The Effect of Silver Nanoparticles on the Healing Process of Excisional Skin Wounds in Adult Albino Rats: Histological and Immunohistochemical Study

Thesis

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وَقُل رَّبِّ

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List of Abbreviations

Ag	:	Silver
AgCl	:	Silver chloride
AgNps	:	Silver nanoparticles
bFGF	:	basic fibroblast growth factor
Caco-2	:	Humanepithelialcolorectaladenocarcinoma
ECM	:	Extracellular matrix
EGF	:	Epithelial growth factor
H2O2	:	Hydrogen Peroxide
HCT-116	:	Human colon cancer cell line
Hep G2	:	Human liver cancer cell line
HT-29	:	Colon Cancercell
IgG	:	Immunoglobulin G
IL-12	:	Interleukin-12
IL-6	:	Interleukin-6
KGF	:	Keratinocyte growth factor
MC	:	Methylcellulose
MCF-7	:	Breast cancer cell line
MMP	:	Matrix metalloproteinase
MPI	:	Matrix metalloproteinase inhibitor
O2-	:	Superoxide
PDGF	:	Platelet-derived growth factor
PMNLs	:	Polymorphnucluear leukocytes
ROS	:	Reactive oxygen species
SSD	:	Silver sulphadiazine
TGF- α	:	Transforming growth factor alpha
TGF-β	:	Transforming growth factor beta
VEGF	:	Vascular endothelial growth factor
vWF	:	von Willibrand factor

Introduction

The skin is the largest organ in the human body. It serves important functions including; protection of internal organs, sensory perception, immunologic surveillance, thermoregulation and control of fluid loss **(Morton and Phillips , 2016**).

A skin wound is defined as a disruption of normal tissue structure and function. There are a variety of causes which give rise to different types of wounds. These include; surgery (planned intervention), trauma (i.e., burns or lacerations), pathological changes in the body (i.e., circulatory vessels associated with leg ulcers), as well as wounds related to pressure (i.e., pressure ulcers) **(Walburn *et al*.,2009).**

Wound healing is a natural restorative response to tissue injury. It is a complex biologic process which involves mainly four different interlacing phases; homeostasis, inflammation, proliferation and maturation **(Rajkumar *et al*., 2017).**

Chronic non-healing wounds present serious drawbacks. It constitutes a substantial economic burden to healthcare system. In addition, it causes significant reduction in the quality of life for those affected and often precedes serious events like limp amputations or even premature deaths**(Järbrink *et al*., 2017)**. Delayed wound healing may result from several causes among them are;

systemic diseases, old age, and some drug treatment **(Basu and Shukla, 2012).**

Mechanism of wound healing has been understood more clearlyduring the last few years but there has been a constant search formethods and materials which can improve wound healing and reduce the quantity ofscar tissue **(Naraginti *et al*., 2016).**

Recently, nanotechnology is becoming of increasing interest in medicine. Nanoparticles (1 to 100 nm in size) have a large surface area-to-volume ratio which increases their interaction with the tissues and offer improved penetration into wounds**(Mordorski *et al*., 2015).**

Silver nanoparticles are the most commonly used nanoparticles in our health care system. They have become of intense interest because of their antibacterial, antifungal, antiviral and anti-inflammatory activity **(Ge *et al*., 2014 and Kumar *et al*., 2013).**

Reviewing the literature, very few studies were available regarding the role of silver nanoparticles in skin wound healing **(Naraginti *et al*., 2016 andBoroumand *et al*. 2018).**

Aim of the Work

The present study was carried out to determine the role of silver nanoparticles in the healing process of excisional skin wounds in adult male albino rats using light microscopic, immunohistochemical and morphometric methods.

Anatomy and Function of Skin

Skin covers the entire external surface of the body, including the external auditory meatus, the lateral aspect of the tympanic membrane and the vestibule of the nose. It is continuous with the mucosae of the alimentary, respiratory and urogenital tracts at their respective orifices, where the specialized skin of mucocutaneous junctions is present. It also fuses with the conjunctiva at the margins of the eyelids, and with the lining of the lachrymal canaliculi at the lacrimal puncta. Skin forms 8% of the total body mass. Its surface area varies between 1.2-2.2 m^2. Its thickness ranges from 1.5–4.0 mm, according to its state of maturation, aging and regional specializations **(Standring, 2016).**

Skin has an important role in the maintenance of body fluid homeostasis, thermoregulation and regulates many metabolic processes **(Jahromi *et al*., 2018).** It also serves as a strong barrier against any harmful environmental insult that may result in loss of the integrity of skin (such as wound) and ultimately lead to morbidity or even death **(Ram *et al*., 2016).**

Microscopic Structure of Skin

Human skin consists of two dependent layers: the outer epidermis and inner dermis which rest on a fatty subcutaneous layer called the panniculus adiposus **(Kolarsick *et al*., 2011).**

Epidermis

The epidermis of thin skin is divided into four layers according to keratinocyte morphology and position as they differentiate into horny cells **(**Fig. I, **Tortora & Nielsen, 2012 and Gartner & Hyatt, 2014).**

I) Stratum basale (basal cell layer)

This layer is generally only one cell thick. The main cell type is the keratinocyte that may be dividing or non-dividing. They are columnar or cuboidal cells that have dark stained oval or elongated nuclei and rest on the basement membrane. Melanocytes are present in the basal layer and make up 5 to 10% of the cell population.

II) Stratum spinosum (spinous or prickle cell layer)

Basal cells or keratinocytes move towards the surface and form a layer of polyhedral cells which are connected by desmosomes. Keratinocytes in this layer are seen as prickles under microscope. Within this layer, Langerhans cells can be identified**.**

III) Stratum granulosum (granular cell layer)

Keratinocytes in this layer are flat. They contain intracellular granules of keratohyalin. The cytoplasm also

contains smaller lamellated granules (Odland bodies). The cells discharge their lipid component into the intercellular space which plays an important role in barrier function and intercellular cohesion with the stratum corneum.

IV) Stratum corneum (horny layer)

This is the outermost layer of the epidermis. It is comprised of cells that have migrated from the stratum granulosum. The cells (now called corneocytes) have lost their nuclei and cytoplasmic organelles. The cells appear flattened and are completely filled with keratin filaments. The time from cell division to shedding from the horny layer is approximately 28 days and is called desquamation.

In the thick skin of the feet and hands, there is an additional layer called stratum lucidium which is superficial to stratum granulosum, it is formed of several rows of clear and dead keratinocytes that protect the underlying layers.

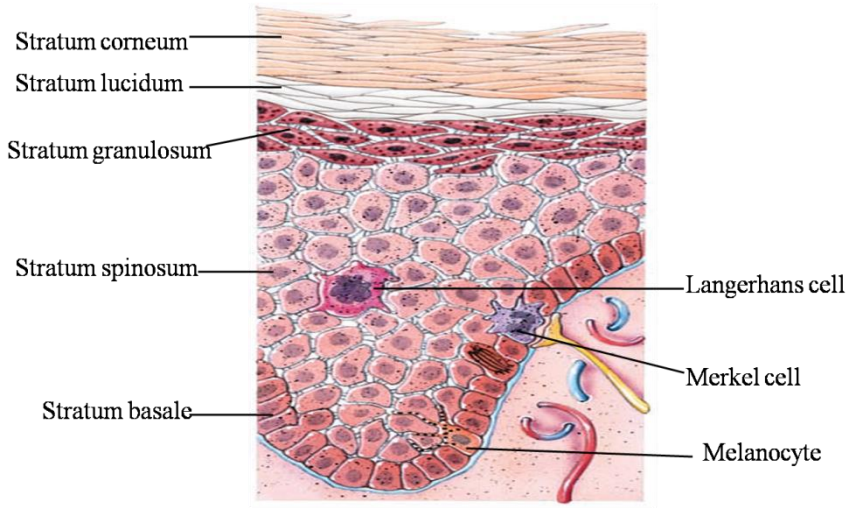

Fig.I : The main features of human epidermis, including different layers and cell types **(Tortora and Nielsen, 2012).**

Dermis

It is the inner layer of the skin that provides its structural integrity, elasticity and nutrition. It contains fibroblasts and extracellular matrix enriched withcollagen and elastic fibers. Also it is enriched with blood vessels, lymphatics, nerve endings, hair follicles, sebaceous and sweat glands **(Zomer and Trentin, 2018).**

About 70% of the dry weight of the dermis is made up of collagen types I and collagen type III. Elastic fibers are less tough than collagen fibers but convey extensible properties to the skin. They account for about 5% of the dry weight of the dermis (**Lai-Cheong and McGrath, 2009).**

The upper layer of the dermis is called the papillary dermis and is composed of thin collagen fibers, while the lower layer of the dermis is called reticular dermis and is composed of thicker and denser collagen fibers **(Mikesh *et al*., 2013).**

The rat skin almost has similar structure to the human skin except for two main things; the first difference is that the epidermis of the rat skin shows more hair follicles associated with sebaceous glands, blood capillaries and connective tissue cells mostly fibroblasts. The second is that the epidermis of human shows more thickness than that of the rat **(Dorsett-Martin, 2004).**

Skin Wounds

A skin wound is defined as a disruption of normal tissue structure and function. It can be categorized by its etiology, location, or duration**(Walburn *et al*., 2009).**As regards duration of wounds, it can be classified as acute and chronic wounds. Acute wounds progress through the normal stages of wound healing and showsigns of healing within four weeks, while chronic wounds often stalks in one phase of wound healing and do not show evidence of healing within four weeks **(Demidova-Rice *et al*., 2012).**

There are a variety of causes which give rise to different types of wounds. These include; surgery (planned intervention), trauma (i.e., burns or lacerations), pathological changes in the body (i.e., circulatory vessels associated with leg ulcers), as well as wounds related to pressure (i.e., pressure ulcers) **(Walburn *et al*., 2009).**

Wound healing

Wound healing involves a complex series of interactions between different cell types and mediators. It has four characteristic phases with considerable overlap; hemostasis, inflammation, proliferation, and remodeling **(**Fig. II,**McCulloch & Kloth, 2010 and Morton &Phillips, 2016).**