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MARINA SKY HÄNSEL

TOWARDS SCAR-LESS SURGERY: A SYSTEMATIC REVIEW OF THE BASAL LAMINA AFFINITY BONDING

DIPLOMA THESIS

Academic year:
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Mentor:
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TABLE OF CONTENTS

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ABBREVIATIONS

IGF-1 – insulin-like growth factor 1
TGF-α – transforming growth factor alpha
TGF-β – transforming growth factor beta
PDGF – platelet derived growth factor
FGF – fibroblast growth factor
VEGF – vascular endothelial growth factor
IL – interleukin
TNF-α – tumor necrosis factor alpha
MMP – matrix metalloprotease
ECM – extracellular matrix
HD – hemidesmosome
NSAIDs – non-steroid anti-inflammatory drugs
BM – basement membrane
BPAG1 – bullous pemphigoid antigen 1
EGF – epidermal growth factor
PGFR – platelet growth factor receptor
bFGF – basic fibroblast growth factor
FGF – fibroblast growth factor
EPC – endothelial progenitor cell
EMT – epithelial-mesenchymal transition
NO – nitric oxide
AgNP – silver nanoparticles
ZnONP – zinc nanoparticles
SARS-CoV-2 – Severe acute respiratory syndrome coronavirus
COVID-19 – Coronavirus disease 2019
SNA – Spherical nucleic acid
AuSNA – gold nanoparticle-based spherical nucleic acids
LSNA – liposomal-based spherical nucleic acids
ZnSNP – zinc sulfide nanoparticles
SPN – smart polymeric nanodrugs
CONP – cuprous oxide nanoparticles
NP – nanoparticle

1. INTRODUCTION

1.1. The function and structure of the skin

The skin serves as the protective layer of the body and consists of three layers (1). The outer layer, the epidermis, is firmly attached to and supported by the connective tissue found in the underlying layer, the dermis. The third layer, called hypodermis, contains adipose tissue.

The epidermis consists of superficial stratified squamous epithelium, which is flattened and filled with keratin. Epidermal ridges connect to the basement membrane of the dermis by interlocking with dermal papillae (*rete pegs*). There is no vascular supply in the epidermis. Constantly dividing cells in its deepest part, the basal layer, and shedding of dead surface squames enable a constant thickness of the epidermis. The basal layer is made of a separate sheet of columnar cells with hemidesmosomes binding it to the *lamina densa* of the underlying basement membrane. This basement membrane lies at the interface between epidermis and dermis and consists of the *lamina densa*, which is rich in type IV collagen, and the *lamina lucida* (1).

The function of the dermis is to structurally and nutritionally, support the epidermis. With age this layer of the skin will get thinner and lose elasticity. Within the dermis three distinct components can be determined: cells, fibers, and an amorphous ground substance. Fibroblasts comprise the main cells of the dermis, with other cells making up a small percentage and mostly serving an immunological function. Most interwoven fibers in the dermis are collagen fibers. With its high tensile strength, collagen prevents tearing. Next to the collagen fibers, elastin performs a crucial role in the dermis, as it allows it to return to its unstretched state. The amorphous ground substance of the dermis primarily consists of two glycosaminoglycans (hyaluronic acid and dermatan sulfate).

Besides the aforementioned layers, other structures, like smooth and skeletal muscle, blood vessels, nerves, and cutaneous lymphatics, can be found. Some areas of the skin contain structures like hair follicles, sebaceous glands, and eccrine sweat glands (Figure 1).

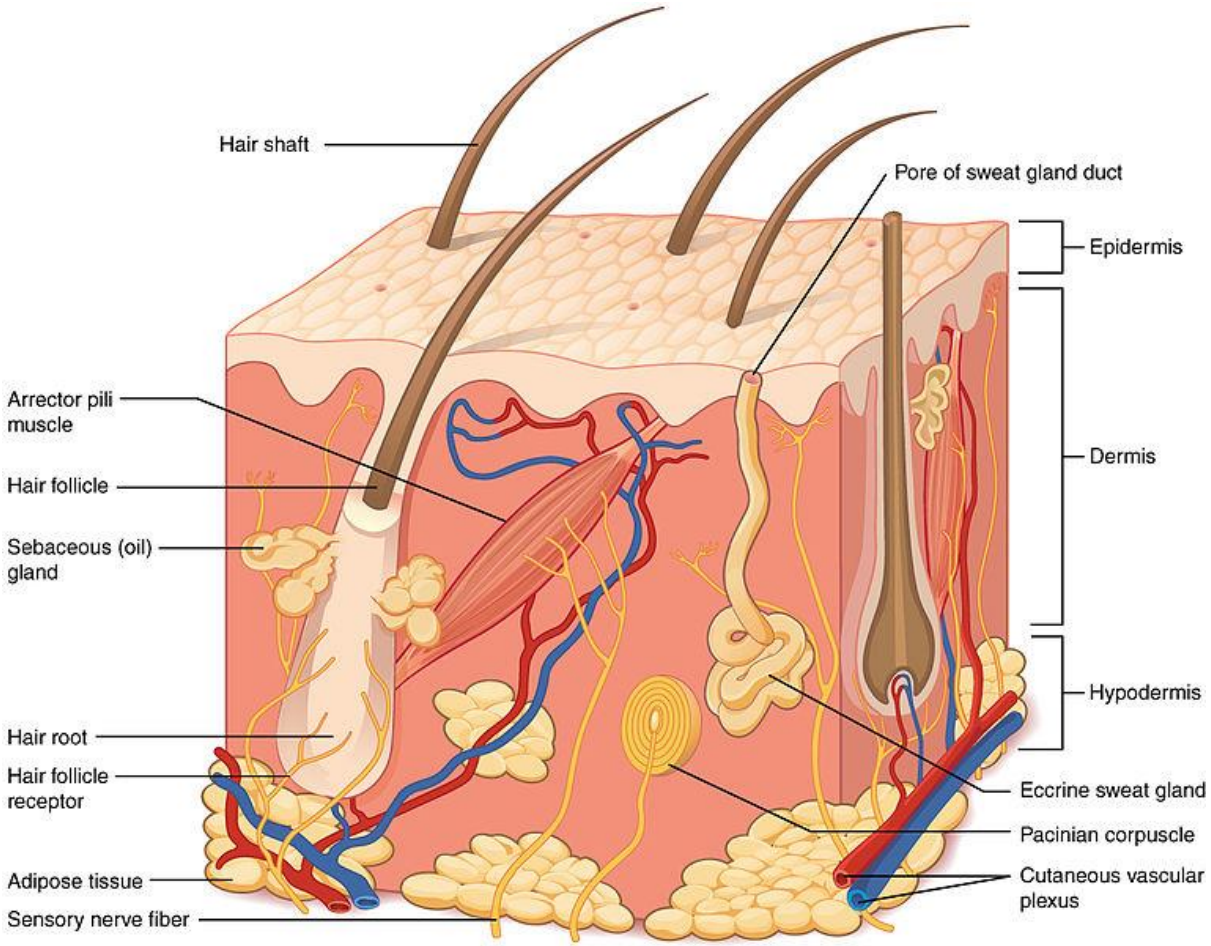

Figure 1: Skin layers and adjacent structures v

(SOURCE: https://commons.wikimedia.org/wiki/File:501_Structure_of_the_skin.jpg)

1.2. Definition of Wound

A wound is an injury that is caused by the transfer of kinetic, thermal, or chemical energy to the tissue, in which the skins anatomical integrity is disrupted (2). We can differentiate between acute and chronic wounds, as there is a difference in the mechanism of healing.

Acute wounds undergo the typical phases of healing and result in complete and sustained repair. This can commonly be observed in healthy and uninjured tissue. The process of wound healing in acute wounds takes between 6–12 weeks. Most surgical wounds fall under this category (1).

Chronic wounds on the other hand, fail to heal within 12 weeks of sustaining the injury. This is due to a pathological prolongation of tissue repair caused by a dysregulation in one of the phases of normal wound healing. Most commonly, this prolongation is linked to an inflammatory process caused by an infection or another chronic irritation of the injured tissue. The most important mechanism in the development of a chronic wound is tissue hypoxia. A second mechanism is failed epithelialization due to repeat trauma or desiccation of the wound. Chronic wounds frequently require surgical treatment, which can be used to convert them into the acute wounds.

1.3. Wound healing

1.3.1. Clinical wound healing

Wound healing can be described as primary and secondary depending on whether the injury has been sustained in a clean or a contaminated environment, respectively (2). Primary healing occurs in the surgical field when the wound is incised in a sterile environment and is anatomically re-approximated. This type of healing commonly proceeds without complication. In contrast, the secondary healing occurs when the wound is left open to heal until it is covered by migration of epithelial cells. This is commonly seen in infected wounds and burns. The granulation tissue that is formed in secondary healing consists of capillaries, fibroblasts, and a provisional extracellular matrix, which forms at the base of the early wound (2).

A third type of healing, which combines primary and secondary, is called delayed primary healing (2). During this process, a wound is left open to heal for 5 days in a controlled, moist environment after which it is closed primarily. This type of healing is recommended over primary closure if the tissue has been contaminated. It is less likely for the tissue to become infected because the bacterial balance can be maintained, and oxygen requirements are improved through capillary formation in the granulation tissue.

1.3.2. The mechanism of wound healing

The mechanism of wound healing is complex and involves several wound healing signals, including peptide growth factors, complement, cytokine inflammatory mediators, and metabolic signals like hypoxia and accumulation of lactate (2).

For a wound to completely heal, the skin must undergo four highly programmed steps. The first step in this process is hemostasis, followed by inflammation, proliferation and finally remodeling (3) (Figure 2).

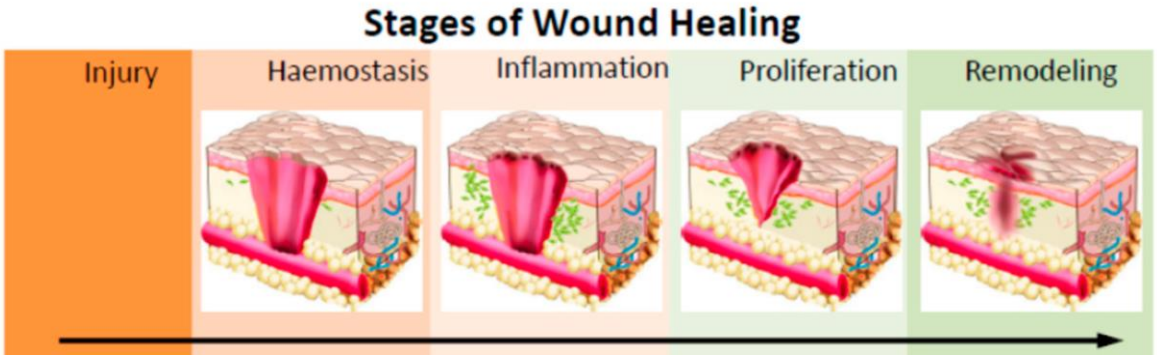

Figure 2. Stages of wound healing (SOURCE: https://www.mdpi.com/1422-0067/22/9/4748/htm)

1.3.2.1. Hemostasis and coagulation

For a wound to heal and for the host to survive, an injury must stop bleeding (3). This is known as hemostasis. It is initiated by vasoconstriction and fibrin clot formation, which includes cellular and molecular components. Besides their importance in hemostasis most cellular and molecular elements involved in this process also play a role in signaling tissue repair (2). Once the skins integrity has been disrupted, a clot starts forming. Coagulation products like fibrin, fibrinopeptides, thrombin split products and complement components then attract inflammatory cells into the wound. Thrombin will activate platelets to release pro-inflammatory cytokines and growth-factors like insulin-like growth factor 1 (IGF-1), transforming growth factor α (TGF-α), transforming growth factor β (TGF-β), and platelet derived growth factor (PDGF). This will promote the inflammatory phase and lead to the migration (*chemotaxis*) of macrophages, neutrophils, and lymphocytes into the wound once the bleeding is controlled (3).

1.3.2.2. Inflammatory phase

The inflammatory phase, which commonly lasts several days, is characterized by an increased vascular permeability which aids the migration of blood plasma and leukocytes to the injured area (3). This limits additional damage, helps close the wound, and removes cellular debris and bacteria (4).

1.3.2.3. Proliferative phase

The proliferative phase involves the formation of granulation tissue, re-epithelialization, and angiogenesis. It typically lasts up to several weeks and is predominated by the proliferation of fibroblasts and endothelial cells (4). Re-epithelialization is induced when cells from the periphery start to migrate into the wound. In the beginning only few cells are laid down forming a very thin superficial layer which gets thicker and more durable with time.

Fibroplasia and matrix synthesis fulfill a vital role in returning the skin to its anatomical structure. While fibroplasia is occurring throughout wound healing it is mostly stimulated by growth factors released by platelets, macrophages, and fibroblasts (2). The most important growth factors and cytokines to stimulate wound healing and fibroplasia include fibroblast growth factor (FGF), IGF-1, vascular endothelial growth factor (VEGF), IL-1, IL-2. IL-8, PDGF, TGF-α, TGF-β and TNF-α. Almost simultaneous to cell proliferation an angiogenic response will be initiated. This response prevents local tissue hypoxia caused by the increased number of cells in the tissue.

The final step of the proliferative phase is the formation of granulation tissue. Once fibroblasts have migrated to the wound site and started proliferating, they will be signaled to start secreting collagen type III, glycosaminoglycans, and fibronectin, forming a provisional matrix. These extracellular molecules become the physical basis for wound strength.

1.3.2.4. Remodeling phase

The last phase of wound healing, the remodeling phase, can take up to 1 year (2). During this phase, a precise balance between tissue synthesis and degradation must be achieved. Fibroblasts replace the provisional fibrin matrix with collagen monomers, which then become polymerized by extracellular enzymes. Collagen type III is replaced by the stronger collagen type I which, with the help of oxygen and vitamin C, increases the newly formed tissues tensile strength. The collagen pattern will be more randomized than in healthy uninjured tissue and thus be more susceptible to mechanical failure. With the help of matrix remodeling enzymes, the matrix is reorganized. Fibroblasts and leukocytes secrete collagenases to lyse collage type III.

The healing is deemed successful when a net excess of matrix substances is deposited compared to lysis. With the remodeling of the matrix and the TGF-β stimulated turnover of fibroblasts to myofibroblasts, wound contraction begins (5). This contraction helps pull wound edges together, therefore closing the wound successfully. The myofibroblasts, once contraction is completed, will undergo apoptosis. If, for any reason, the myofibroblasts fail to undergo apoptosis or are excessively active, the affected area will undergo fibrosis and a scar will be formed. Once the wound has closed, the angiogenic response will cease and blood flow diminishes. Any metabolic activity within the tissue ceases.

Scar formation is the final step of wound healing. This step comes with the disadvantage of the tissue reaching only about 80% of the skin's original tensile strength. Additionally, sub-epidermal appendages like hair follicles or sweat glands will not regenerate after severe injuries (5).

1.3.3. Factors affecting wound healing

Wound healing is a complex process involving strictly regulated steps that need to be completed correctly in order, for the injured tissue to regain integrity. The complexity of wound healing brings many obstacles which can be divided in local and systemic factors. Local factors that influence the character of the wound itself can impair wound healing (3). Systemic factors, on the other hand, are concerned with the general health of the individual affecting their ability to heal appropriately. Commonly these factors will occur in a combination of local and systemic factors because systemic factors often act by affecting the local tissue.

1.3.3.1. Local factors

The most important local factors influencing wound healing include hypoxia and infections. Hypoxia due to inadequate oxygenation or impaired perfusion is the most frequent cause of healing failure (2).

Oxygen plays an important role in the cells metabolism and is essential in almost all wound healing processes. Its importance in wound healing is especially evident as it helps prevent wound infection, induces angiogenesis, increases fibroblast proliferation and collagen synthesis, and promotes wound contraction (3). Factors decreasing oxygen concentration in injured tissue, and thus impairing wound healing, include hypovolemia, catecholamine infusions, stress, fear, and cold temperature (2). Wounds affected by hypoxia are commonly found in systemic conditions like diabetes mellitus and can turn into chronic wounds (3). In opposition tissues that are highly vascularized, and in turn highly oxygenated, heal more readily and are less prone to developing infections. When a tissue is hypoxic the release of growth hormones is stimulated and angiogenesis is induced in order, to improve oxygen delivery to the tissue and thus wound healing.

Another common local factor reducing wound healing is a dysregulated inflammatory response. While some degree of inflammation is beneficial and even necessary to promote wound healing, excess or lack of inflammatory stimuli will impair the tissue's ability to regenerate. A prolonged inflammatory response, due to the absence of effective decontamination, can cause the wound to become chronic. With time the levels of matrix metalloproteases (MMPs), a protease family that degrades the extracellular matrix (ECM), increase. This increase leads to a shift in the protease balance, which ultimately causes the degradation of growth factors present in the chronic wound tissue. The progress of the pathological cycle may result in an impaired quality and quantity of the wounds scar formation (2).

Physical stress, like friction, pressure, or tension in the wound area, can impair wound healing significantly. It is important to pay close attention to the wound site, as certain areas of the body may exhibit an anatomically increased physical stress on the wound.

1.3.3.2. Systemic factors

Different systemic factors play an important role in the success of wound healing, some are modifiable while others are not (3). Since the elderly population has been growing in the last century, age has become more and more important in the medical field. As a non-modifiable risk factor, age must be considered when treating a patient with a chronic wound. Not only does the injured tissue of an elderly patient heal more slowly, but its wound strength is decreased as well. An altered inflammatory response has been linked to the delayed wound healing observed in elderly patients. T-cell infiltration into the wound tissue is delayed, with changes in the chemokine production and decreased macrophage phagocytic ability. In animal models a slowed re-epithelialization, collagen synthesis, and angiogenesis has been observed in older compared to younger specimens. It has also been noted that platelet aggregation is enhanced, growth factors are secreted in smaller amounts, and scar strength is overall decreased.

In elderly patients, sex hormones influence the tissue's ability to recover additionally. Male patients have been shown to be more prone to delayed wound healing in acute wounds than females. This observation indicates that hormones like estrogen, androgen, and their precursor dehydroepiandrosterone play an important role in the wound-healing process. Estrogen regulates different genes related to regeneration, matrix production, protease inhibition, epidermal function, and inflammation. Studies have shown that estrogen supplementation potentially improves wound healing in the elderly population (both men and women). On the contrary androgens seem to have a negative effect on cutaneous healing (3).

Stress is another systemic factor that impacts not only human health but also social behavior. Many diseases and disorders besides compromised wound healing are related to stress. Different studies have shown that stress causes a visible delay in wound repair. This delay in healing is attributed to the anti-inflammatory response to stress. The release of glucocorticoids is up-regulated, and the secretion of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α is reduced. Chemotactants IL-1α and IL-8, which are essential for the initial inflammatory phase of wound healing, are also expressed less at wound sites due to stress (2).

One of the most prevalent causes of delayed wound healing is diabetes. In about 15% of diabetic individuals development of chronic non-healing diabetic foot ulcers (DFUs), a serious complication of the disease can be observed. The mechanism behind the development of DFUs is complex and involves many pathophysiological mechanisms. These mechanisms include hypoxia, dysfunctions of fibroblasts and epidermal cells, impaired angiogenesis, high levels of metalloproteases, damage from oxidative stress, decreased host immune resistance and neuropathy (4).

Obesity is becoming more prevalent among all age groups and many diseases and health conditions are linked to it. Obese patients are more likely to develop wound infections, dehiscence, hematoma and seroma formation, pressure ulcers and venous ulcers. They are also more prone to wound complications following surgical procedures. The impaired healing is related to a decreased perfusion and even ischemia in subcutaneous adipose tissue. An increased tension on surgical wounds increases tissue pressure, thus reducing micro perfusion and oxygen delivery to the tissue (5). Many more factors influence wound healing in obese patients, but they are beyond the scope of this review.

Other systemic factors include medications, like glucocorticoids, which inhibit wound repair via a global anti-inflammatory effect, non-steroid anti-inflammatory drugs (NSAIDs) used long term, some of which have an anti-platelet effect, and chemotherapeutic drugs, which inhibit rapid cell division, metabolism, and angiogenesis. Nutrition, smoking, and alcohol consume also influence wound healing.

Overall, can be said that wound healing is a complex biological process that is influenced by many factors. While not all factors are equally influential, they all affect the outcome of the healing process to some degree.

1.4. Scar formation

1.4.1. Definition and characteristics of a Scar

A scar is an undesirable natural result of the bodies healing process (6). Scars form when injured tissue is replaced with newly formed cells leading to an excess of new cells. Frequently, they cause some degree of functional impairment of the affected tissue and can even lead to emotional and social distress, especially if the scar is hypertrophic or keloid.

Hypertrophic scars are scars that are more extensive than normal scar tissue but do not extend beyond the wound borders. These scars are commonly caused by wound infection or high tension on the wound while healing (7). They can regress spontaneously (6).

Keloids are less favorable as they extend beyond wound borders, thus being harder to manage. Some pain has been reported with keloid formation, and collagen alignment is thicker and more irregular than in healthy scar tissue.

Once a cutaneous scar has matured, it consists of a large amount collagen, 80-90% of which are type I collagen while the remainder is type III (7). Unlike collagen in normal skin, which is arranged in a "basket-weave" pattern (Figure 3A), collagen of scar tissue is arranged in bundles parallel to the skin surface (Figure 3B). Typical for cutaneous scarring is the lack of dermal appendages like hair follicles and sebaceous glands (Figure 4), and their associated stem cells. Another characteristic feature is the decreased elasticity caused by a smaller amount of elastin within the scar's extracellular matrix. Scars tend to be hyperpigmented and raised which leads to a worse aesthetic result. Finally, one of the least desirable traits of scars is the contracture, which can cause pain and restrict movement, especially when located over a joint.

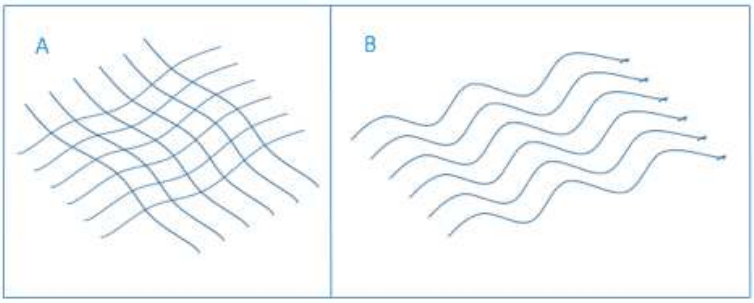

Figure 3. Collagen alignment. A. "Basket-weave" pattern in normal skin. B. Parallel collagen bundles in scar tissue. (SOURCE: https://en.wikipedia.org/wiki/File:Collagen_structure_in_scar_and_scar_free.png)

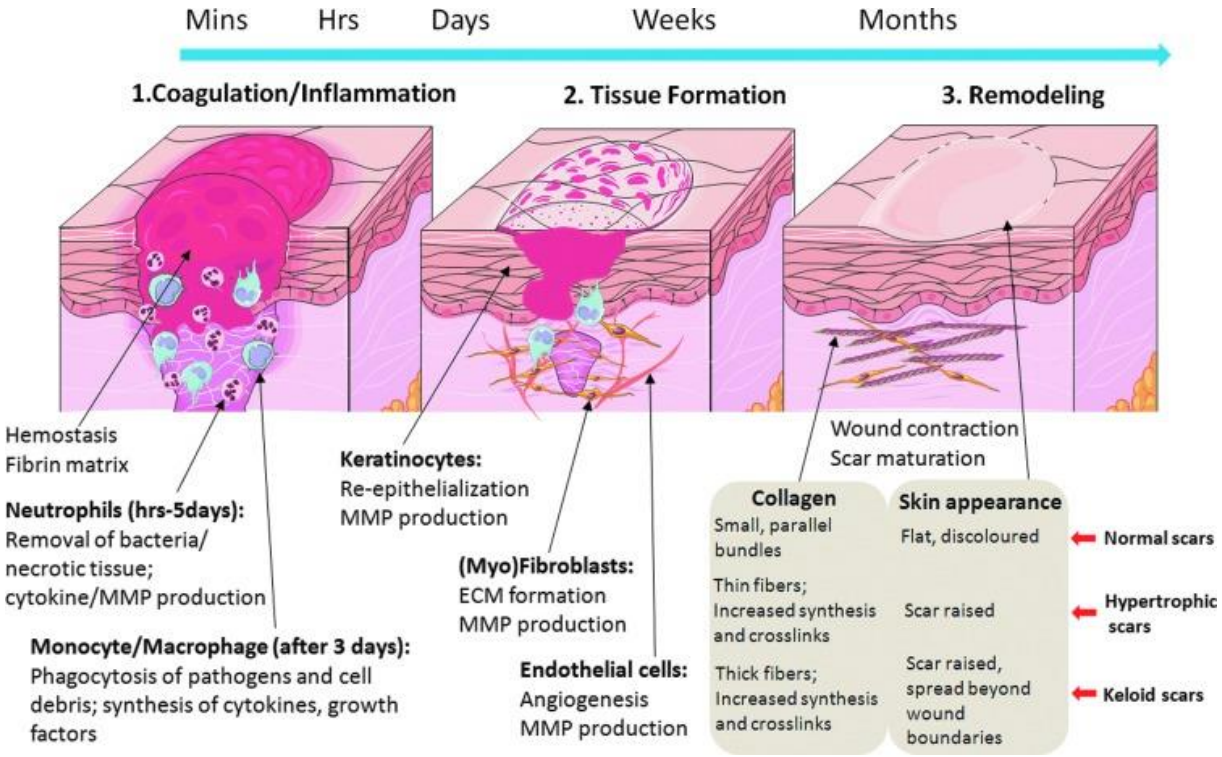

Figure 4. Stages of scar formation.
(SOURCE: https://europepmc.org/articles/PMC4352699/figure/f1/)

1.4.2. The effect of scarring

The importance of achieving as little scarring as possible lies in its effect on the overall quality of life of a patient (8). Not only does scar tissue break more readily than healthy skin does the psychological burden of having a scar can be overwhelming for some patients as well. For example, a psychosocial impact can be observed in patients with facial scars as they can cause increased anxiety and self-consciousness, especially when scars are hypertrophic.

Functional impairment is caused by the reduced failure properties (load, displacement, and energy) found in scar tissue, which makes it more likely to be compromised and burst (9). This considerable compromise in biomechanical durability and decreased failure resistance provides the basis for research efforts in this field. By preventing scarring load transfer and strain compatibility between skin and scar tissue could be increased and thus lead to an improved functionality and aesthetic results.

1.5. Scarless wound healing

Some animals can heal without forming scars (7). The knowledge of what differentiates their healing process from cutaneous healing in humans is essential in finding strategies to reduce or even prevent scar formation in humans.

1.5.1. Fetal wound healing

Fetal wound healing is one of the most remarkable findings in the search for scarless healing. After Burrington *et al*. proved in 1971 that surgical incisions placed on a fetal lamb healed rapidly with almost no scar formation many experiments have been done in different animal models that demonstrated the same phenomenon (7). With successful experiments in animal models, Lorenz *et al.* developed a similar experiment on human fetal tissue which also demonstrated scarless healing. The healed skin of the fetus was nearly identical to uninjured tissue. Collagen, epidermis, and epidermal appendages of the fetal skin appeared normal in contrast to adult skin, which consisted of disorganized collagen bundles, a thin epidermis, and no skin appendages within the scar tissue.

It was found that the scarless healing in fetal tissue is attributable to the tissues intrinsic properties and not like previously assumed caused by the intrauterine environment. With fetal maturation the incidence for scar formation rises, with the transition from scarless to scarring healing occurring at about 24 weeks of gestation. Fetal skin starts to differentiate more and becomes similar to adult tissue (10). It is noteworthy that the bigger the injury to fetal tissue is the earlier the tissue will heal with scarring (7).

When comparing fetal scarless healing to adult scarring healing one can find several differences. While we can find an initial phase of inflammation with migration of neutrophils and macrophages as well as the release of cytokines in adult tissue, fetal tissue has almost no involvement of these inflammatory mediators. The production of ECM in fetal tissue, as well as the ratio of collagen type III to collagen type I, is higher than in adult tissue. The ECM is richer in hyaluronic acid, while myofibroblasts are absent in fetal wounds.

1.5.2. Oral mucosa

Scarless healing can be found in certain human adult tissues as well. Once an injury to the oral mucosa has been sustained, it will heal with almost no scarring. This is attributable to a decreased inflammatory response early on as well as an overall increased rate of healing. It has also been suggested that the presence of saliva accelerates wound healing (1,2).

1.6. The molecular biology of wound healing and scarring

Over the past thirty years, the development of molecular biology has enabled a better understanding of many physiological processes on a deeper level. One of which is the dynamic and complex process of wound healing (11). The increasing understanding of growth factors and their critical role in wound healing offers the potential for future treatment alternatives that are more targeted and effective.

1.6.1. The basement membrane

The basement membrane (BM) of the skin is a highly specialized ECM that lies between the epidermis and the dermis (12). It is composed of glycoproteins and proteoglycans and binds a variety of growth factors and cytokines for controlled release if necessary. This function of the BM plays an important role during repair processes after injuries when epithelial cells are brought into contact with newly accessible ECM molecules, proteolytic fragments of the molecules or cleavage sites in the surrounding stroma.

1.6.1.1. The molecular architecture of the basement membrane

Basement membranes consist of members of four protein families (laminin, type IV collagen, nidogen, and perlecan) (12). Laminin and type IV collagen form distinct networks which become interconnected by mono- or oligomeric nidogen and perlecan to form irregular polymers. Besides the four main components, other proteins are part of the BMs architecture including fibrillin and type V collagen. In the skin collagen IV and VII, laminin-332, as well as perlecan are synthesized by epidermal keratinocytes and dermal fibroblasts, while fibroblasts produce nidogen.

Laminin consists of five α, four β, and three γ subunits connected through a coiled-coil domain (13). The α-subunits are mostly involved in cell surface adhesion and receptor interaction and can contribute to self-assembly. Structural support with only moderate receptor binding is the β– and γ-subunits primary function. Laminin can self-assemble its α, β, and γ chains by noncovalent bonds at the N-terminal globular domains and form large two-dimensional sheets (12). This process is reversible which is essential during tissue remodeling as the sheets can be disassembled. Cell adhesion to laminin is important for cell or tissue fate and functions.

Collagen IV molecules are covalently cross linked by disulfide bridges, which gives them their "chicken-wire"-like meshwork with high chemical resistance. This strength makes type IV collagen the most important structural component of the BM.

Nidogen and perlecan have several binding sites for each other as well as for both laminin and collagen IV and serve as a bridge between both types of scaffolds. Nidogen acts by cross-linking collagen IV and laminin primarily. Perlecan is said to have a regulatory role as it provides a high negative charge in BMs implementing a diffusion barrier and anchoring ports. Hemidesmosomes (HDs), consisting of the intercellular plaque proteins plectin and bullous pemphigoid antigen 1(BPAG1), span the whole BM and anchor the epidermis to the BM. BPAG1 connects the keratin filaments to the transmembrane proteins integrin α6β4, tetraspanin CD151 and collagen XVII. The dermis connects to the BM via anchoring fibrils, which are loop structures of collagen VII that bind to laminin-332. They are interwoven with collagen I and III fibrils. All adhesion complexes are important for the structural and functional integrity of the skin (12).

1.6.1.2. Epidermal-dermal junction

The role of the basement membrane is to form stable links through hemidesmosomes and thus to protect the tissue from disruptive shear forces and prevent blistering (13). Laminin-332 is one of the main components of the BM at the epidermal-dermal junction. It is binds to α6β4 integrin (allowing it to bind to HD plectin that links keratin) and BP180. It also forms an important link between the cells HDs and the stromal anchoring fibrils. If this linkage is lost, the BM will split causing blistering.

Once an injury occurs to the skin, keratinocytes detach from anchoring HD and migrate to restore epithelial unity. HDs are believed to undergo structural disassembly that is dependent on proteolytic enzymes that enable them to switch from stable anchorage to migration. A cryptic site that promotes migration seems to be exposed by cleavage of the γ2 short arm by T1-MMP or MMP2. This might occur due to the release of an EGF-containing fragment that binds to an EGF receptor. Cleavage of the α3LG domains has been linked to changes in laminin-3A32 and its transition from an anchorage-promoting mode to a migration-mode (13).

1.6.2. Growth factors

Growth factors are cytokines, proteins that allow messaging from one cell to another (12,13). The specific function of each growth factor depends on the receptor it attaches to. While initially named after their known function at the time, to cause growth, it has been found that many different types of responses are signaled in cells when activated by growth factors. Especially in wound repair growth factors play a critical role for successful resolution because they regulate many activities involved in the healing process.

1.6.2.1. Growth factor receptors

Growth factors affect target cells through specific receptors (14). These receptors can be categorized into direct catalytic receptors and G-protein-coupled receptors. Receptors consist of a cytoplasmic region, a hydrophobic transmembrane region, and an extra-cellular ligand-binding domain. While the exact mechanism of how receptor-cell stimulation functions is unknown, several different actions have been proposed.

For direct catalytic receptors has been suggested that occupation of a receptor site triggers intracellular reactions by aggregating other receptors on the cell surface. This, in turn, may provide a pathway for intracellular enzymes to be activated. Activation of target cells is related to the phosphorylation of intracellular serine, threonine, or tyrosine residues by protein kinases, which in turn lead to conformational changes within the cell. The transference of extracellular physical activities to intracellular chemical changes is called cell transduction. G-protein-coupled receptors are linked to GTP-binding proteins (G-proteins), which are present on the inner surface of target cells. G-proteins induce different intracellular activities like the regulation of enzymes and ion channels within the cell membrane (14).

1.6.2.2. Growth Factor Transmission

During wound healing growth factors can be found in all phases of the repair process (14). They are transported to the injured site via three mechanisms. The endocrine mode of delivery acts by secreting factors into the blood. These factors travel to a distant site to act there. Examples of factors for the endocrine mode of delivery are insulin-like factor I and II. Another mechanism, the paracrine mode of delivery, involves cells secreting factors like PDGF, TGF-α, and TGF-β, which directly act on the adjacent cells. For this mode of delivery, cell to cell distance plays a crucial role. The third mode of delivery is called autocrine secretion. Cells perform self-regulatory functions being both the source and target of secretion. TGF-β is a common growth factor for autocrine regulation. During the wound healing phases multiple modes of delivery can be facilitated by the body and lead to varying amounts of growth factors in the tissue.

1.6.2.3. Epidermal growth factor and TGF-α

Epidermal growth factor (EGF) is a mitogen and maturation factor for epidermal cells (14). It is closely related to TGF-α. EGF and TGF- α possess similar activities. Both growth factors are present in most body fluids and occur at very low levels in healthy tissue. They have a molecular weight of 6kDa and affect mesenchymal and epithelial cells through paracrine secretion from transmembrane proteins that act on EGF receptors. Large amounts of TGF-α are found in wound macrophages, making this type of cell essential in the early phases of wound healing. EGF and TGF-α mainly affects granulation tissue development. Only TGF-α induces epidermal re-growth and modulation of angiogenesis.

1.6.2.4. Platelet-derived growth factor

The dimeric glycoprotein platelet-derived growth factor (PDGF) consists of A and B chains (14) and has a molecular weight of 30kDa. The chains can be arranged in three different ways (AA, AB, BB) of which AB is the most common. The growth factor is stored in platelets and released after their activation at the site of injury. Other cells, like macrophages, endothelial cells, vascular smooth muscle cells, and fibroblasts express PDGF receptors as well. This makes it one of the most potent growth factors present in wounds. PDGF is active in the first two phases of wwound healing. Its chemotactic effect on fibroblasts and monocytes and its mitogenic effect on fibroblasts and vascular smooth muscle make this growth factor essential in wound healing. The factors concentration at wound sites is not always constant and increases with augmented connective tissue formation. Acting through autocrine and paracrine mechanisms, PDGF is not only a stimulator of cellular activity but also plays an important role in the induction of homeostatic feedback.

1.6.2.5.Transforming growth factor-β

Transforming growth factor β (TGF-β) (molecular weight 25kDa) exists in three separate isoforms in humans (14). While their functional activity is similar, the difference lies in their area of distribution. The growth factor plays an essential role in embryogenesis, especially the tissues of the embryo that are derived from the neural crest mesenchyme.

With its diverse origin (being secreted by platelets, macrophages, bone cells, monocytes, and lymphocytes) TGF-β is involved in many different functions of matrix production which are mitogenic as well as regulatory. It is not only chemotactic for fibroblasts and monocytes but can also inhibit and stimulate fibroblasts, promote cellular influx into the wound and increase the synthesis of ECM proteins. Through the induction of interleukin-1 and TNF-α it also stimulates angiogenesis. A dysregulation of TGF-β1 signaling protein has been linked to abnormal scarring pathogenesis leading to keloids or hypertrophic scars (15).

1.6.2.6.Basic fibroblast growth factor

Basic fibroblast growth factor (bFGF) (with a molecular weight of 150kDa) belongs to a family of homologous peptides (14). bFGF has a high affinity for heparin and is released in response to the action of the enzyme heparinase which is found in platelets. The growth factors are found in endothelial cells, macrophages, and fibroblasts. FGFs are chemotactic toward endothelial cells and leukocytes and mitogenic for endothelial cells. Like many other growth factors, bFGF plays an important role in angiogenesis. It is also involved in the initiation of the release of BM degrading enzymes that help remove endothelial cells prior to new vessel formation.

1.6.2.7.Tumor necrosis factor-α

Tumor necrosis factor-α (TNF-α) is expressed by macrophages to stimulate angiogenesis (14). It is a mitogen for fibroblasts.

1.6.3. Platelets

Platelets play an influence the initial phase of wound healing by forming a clot to achieve hemostasis (11). Once they arrive at the injured site platelets will release cytokines like PDGF, TGF-β, VEGF, etc. These cytokines will then act on local cells via a cell surface receptor.

1.6.4. Inflammatory cells

As the initial stage of wound healing proceeds chemokines start attracting circulating inflammatory cells to the injured tissue by acting on membrane bound receptors (11). Early on (48h after the injury) neutrophils are the most prominent cells in the injured site. The neutrophils start secreting IL-8 which attracts more inflammatory cells to the tissue. After the neutrophils undergo apoptosis circulating monocytes accumulate in the tissue and mature into macrophages. This step is essential not only for the inflammatory stage of wound healing but also the repair process. The role of the macrophages is to remove debris by secreting collagenases and elastases. They also act in removing bacteria from the tissue and produce inflammatory cytokines like TNF, IL-6, IL-1, and bFGF, among others.

As the inflammatory phase continues IL-1 attracts lymphocytes into the injured tissue (around 72h after the injury). They start secreting lymphokines, EGF, bFGF and other factors. With the inflammatory phase slowly subsiding macrophage-derived growth factor as well as macrophage-derived angiogenic factor reach optimal levels. This causes an influx of fibroblasts, endothelial cells, and keratinocytes to the wound site. Thus, macrophages can directly and indirectly stimulate the proliferation of connective, endothelial and epithelial tissue by changing the composition of the ECM during angiogenesis and remodeling. Mononuclear cells replace macrophages and other inflammatory cells. The proliferative phase starts.

1.6.5. Repair cells

As the healing phase progresses to a proliferative state, fibroblasts migrate, proliferate to the site, and produce components of the ECM (11). In this step pro-collagen is secreted into the extracellular space and cleaved. The cleaved terminal segments are called tropocollagen. These molecules accumulate and form collagen filaments rich in hydroxylysine and hydroxyproline. Depending on their intra- and intermolecular cross links the stability and bursting strength is determined.

During normal wound healing angiogenesis is an essential step. Endothelial progenitor cells (EPCs) express markers for hematopoietic stem cells (CD34 and CD13) and endothelial cells (CD146, vWF, and VEGFR2). EPCs migrate from the bone marrow to healing tissue where they proliferate, migrate, and differentiate. By releasing pro angiogenic cytokines they enable wound revascularization and quick repair.

Growth factors are important during cell differentiation processes like epithelial-mesenchymal transition (EMT). In EMT growth factors help in the communication of establishing the emerging basal membrane and guide reepithelialization. An abnormal regulation of EMT is said to cause hypertrophic scarring and tissue fibrosis. In EMT epithelial cells gain mesenchymal phenotypic features and are able to migrate and move more easily. An important molecule of EMT is β2-AR.

Repair cells are found in the ECM. At their site of contact with the ECM, big protein complexes, known as focal adhesions form due to accumulation of integrin receptors. Once integrin binds with the ECM, cytoplasmic actin filaments engage myosin II and other contractile proteins. This leads to the stabilization and shortening of the membrane-tethered actin filaments. By shortening the actin filaments cells start to contract. Epidermal cells move across the wound surface when intracellular actin micro-filaments are formed and secrete collagenases. The collagenases, in turn, break down collagen and plasminogen activator thus stimulating the production of plasmin. The function of plasmin is to break down the clot along the path of epithelial cell migration. On their path of migration, epithelial cells interact with a provisional matrix consisting of fibrin cross-linked to fibronectin and collagen. For the whole process of re-epithelialization EGF and stem cells play an important role in aiding migration, proliferation, and differentiation.

Once epithelialization is completed, epidermal cells take their original form. New desmosomal linkages to other epidermal cells are formed and hemidesmosomal linkages to the basement membrane are restored (11).

1.6.6. Fibroblasts

Fibroblasts are involved in several important processes of wound healing and scar formation (16). Breakdown of fibrin clots, creating new ECM and collagen structures, and wound contraction are all influenced by fibroblasts. Especially Engrailed-1 lineage-positive fibroblasts (EPFs) have been linked to scar formation, while until now little was known about Engrailed-1 lineage-negative fibroblasts (ENFs) (17). A study by Mascharak *et al.* recently uncovered that inhibition of Engrailed-1 activation by Yes-associated protein (YAP) encourages ENF mediated wound repair, thus, enabling skin regeneration without fibrosis and regeneration of lost skin appendages (e.g., hair follicles, sebaceous glands).

1.7. Nanoparticles

Nanomedicine is one of the fastest developing fields in medical science (18). With nanomedicine structures and functions of living cells can be targeted directly at the genetic and molecular level. Nanoparticles (NPs) are particles in a nanometer scale that can be won form different materials like metal, lipid, polymer etc. Their physical properties change with size and thus different sizes can be applied for varying indications. NPs can facilitate the external delivery of wound healing substances which are commonly produced at a site of injury, like nitric oxide (NO) (17). Another use for nanotechnology is the delivery of antimicrobial plant components to the affected tissue. Often these plant components have wound healing properties and improve the overall outcome of the healing process. The application of nanoparticles in form of wound dressings has proven especially promising in cases of delayed wound healing and burn treatments.

Metal nanoparticles have bacteriostatic and bactericidal properties, thus enabling a swift recovery of the tissue. With the field of nanotechnology rapidly expanding in recent years, many new therapeutic options have arisen which show promising results. This review will include some of the nanoparticles potentially important for the process of wound healing.

1.7.1. Metal nanoparticles

1.7.1.1. Silver

Silver compounds have been used in wound management for many years (19). The application of silver nanoparticles (AgNP) may overcome certain limitations associated with silver compounds. These limitations are mostly related to the surface-to-volume ratio which is optimized by the application of AgNP. With their increased surface-to-volume ratio AgNPs are more potent at smaller concentrations which in turn lowers their tissue toxicity. A wide range of antimicrobial activity and wound healing properties enables AgNPs to accelerate the wound healing phases and improve the overall outcome (17). The use of pure AgNPs can enhance re-epithelization and promote wound healing by modulating inflammatory cytokines and decreasing lymphocyte infiltration (17,19). AgNPs also play a role in the differentiation of myofibroblasts from fibroblasts therefore promoting wound contractility. In combination with other wound dressing materials, like biocompatible bacterial cellulose, an increased attachment and proliferation of keratinocytes at the wound site has been noticed (17).

1.7.1.2. Zinc oxide

Another nanoparticle improving wound healing is zinc oxide (ZnONP) (19). Zinc is a cofactor for metalloproteinase therefore it is involved in the regeneration of the ECM (17). The effect of ZnONPs on wound healing depends on their size and concentration.

When ZnONPs are added to a hydrogel-based wound dressing keratinocyte migration and re-epithelization are enhanced (19). A recent study by Rajendran *et al.* has shown that wound dressings containing hydrogel and ZnONPs had an increased absorptive capacity for wound exudates and enabled the formation of hemostatic blood clots. At the same time such wound dressings appeared to have antibacterial properties while exhibiting a reduced amount of cytotoxicity.

One issue that limits their use in wound treatment is that with excessive use ZnONPs can be toxic to the tissue. High concentrations of ZnONPs can cause mitochondrial dysfunction in keratinocytes and have even shown a tendency to induce carcinogenic transformations.
Thus, the use of ZnONPs in wound dressings needs to be carefully planned and executed to achieve the best possible outcome.

1.7.2. Peptide nanoparticles

Peptide nanoparticles can be used widely in tissue repair due to the fact, that the peptide scaffolds closely resemble the natural ECM (17). These nanoparticles do not hold any immunogenic activity and are free from graft rejection making them an optimal alternative in wound management.

1.7.3. Polymeric nanoparticles

Polymeric nanoparticles have proven successful in skin regeneration because they can be modified according to tissue needs (17). They are commonly incorporated in surgical tools, implantable devices, and vascular grafts. Additionally, adding cells, cytokines or growth factors to polymers can induce blood vessel formation, neovascularization and improve the microenvironment around the wound tissue.

1.7.3.1. Nanohydrogel

Nanohydrogel is an ideal formulation to apply in wound dressings because it has the ability to absorb aqueous fluid while preventing wound dehydration and creating an optimal moist environment for wound closure (20). Moreover, it maintains the wound bed due to its non-adhesive properties while simultaneously allowing oxygen penetration.
With the addition of certain drugs or other nanoparticles, which are encapsulated by hydrogel, impressive effects can be observed on skin regeneration.

Incorporating VEGF into nanohydrogel formulations has shown enhanced cell adhesion, reduced blood clotting time, and facilitated tissue regeneration in an in vitro setting. Further investigations are required to prove the therapeutic efficacy of this formulation on wound repair.

1.7.4. Liposomes

Liposomes present promising nano-carriers for topical drug delivery (21). They are amphiphilic, biodegradable, and biocompatible with human skin. Their ability to transport both hydrophilic drugs and hydrophobic agents provides the optimal prerequisite for a drug delivery medium. Liposomes can cover wound surfaces and thus provide additional protection. By creating a moist environment liposomes aid wound healing. With their merits, liposomes have become heavily used in wound treatment. Certain limitations need to be considered, however. Drug leakage is a common issue related to liposome application, which can often be unavoidable and rapid. Its low stability as well as reproducibility pose major obstacles for routine clinical use.

1.7.5. Lipid nanoparticles

Lipid nanoparticles have been found to overcome the obstacles associated with liposomes (21). They have a nontoxic colloidal dimension that helps release drugs and other agents in a controlled manner. Several lipid nanoparticles can already be found on the market for cosmetic or topical therapeutic purposes. Loading lipid nanoparticles with certain agents will allow a controlled and slow delivery to the affected tissue.

2. OBJECTIVE

The objective of this review was to perform a literature search and review the basal lamina and the effects of the affinity nanoparticles bonding on scar formation. It was important to establish whether there are certain factors influencing the wound healing process. By finding these factors, we could potentially apply the knowledge to decrease the risk of the scar formation in the surgical setting and possibly even in non-surgical wounds in the future.

Hypothesis. This was a hypothesis-free systematic review, aiming to explore the possible use of nanoparticles in reduction of the scarring in the skin, by employing the affinity bonding to the basal lamina, which could create a functional bridge for the epithelial cells to migrate and close off the wound in a scar-free manner.

3. MATERIALS AND METHODS

3.1. Data collection

This study was planned and executed as a systematic review, a secondary study design, which aims to summarize published data in a qualitative approach. The focus was set on exploring the extent of papers that discuss nanoparticles for the basal lamina affinity molecules.

3.1.1. Literature search

A detailed systematic search was performed. Published data were compared and summarized related to factors affecting the basal lamina affinity molecules and nanoparticles using the database PubMed (https://pubmed.ncbi.nlm.nih.gov/). The search was performed on 28th of May 2021. Initial research efforts including the terms basal lamina and affinity molecule did not yield any results. The extension of the search terms and addition of more refined keywords did not yield any more results ((basal lamina affinity molecule AND (surg* OR operat* OR scar*)). Therefore, no synthesis of the initial topic was performed, and the synthesis of the related material was qualitative only.

Finally, a complex search strategy was developed that included several search terms and yielded several papers related to the search topic. These papers were used in this review (Table 1). Keywords included were basal lamina affinity molecule; basal lamina affinity molecule AND (surg* OR operat* OR scar*); (nanomedicine OR nanomolecu*) AND (scar OR scarring), without any time restriction.

All identified articles were first categorized for usefulness as relevant/maybe/not relevant by two reviewers independently by assessing the title and abstract. Articles marked as relevant and maybe were read in full text by two reviewers independently and evaluated based on specific inclusion and exclusion criteria. The systematic review was conducted and written according to the PRISMA reporting guidelines (Appendix 1).

Since the study was based on secondary data, no ethical approval was sought.

Table 1. Search terms and results

Keywords	Search results	Relevant
basal lamina affinity molecule	213	0
basal lamina affinity molecule AND (surg* OR operat* OR scar*)	11	0
nanomedicine OR nanomolecu*) AND (scar OR scarring)	134	0 (related to the topic : 9 - PMID: 33787467, 33709070, 32645446, 32423071, 31534333, 30901304, 30863067, 27597828, 26295361)

3.1.2. Eligibility criteria

To be included in this thesis, studies needed to fulfill certain requirements. Studies not published in the English language were excluded. Studies not including all relevant search terms were excluded.

3.1.3. Data extraction and presentation

Before starting the review, a data collection form was planned. Two reviewers collected research data independently and compared their results afterwards. Any disagreements were cleared by a mentor of the Thesis. Extracted data contained article details (title, authors, year of publication, journal name, PMID), and study details (types of nanoparticles used, affected tissues, test subjects).

The main results were presented as a narrative review based on various combinations of basal lamina, nanomolecules and scar formation. Due to diversity of the studies, only the narrative synthesis was performed.

Funding: none.

Conflicts of interest: none reported.

4. RESULTS

There were no identified published studies that met the inclusion criteria, rendering the systematic review as empty. However, nine studies were retained in the analysis, which were laying the grounds for the future use of the nanomolecules, out of 358 articles identified through literature search.

Nanoparticles have found their use in various medical fields. Polymeric nanomedicine is versatile due to its low cytotoxicity, biodegradability, bioavailability, biocompatibility, and specific delivery at the target tissue (22). Therapeutic agents can be delivered to tissue sites and support tissue growth at many sites, not only including the skin but also the lung. With the current SARS-CoV-2 pandemic, many researchers have started focusing on drug delivery systems that aid in moving therapeutic agents to tissue sites. Especially, since other modes of delivery have failed in proving successful in treating inflammatory lung injuries have nano-therapies become an attractive alternative. Polymer chemistry enables the development of a wide range of nanocarriers with a broad functional capacity to customize treatment at injury sites while bypassing the conventional limitations of respiratory injury treatments. While a great number of different drug delivery systems has been proposed for pulmonary or respiratory applications, biodegradable polymeric nanocarriers have appeared most promising. Their ability to overcome limitations at the tissue site have helped increase the biodistribution of therapeutic agents while limiting their tissue toxicity. Nanomaterials not only aid in delivering drugs to the affected tissue, with their potential to support the production of bioengineered lung tissue, they could potentially reverse lung damage.

One specific type of nanoparticles used in surgery are the spherical nucleic acids (SNAs). These molecules are able to penetrate skin and regulate gene expression at tissue sites (15). A reduced expression of TGF-β1 caused by specifically targeted SNAs, which have been applied topically, has shown to reduce scar elevation significantly and change collagen deposition in healing wounds in a rabbit ear model. Special focus should be put on gold nanoparticle-based SNAs (AuSNAs), which have been in use for some time, and liposomal-based SNAs (LSNAs), which has shown more recent success in small animal models and skin equivalents.

A study by Ponedal *et al*. has provided prove that SNAs enter cells via scavenger receptor-mediated endocytosis. With the use of confocal microscopy, it was demonstrated that Cyanine 3 (Cy3)-labeled AuSNAs and LSNAs penetrate rabbit fibroblasts (Rab9), human hypertrophic scar-derived fibroblasts (HSF), and human keloid scar-derived fibroblasts (KF) (Figure 5).

The same study assessed TGF-β1 expression in rabbit scars treated with SNAs via Western blot analysis. The results were then normalized to match untreated conditions of all rabbits. Figure 6 shows that only treatment groups who received TGF-β1-targeting AuSNAs and LSNAs had a significantly lower expression of the protein compared to the untreated control groups. Subsequently, a qualitative assessment of the scar tissue was conducted, showing a less dense and less bundled collagen organization for TGF-β1 AuSNA- and LSNA-treated scars compared to other groups.

Overall, the data suggests that topically applied TGF-β1-targeting SNAs have a significant impact on reducing TGF-β1 expression and thus decreasing scar elevation. Further testing needs to be done in order, for these findings to be applied to the human model.

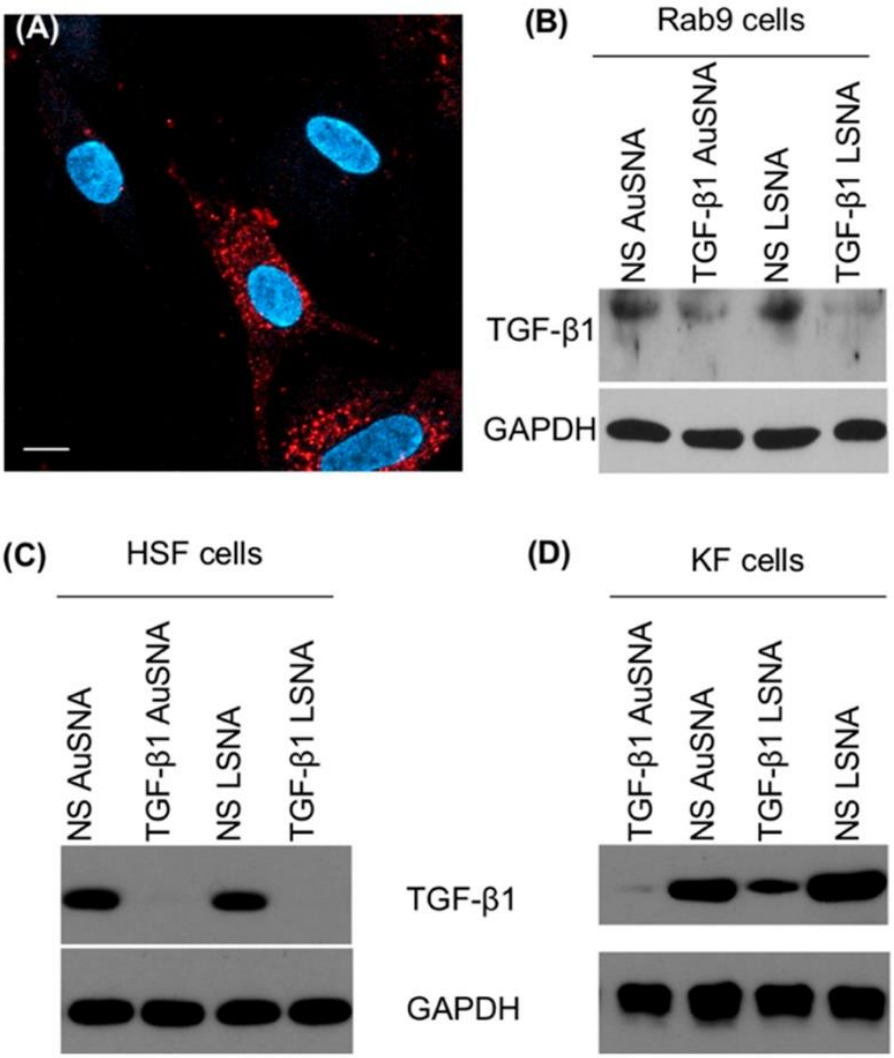

Figure 5. Cellular uptake of SNAs and TGF-β1 regulation in fibroblasts. A. Cellular uptake of Cy3-labeled AuSNAs in Rab9 cells visualized with confocal microscopy. Fluorescence from SNAs is shown in red and blue is a nuclear stain (DAPI). Scale bar = 10 μm. Downregulation of TGF-β1 by TGF-β1 AuSNAs and LSNAs in B. Rab9 cells, C. HSF cells, and D. KF cells. NS = nonsense (source: PMC, taken with U.S. fair use guidelines as non-commercial, educational purposes, with reference to the original material).

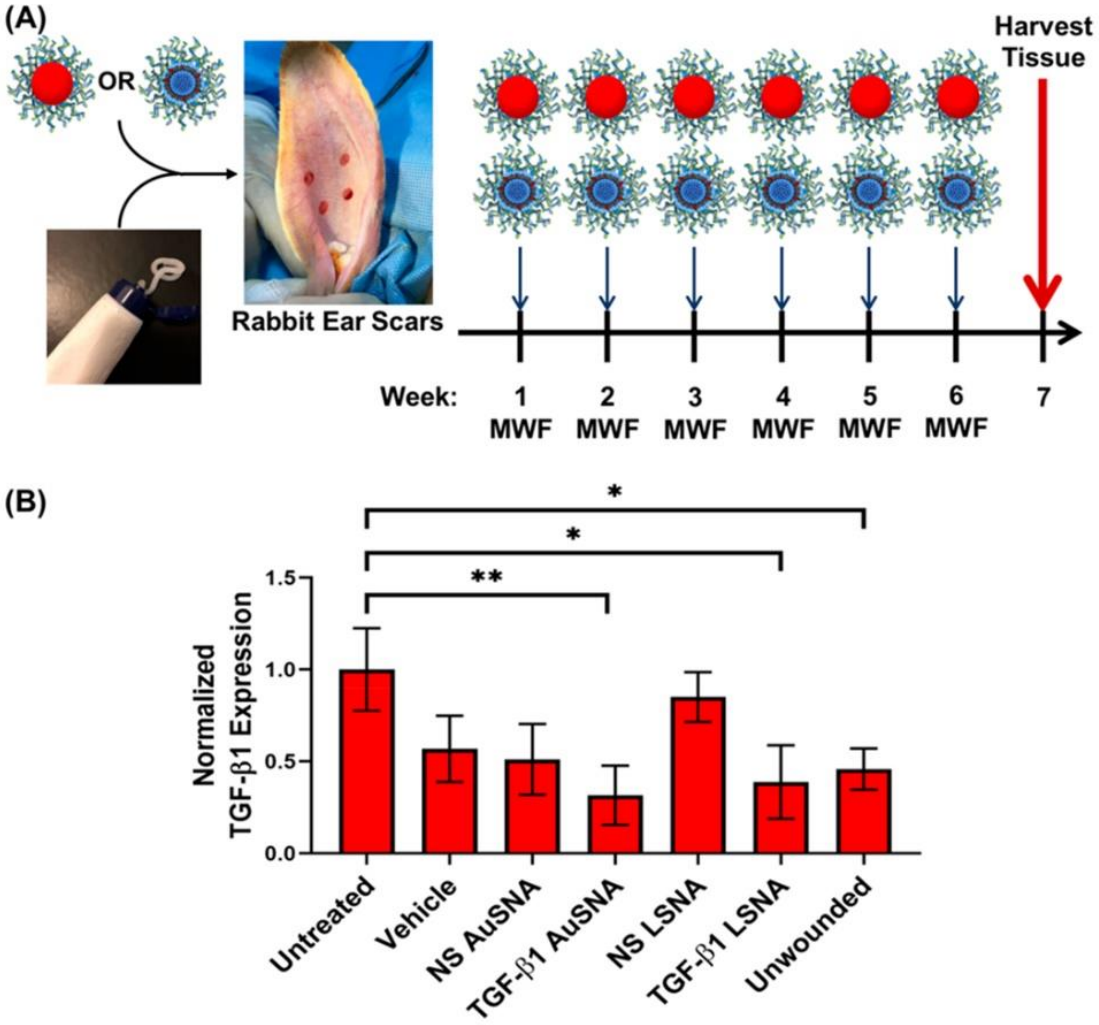

Figure 6. Assessment of SNAs regulating TGF-β1 expression level in vivo. A. Treatment scheme of topical treatment of rabbit ear scars with SNAs. MWF - Monday, Wednesday, Friday (treatment was given 3 days a week). B. Average TGF-β1 expression level, as quantified by densitometry of Western blot protein analysis. Data is expressed as the mean, normalized to the untreated group, ± SEM (N = 6) (source: PMC, taken with U.S. fair use guidelines as non-commercial, educational purposes, with reference to the original material).

A recent study by Han *et al.* has proven that zinc sulfide nanoparticles (ZnSNPs) can promote skin regeneration instead of causing fibrotic changes when added to wound tissue (23). By applying ZnSNPs and thus inhibiting fibroblast collagen synthesis, collagen contraction can be decreased and a lower density of collagen in the newly formed tissue can be achieved. Additionally, the formation of skin appendages in healing wound tissue can be deemed promising in this field. It is essential to control wound contraction and reduce fibrous scar tissue formation in order, to regenerate the injured tissue. This has been achieved in the study, both *in vivo* and *in vitro*. It can be concluded that the application of ZnSNPs to wound tissue shows promising in scar-free wound repair.

Smart polymeric nanodrugs (SPN) are based on natural and synthetic materials (24). Their medical efficacy was evaluated by Silina *et al*. by comparing wounds treated with SPNs compared to untreated wounds (control group) in the rat model. The study showed that applying SPNs accelerates wound healing compared to untreated wounds. The size of the wound area decreased significantly when compared to the control group. An inhibition of the inflammatory reaction, which was proven by measuring the number of non-resident cells, enabled an accelerated regeneration rate by allowing space for a higher number of regenerating cells. An earlier formation of immature fibroblasts and a decelerated collagen maturation have both been linked to application of SPNs and lead to good aesthetic postoperative results. A less evident post-surgical scar could also be accredited to a more evenly distributed epithelial layer differentiation. Additionally, neoangiogenesis was observed in the group treated with SPNs, which aids in tissue regeneration. Overall, it can be assumed that SPNs are promising agents used in scar prevention, but further research should be done to assess their clinical use.

A study by Xiao *et al.* has aimed to establish a treatment for the prevention of hypertrophic scar formation (25). Hypertrophic scars are caused by an increased growth of fibroblasts and a decreased apoptotic action. In this study cuprous oxide nanoparticles (CONPs) have been examined in detail for their ability to induce apoptosis, inhibit tumor cell proliferation, and promote wound healing. The experiment was conducted on rabbits that were injected with 0.1mL of CONPs diluted with a 5% glucose solution at the wound site. On gross examination it was found that CONPs-treated scars showed significant improvement compared to the control group treated with glucose only, on day 35 (Figure 7). While control group scars started getting raised, red, and stiff, scars treated with CONPs became softer and less visible. On microscopic examination a Masson trichrome staining was done that compared the collagen deposition in both groups. In subjects treated with CONPs a marked reduction in density and irregularity of collagen arrangement was noted compared with the control group. Several other investigations were conducted to prove the efficacy of CONPs. While not without limitations, it can be concluded that CONPs show great therapeutic potential in the treatment and prevention of hypertrophic scar formation by inhibition of fibroblast proliferation and activation of apoptosis of tumor cells.

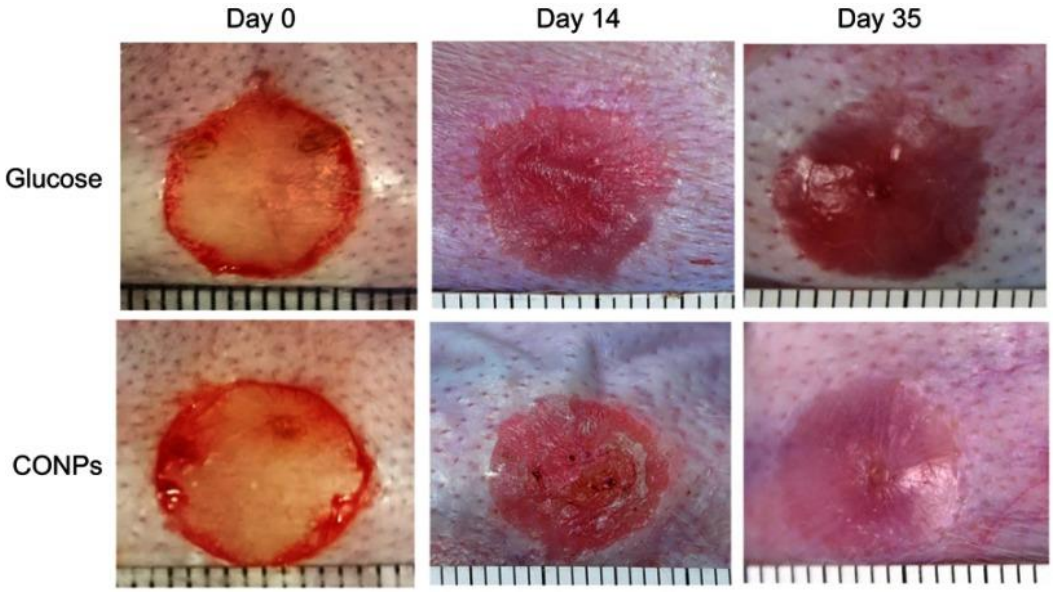

Figure 7. Gross examination images. CONPs-treated scars improved visibly on gross examination and were softer and less pronounced compared with their controls group (source: PMC, taken with U.S. fair use guidelines as non-commercial, educational purposes, with reference to the original material).

Nanotechnology-based drug-delivery systems can improve bioavailability through controlled drug release and targeted delivery (18). A study by Al-Mashahedah *et al*. investigated the efficacy of these drug-delivery systems in corneal scarring. Using nanoparticles to deliver certain drugs to the cornea proved to extend the duration of the drug on the corneal epithelial layer by 1.5-fold. It also helped provide a steady release of drugs at the site thus leading to a steady bioavailability. Nanofibers, another approach, can provide ideal conditions for cell and tissue regeneration. Thus, they can potentially reduce complications of acute corneal injuries. Nanodevices, like soft lenses, enable drugs to remain on the surface of the cornea for a longer time. Their high cost, discomfort, and drug clearance issue constitute their main limitations for clinical use.

Despite certain limitations, like the high cost of producing nano-formulations and the swelling behavior of the drug-delivery systems, which impacts the solubility and bioavailability of the drugs, nanotechnology-based drug-delivery systems have a promising outlook in treating corneal scaring. Further testing needs to be done on the human model for this treatment to become applicable in the clinical setting.

Nanoparticles have been shown to have a great variety of application possibilities in wound treatment. A study by Wang *et al.* has focused on using NPs to facilitate the delivery of paclitaxel (PTX) to prevent keloid formation (26). PTX is an effective chemotherapeutic agent that has been shown to have anti-fibrotic properties. Due to its strong hydrophobic nature a clinical application in wound healing has proven difficult. By combining PTX with a cholesterol-loaded liposome (PTXL) these limitations were bypassed. The study proved that PTXL could be taken up into human keloid fibroblasts (HKFs) and that PTX was released in a slow and sustained mode. *In vitro*, compared to PTX alone, PTXL showed an improved ability to inhibit cell proliferation, migration, and invasion, as well as keloid growth. An inhibited keloid growth was also observed *in vivo* in mice. PTXL could, with further testing, become a promising agent in anti-keloid therapy.

With a limited regeneration potential, tendon tissue, when injured, often results in persisting mechanical impairments (27). For this reason, researchers have started searching for treatment options to enable tendon tissue regeneration. Especially nanotechnology has shown promising results. Tendons are structurally composed of nanostructured material, as are their associated extracellular matrices. By using NPs with a size of < 100 nm, a bridging between tendon structures and orthopedic materials can be achieved and aid in regeneration of injured tissue. NPs could be used in a variety of ways to achieve the desired results. By labeling tendon stem cells (TSC) with nanoparticles and improved MR imaging quality can be achieved. NPs can also work as gene therapy and drug delivery carriers. Additionally, NPs can aid in the development of a new generation of bioactive scaffolds and even modulate the ECM and cellular response. All these possibilities are promising, but further investigations need to be conducted in order, to provide information on the safety of using these NPs in the clinical setting.

Nanotechnology has been revolutionizing wound management for the past few years (28). It has become a promising treatment option for curing chronic wounds and improving surgical wound healing. The outcome of cutaneous wound healing depends strongly on the nanomolecular formulation, doses, and methods of application. Nano-molecules can modify any wound healing phase, which can be accredited to their anti-bacterial, anti-inflammatory, proangiogenic and proliferative properties (Figure 8). An enhanced wound healing is also achieved by their ability to improve the expression of several important proteins and molecules. The full mechanism and signaling-pathway of how nano-molecules act is not yet fully understood but a positive outcome has been observed. Further investigation needs to be conducted into the mechanism of action to fully exploit the therapeutic possibilities that are linked to nanomedicine. By understanding the cellular response new routes may open in the field of nanomedicine. It is important to consider certain limitations, like tissue toxicity before nanotechnology can be fully implemented in every-day practice.

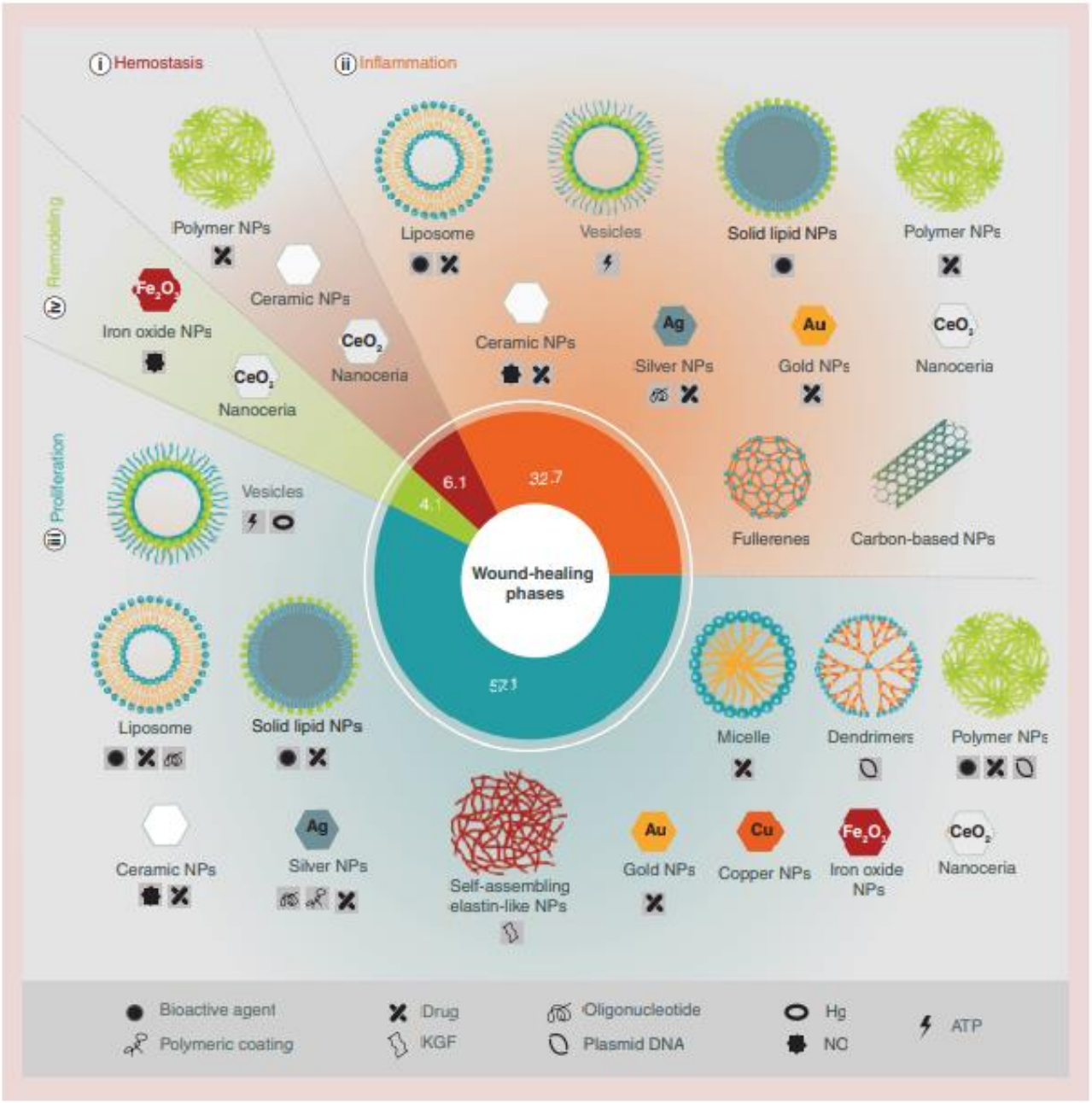

Figure 8. Various nanomaterials affecting different phases of wound healing. NP – nanoparticles (source: PMC, taken with U.S. fair use guidelines as non-commercial, educational purposes, with reference to the original material).

5. DISCUSSION

The purpose of this review was to explore the current research status and the effectiveness of nanoparticles on tissue regeneration to ultimately decrease scar formation of surgical wounds. There were no primary studies identified, meaning that there were no attempts to use the nanoparticle with the strong affinity for the basal lamina, which could be used in the control of the post-surgical scarring. Despite no such studies that met the planned inclusion criteria were identified, the analysis of the related papers managed to provide a general overview of this field of research.

The field of nanotechnology is developing rapidly with many studies investigating the efficacy of NPs in tissue regeneration and drug delivery. A noted range of NPs and a broad variation of application possibilities makes this field of medicine very interesting. Several studies are being conducted that examine the efficacy of NPs in tissue regeneration. Interestingly, these studies seem to focus on a diversity of approaches, some of which do not seem to use the specific mechanisms, but instead aim to deliver the target molecules to the wound site. This makes an affinity bonding molecule search even more interesting, since it may indeed present a truly novel approach to this problem.

After carefully reviewing relevant articles on PubMed, it was evident that the application of NPs and facilitating NPs as drug-delivery systems improved tissue regeneration significantly. With a wide variety of NPs, many different effects at tissue sites can be noticed. The type, size and concentration of NPs plays an important role in how the target tissue is affected. Some tissue toxicity was observed warranting a carefully planned administration of NPs to injured tissues. Notably, the majority of these studies were performed in animals, and there are only a few molecules that are used in humans, mostly in the trial stages.

Several limitations can still be found when discussing the efficacy of NPs regarding tissue regeneration. The first is the lack of proper primary studies, especially in the form of the head-to-head comparisons, which are required for the safety and efficacy assessment. Only nine studies were included in this review pointing out one of the biggest limitations in this field, which is a lack of relevant papers that have been published. Additionally, only a small sample size of in vivo human trials has been available so far. With a larger sample size, a more profound and generalised conclusion regarding the effectiveness of NPs on tissue regeneration could be drawn.

A lack of statistical data made a conclusion about potential correlations and effects limiting. This limitation can likely be attributed to the small sample size as well. The lack of research on the efficacy of NPs in patients with co-morbidities are additionally limiting. The reviewed studies did not differentiate between genders, age groups, and ethnicities. These limitations could be easily removed and present the opportunity for future research. Removing these limitations could include having a larger sample size, as well as providing a detailed follow up of patients treated with NPs.

Differentiating between age groups could provide a better understanding of NP-based tissue repair as older patients generally tend to have poorer wound healing abilities and a decreased tensile strength of the scar tissue. Similarly, gender plays an important role, since women seem to be less predisposed to delayed wound healing than men. Obesity has also been linked to poor tissue repair. Taking these and other factors into account can help improve our understanding of wound healing and the role of NPs in tissue regeneration.

After carefully reviewing relevant studies, an overall success has been noted in the animal model promising the potential for future testing on human tissue. It can be said that nanotechnology appears to be a solution for scar free wound healing. Not enough is yet known to indicate a risk-free implementation into everyday practice. Further investigations are necessary to prove a safe and significantly improved healing process in the human model and to lead us towards scar-free surgery.

6. CONCLUSION

Despite the lack of primary studies and effectively an empty result of the systematic review, results from nine published related studies on nanoparticle-aided wound healing have been identified in the literature. They examined the efficacy of nanoparticles in tissue regeneration of skin, lung, eye and tendon tissues. After reviewing relevant studies, a clear improvement can be noted when NPs were included in the treatment of tissue injuries compared to the control group.

Not enough is yet known to prove the efficacy of NPs when applied to surgical wounds because the studies are currently in their early stages of *in vivo* animal testing. An overall promising outcome has been observed in all nine studies.

With the studies performed mostly *in vitro* and on animal models *in vivo*, further investigations are necessary to permit an application in clinical practice. Possible future directions would include replicating current studies with a larger sample size and detailed follow ups.

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8. SUMMARY

Objectives: The aim of this study was to perform a systematic review of nanoparticle aided tissue regeneration and establish whether wound healing can be improved to decrease scar formation in surgical wounds, based on the basal lamina affinity molecule.

Materials and methods: A detailed systematic search was performed to summarize published data related to nanoparticle-aided tissue regeneration using the database PubMed. A focus was put on scar formation and link to the molecular feature of the basal lamina. The search was performed in PubMed.

Results: The review did not identify a single study that met all the inclusion criteria. Nevertheless, nine related papers were selected to provide a current overview of the research field and discuss the future possibilities. These studies employed a variety of approaches, which signified the developing and promising field of research.

Conclusion: Nanotechnology shows promise for improved tissue regeneration. Further investigations are necessary to assess the efficacy of nanoparticles in clinical practice. Risks and benefits need to be assessed and compared.

9. CROATIAN SUMMARY

Naslov: Razvoj kirurgije bez ožiljaka: sustavni pregled afinitetnog vezanja za bazalnu membranu

Ciljevi: Cilj ovog istraživanja bio je provesti sustavni pregled regeneracije tkiva potpomognutog nanočesticama i utvrditi može li se zacjeljivanje rana poboljšati kako bi se smanjilo stvaranje ožiljaka u postoperativnim ranama, na temelju afinitetnog vezanja bazalne lamine.

Materijali i metode: Proveden je sustavni pregled objavljenih radova koji se odnose na regeneraciju tkiva uz pomoć nanočestica pomoću baze podataka PubMed. Fokus je stavljen na stvaranje ožiljaka i vezu s molekularnim obilježjem bazalne lamine.

Rezultati: Pregledom nije utvrđena niti jedna studija koja je zadovoljila sve kriterije za uključivanja. Unatoč tome, odabrano je devet srodnih radova koji su pružili trenutni pregled područja istraživanja i podlogu za izradu rasprave. Te su studije koristile različite pristupe koji su označavali razvojno i perspektivno područje istraživanja.

Zaključak: Nanotehnologija je vrlo obećavajuća tehnologija za postizanje kontroliranog stvaranja ili potpunog izostanka postoperativnih ožiljaka. Potrebna su daljnja ispitivanja kako bi se procijenila učinkovitost nanočestica u kliničkoj praksi, posebice iz domene rizika i koristi.

10. CURRICULUM VITAE

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11. APPENDICES

Appendix 1. PRISMA sheet

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	p.1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	p.48
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	p.2-25
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	p.27
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	p.30
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	p.29
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	p.29,30
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	p.29
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	p.29
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	p.30
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	p.29,30
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	p. 29
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	p. 29
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	p. 29
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe	

Section and Topic	Item #	Checklist item	Location where item is reported
		the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	p. 29
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	p. 29
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	p. 32
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	
Study characteristics	17	Cite each included study and present its characteristics.	p. 32-40
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Not applicable
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Not applicable
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	p. 32-40
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	p.32-40
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Not applicable
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Not applicable
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	p.42,43
	23b	Discuss any limitations of the evidence included in the review.	p.42,43
	23c	Discuss any limitations of the review processes used.	p.42,43
	23d	Discuss implications of the results for practice, policy, and future research.	p.43
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Not applicable
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	

Section and Topic	Item #	Checklist item	Location where item is reported
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	p. 30
Competing interests	26	Declare any competing interests of review authors.	p. 30
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Not applicable

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

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