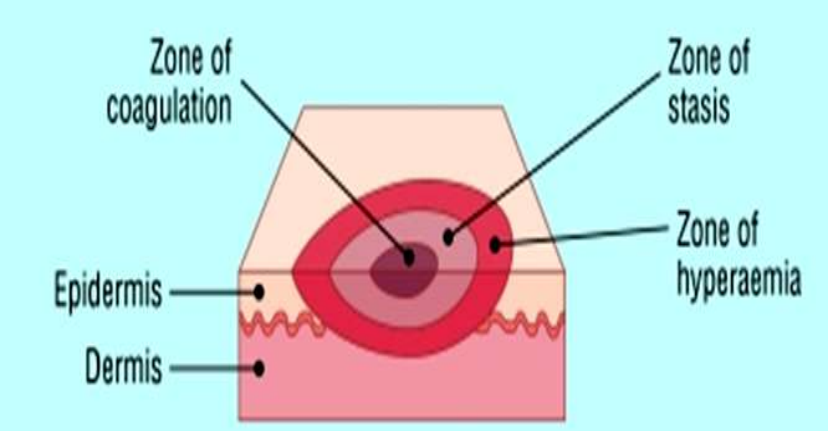

Figure (1): Jackson's burns zones ***(Hettiaratchy and Dziewulski, 2004)***.

Evers et al. (2010) mentioned the international classification of skin burn injury which was categorized into three degrees (I-III) depending on the invasion depth of burn. In the first-degree burn (epidermal burn), the injury was limited to the epidermis. Second-degree burn was classified into superficial partial and deep partial burn. They added that the third-degree burn (deep burn), involved the subcutaneous tissue and caused cellular death.

Park et al. (2001) carried out a deep burn model on rats by applying a brass rod heated in 75 °C hot water on their

contralateral sides. Histological examination of thermally damaged skin revealed that the collagen fibers lost their linearity, fused with each other and created a glass-like homogenous appearance from the dense coagulations of collagen.

Dasu et al. in (2003) performed a full-thickness scald burn on the dorsum and ventrum of the rats of 40% total body surface area (TBSA). They found that major thermal injury caused tissue damage by membrane destabilization and energy depletion at the cellular level, resulting in tissue necrosis, ischemia and delayed cell death.

Oliveira et al. (2008) studied the effect of scalding thermal burns performed on the dorsum of rats, 30% TBSA. At the 7^{th} day post injury, histological examination of the skin showed lost epidermis and the ratio between collagen fibers type I and III were altered due to the increase in the immature type III collagen fibers.

Younan et al. (2010) added that a second-degree scald burn in mice showed cytoplasmic vacuolation in the epidermis, and disruption of the tight junctions between the basal cells. The hair follicles showed mild hydropic swelling and disruption of their epithelium. Also, the dermis at the burn site showed coagulative necrosis and the collagen fibers were denaturated (lost linearity and birefringence resulted in hyaline appearance).

In ***Campelo et al. (2011)*** experiment, skin burns were inflicted by direct heat transfer conduction from copper to skin utilizing gradually different increasing temperatures, 100°C, 150°C and 200°C. Burn injury was induced on the back of rats on a 4 cm^2 area. Histological examinations of burns showed damaged to lost epidermis. The dermis presented obliterated vessels and damaged pilosebaceous units.

Tanaka et al. (2013) performed a burn 20 mm in diameter by immersing the back of male Wistar rats in hot water for 10 seconds. They controlled the degree of the skin burn by the temperature of the hot water, 70°C for a superficial dermal burn, 78°C for a deep dermal burn, and 98°C for a deep burn. Dermal collagen fibers in skin burn visualized by collagen sensitive second harmonic generation microscopy showed that the fibrous structures of the dermal collagen in deep burn were almost completely lost and changed into amorphous structures. They also added that by Masson trichrome stain in deep burn, the boundaries between collagen bundles and collagen fibers completely disappeared.

Yang et al. (2014) studied the effect of the second degree burn on the back of rats exposed to boiling water for 20 seconds. They found that the collagen and elastic fibers in the dermal layer were destroyed. Few residual hair follicles were visible, and also showed necrosis of the sebaceous glands.

A scald burn injury was induced by ***Kiyan et al. (2015)*** on the back of the rats using boiling water for 10 seconds. Histological evaluation of the skin revealed thinned epidermis and the collagen in the dermis gained a denaturated hyaline appearance. They also noted degeneration and apoptosis in hair follicles and sebaceous glands.

Regarding skin burn complications, ***Blaha (2006)*** observed that in patients who had burn injury, they developed burn induced scars which was characterized by delayed restoration of stratum granulosum of the epidermis while the hair follicles and sebaceous glands almost did not regenerate. The papillary layer of the dermis was not completely formed and it was partially restored during the first months of scar maturation. In burn induced scars, collagen fibers in the dermis were formed almost without the elastic fibers.

Zhang and Fu (2008) reported that healing of burns occurred with high risk of infection and hypertrophic scarring. Superficial burns that left hair follicles intact healed rapidly with the regeneration of epidermal appendages. Deep burns that affected the hair follicle bulge healed with a scar and without regeneration of epidermal appendages.

Van der veer et al. (2009) reported that burn injury altered the pH and temperature of the skin thus it subjected the wound to infection and development of chronic non-healing ulcers and hypertrophic scarring.

In a clinical study, bacteriological skin samples were collected on admission of burn patients. Data collected showed that burn wounds lacked epidermis and showed obliterated blood vessels. Few hours after the burn, wound surface became contaminated with many bacteria that started to grow and multiply ***(Essayagh et al., 2014)***.

Burn Injury caused the immediate onset of acute inflammation mediated by cytokines secreted from resident plasma proteins, recruited hematopoietic cells, extracellular matrix and bacteria. Resolution of the inflammatory response was essential to achieve successful wound healing. Resolution of inflammation was directed by the decrease in proinflammatory mediators and the return of normal microvascular permeability. That was followed by stopping of the secretion of local chemo-attractants, synthesis of anti-inflammatory mediators and lymphatic drainage. In case of prolonged and excessive inflammatory response, increased tissue injury and poor healing occurred ***(Eming et al., 2007)***.

Keloids were described as raised overgrowth of skin tissue in the form of reddish nodules that were developed at the site of the burn injury. After burn, both the epidermal cells and connective tissue cells (fibroblasts and myofibroblasts) multiplied in order to repair the injury. The connective tissue fibers were deposited by the fibroblasts to hold the wound closed. In keloid, the fibroblasts continued to multiply even after the wound was filled in and the keloid projected above the

surface of the skin and formed large piles of scar tissue ***(Brewin and Lister, 2014)***.

Concerning spontaneous regeneration after skin injury, ***Taylor et al. (2000)*** studied involvement of long-lived epithelial stem cells in the bulge region of the hair follicle in renewal of follicle and epidermis after wounding. They detected follicular stem cells that were actively involved in supplying progenitor cells to the epidermis when needed, such as during neonatal expansion of the skin and adult wound repair.

Fathke et al. (2004) mentioned that after skin injury multipotent stem cells mobilized from the bone marrow into the pool of circulating cells. These cells migrated to the site of skin injury and regulated the migration and proliferation of epithelial and dermal mesenchymal cells during the early inflammatory phase.

Further investigations were done by ***Ito et al. (2005)*** to determine whether bulge cells of hair follicles were necessary for epidermal renewal by ablation of these cells through targeting them with a gene encoding herpes simplex virus thymidine kinase. They concluded that after epidermal injury, cells from the bulge region of the hair follicle recruited into the site of injury of epidermis. These bulge-derived cells acquired an epidermal phenotype and responded rapidly to epidermal injury by generating cells responsible for acute wound repair.

Gaelle and Cedric (2008) noticed in their study the release of soluble mediators from the platelets and injured blood vessels, leading to formation of a blood clot. Later on, they noticed that the invasion of neutrophils, monocytes and leukocytes promoted migration and proliferation of various cell types including keratinocytes, leading to the beginning of the reepithelialization process. However, they concluded that major skin injuries which resulted from extensive burns could not be completely repaired alone and required medical interventions to heal properly.

In a trial for successful treatment, ***Burd et al. (2007)*** reported that during the healing process of deep partial or full-thickness burns treated by autologous skin grafts or tissue engineered skin, wound contraction and scar formation were unavoidable. Furthermore, ***Zhang and Fu (2008)*** stated that transplanted skin from donors was not an option due to rejection; however, augmenting immune tolerance via stem cell therapy might overcome that problem.

Treatment of burns required rapid removal of the scab with supportive therapy. This was achieved through surgical scab excision and split thickness autografts from healthy skin areas of the same patient. It was found that this treatment did not restore the functional dermis and epidermal appendages despite providing epidermis and a thin layer of dermal tissue ***(Bargues et al., 2010).***

Despite the use of many different wound dressings, ointments and devices, wound healing still remained a clinical challenge in burned patients ***(Wasiak et al., 2013).*** Therefore, new strategies are needed to promote and help in wound healing and repair ***(Arno et al., 2014).***

Liu et al. (2014) stated that bioengineered skin substitutes were still in the experimental stage. They suggested that novel and effective therapies for promoting wound healing needs investigations such as mesenchymal stem cell therapy.

Mesenchymal Stem Cells (MSCs)

Vats et al. (2002) classified stem cells by origin into embryonic and non-embryonic stem cells. The latter were referred to as adult or somatic stem cells. ***Baharvand and Kazemi (2005)*** added that embryonic stem cells were identified as pluripotent cells in embryos, and are defined by their origin in the inner cell mass of the blastocysts.

Leeb et al. (2010) stated that adult stem cells (ASCs) were found in low abundance in almost all adult tissues and in high abundance in the umbilical cord. They were found in special regulatory niches as self-renewing progenitor cells that were able to produce one or more specialized cell types. ***Gomillion and Burg (2006)*** mentioned that adult stem cells were considered to be tissue specific, self-renewing populations

of cells, which could differentiate into cell types of the tissue or the organ in which they resided.

Mesenchymal stem cells (MSCs) were first isolated and characterized by ***Friedenstein et al. in (1974).*** They were non-hematopoietic stromal cells that were present in the bone marrow. They composed only 0.001% to 0.01% of the total nucleated cells in the marrow.

Kastrinaki et al. (2008) defined MSCs as multipotent adult stem cells which are abundant in human tissue. Mesenchymal stem cells are easily isolated from adult tissues, not ethically restricted and had low immunogenicity ***(Le Blanc, 2006)***. They are classified according to their tissue of origin, as bone marrow MSCs (BM-MSCs), umbilical cord MSCs and adipose MSCs ***(Al-Nbaheen et al., 2013)***. Bone marrow cell types were harvested from the bone cavities by aspirating the bone marrow using a thick needle ***(Lorenzi et al., 2008).***

Dominici et al. (2006) reported three criteria to define MSCs. These criteria were adherence to plastic, specific surface antigen expression, and multipotent differentiation potential. Population of MSCs isolated from bone marrow expressed CD105, CD73, CD44, and CD90. However, MSCs did not express CD34, CD31, CD45, and CD11 that typically identify the hematopoietic cells and endothelial cell lineages only.

Hematopoietic cells in the bone marrow were found to express certain surface antigen marker, thus lack of expression of these antigens was a way to validate the presence of MSCs in the bone marrow ***(Xiao, 2012)***. Mesenchymal stem cells have the ability to expand many times in culture while retaining their growth and multilineage potential ***(Chamberlian et al., 2007).***

Badiavas et al. (2003) mentioned that MSCs propagated in vitro and retained their multipotency through multiple passages and could differentiate into adipose tissue, cartilage, bone, muscle, neurons, liver cells and cardiocytes, among other cell types.

Leclerc et al. (2011) defined stem cell therapy as a therapeutic administration of living cells for tissue regeneration, support for any defective function, such as wound healing and modulation of pathophysiological processes, such as hyperinflammation and immune dysfunction**.**

Mesenchymal stem cells have gained considerable attention due to their potential use for cell replacement therapy and tissue engineering ***(Ciapetti et al., 2006)***. The rationale for use of MSCs in different models of tissue healing was due to their ability to differentiate to several cell types and their homing capacity towards injured tissues in response to various chemokines ***(Chamberlain et al., 2007)***.

Mesenchymal stem cells were considered 'immunoprivileged' and permit allo-transplantation without