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RESEARCH HIGHLIGHTS ON AN ARTIFICIAL WOUND HEALING METHODOLOGY IN AN *IN VITRO* SCIENTIFIC STUDY AND MEDICINAL PLANTS IN EFFECT; ADVANCEMENTS IN *IN VITRO AND IN VIVO* WOUND HEALING TECHNIQUES: ACCELERATING RECOVERY AND MINIMISING COMPLICATIONS

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ABSTRACT

Background: Highlights of *in-vitro* scientific research on an artificial wound healing techniques that have been utilised to study wound healing activity have been a significant part of this study, which is utilised for the scientific research methodologies where wound healing is an important aspect of the study. Studying such methodologies has been very important to understand the values of *in vitro* studies with the minimum amount of invasiveness required to perform such evaluation parameters. **Main body of the abstract:** Important debates that will be had in this research study include re-epithelialisation, skin transplant in severe burns like acid burns, neoepidermis development, tissue-engineered human skin, grafted dermal substitutes, and corroboration in rodent wound healing models. These are significant ideas and abstract principles with vivid studies. Scientific literature and theories based on an artificial wound healing techniques have been a majour focus and concern of the studies. **Short conclusion:** This scientific concept has as its main focus a study model for *in vitro* wound healing used to evaluate dermal substitutes. It also includes snippets through which different *in- vitro* techniques that have been documented are focused, as well as analyses of the state of development in specific areas and critical evaluations of problems.

KEYWORDS: Wound healing, medicinal plants, anti-inflammatory, anti-bacterial.

I. BACKGROUND

Wound healing is crucial for restoring the skin's barrier function. In this process, cells at the wound margins proliferate and migrate, facilitating the re-epithelialisation of the wound surface. Wound healing assays are employed to explore the molecular mechanisms involved in wound repair and to investigate potential therapies and treatments that could enhance healing. Recently, various models of wound healing have been developed for these purposes.[90]

II. Mechanical wounding

The scratch assay's simplicity is one of the main reasons for its widespread use. This assay involves creating a scratch in a confluent cell monolayer using a sharp tool such as: a pipette tip, though other tools like special cell scrapers, metallic micro-indenters, and toothpicks are also employed. After wounding, the migration of cells into the scratched area is tracked through micrographs, which are later analyzed. As the scratch assay is a well-established method for studying wound healing, it often serves as a benchmark for newer techniques.

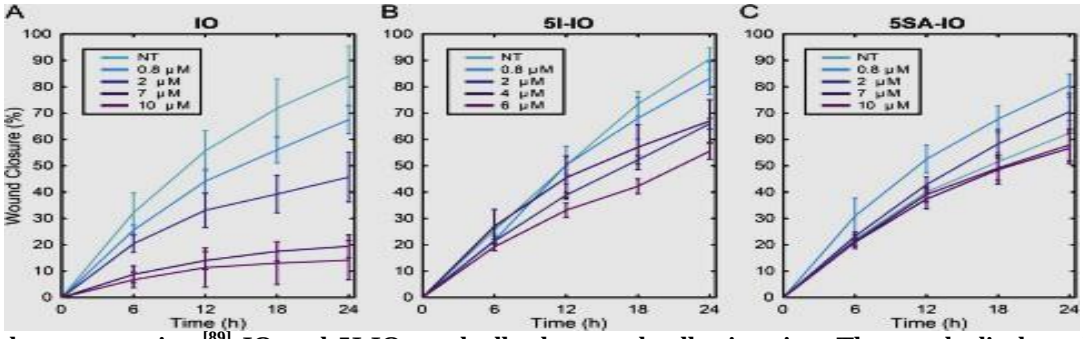
Many current variations of the scratch assay integrate automation to enhance throughput and reproducibility. However, the scratch assay has notable disadvantages, such as irregular scratches from manual wounding and potential damage to the extracellular matrix coatings on the culture dish. Addition to this, dislodged cells can accumulate at the edges of the scratch, complicating data analysis and affecting cell proliferation and migration.

An alternative method of mechanical cell destruction is stamping. This involves placing a stamp mould on a confluent cell monolayer and applying pressure either manually or automatically, which destroys the cells beneath the old. The resulting cell debris can be either removed by shifting the mould or left in place. Most stamping assays use moulds made from rubber or polymers like poly (dimethyl)siloxane (PDMS), and

these moulds can be engraved with patterns such as squares, concentric circles, or parallel lines. If debris remains, its effect on cell migration can be studied. While stamping also suffers from irregular wounding, especially with manual pressure application, it has the advantage of not disturbing the cell culture matrix coating.

Thermo-mechanical damage is a common skin injury caused by electrocautery or contact with hot objects. An *in vitro* model that simulates this type of damage is valuable for studying its cellular effects. For instance, temperature-controlled stamps can be used to distinguish between thermo- mechanical and purely mechanical damage. However, this method's drawbacks include possible heat transfer beyond the targeted area, leading to lower reproducibility in creating defined wounds.[95-107]

The wound healing assay, also known as the scratch assay, is a technique used to assess two- dimensional cell migration. In this method, a confluent monolayer of cells has an artificial gap created, and the movement of cells to fill the gap is monitored using microscopy or other imaging techniques. For this experiment, U2OS cells were seeded into 24-well plates at a density of 2 × 10^5 cells/mL and incubated for 24 to 48 hours. Upon reaching full confluence, wounds were made using a 1 mL micropipette tip. The medium was removed, cells were washed with 500µL of PBS, and 500µL of complete culture medium containing various compounds was added to each well. Images were captured immediately after media replacement (T = 0) and every six hours over a 24-hour period using a multi-mode plate reader at 10× magnification. The wound areas in the exported images were measured using Image J. Using the polygon selection tool, the wound areas were marked and quantified (via Analysis > Measure). The extent of wound closure at T6, T12, T18, and T24 was calculated by subtracting the area measured at T0, and the percentage of closure was determined by normalizing this difference to the area at T0. Both IO and 5I-IO significantly inhibited cell migration, a finding confirmed by a two-tailed Student's T-test at t=24h with Bonferroni-corrected P-values of less than 0.05. Conversely, 5SA-IO did not significantly affect cell migration, although the lowest dose resulted in a statistically significant increase in migration, higher doses did not show a substantial effect. This is consistent with circadian effects, where IO and 5I-IO influenced periods, but 5SA-IO did not. Despite the small sample size (N = 3 or 4), the variance across treatments was consistent, and the detected effects were large enough to ensure that the student's T-test had sufficient power (greater than 0.95).[88]



Graphical representation:[89] IO and 5I-IO markedly decreased cell migration. The graph displays the mean (line) and standard deviation (error bars) of wound closure over time for both untreated and treated cells.

The skin is the largest organ in the human body, and its barrier function is vital for the overall health of the organism. The wound healing process is dynamic and can be categorized into five stages: hemostasis, inflammation, proliferation, migration, and maturation or remodeling. This process can encounter complications such as: infections, chronic wounds, and excessive healing, which can result in hypertrophic scars and keloids. Studying wound healing not only uncovers the fundamental mechanisms behind tissue repair but also aids in enhancing current clinical treatments. Throughout healing, cells proliferate and migrate to the wound site, with migration being the critical limiting step in the process. As a result, wound healing assays often focus on cell migration. In these assays, a monolayer of confluent cells is intentionally wounded under controlled conditions, and the migration of cells into the previously cell-free area is monitored and analysed. To mimic cell-matrix interactions, the cell culture surface is often coated with extracellular matrix components. These *in vitro* assays are valuable for screening potential medical treatments and understanding the effects of specific compounds and genetic modifications on the healing process before moving on to *in vivo* experiments.[91-94]

For the skin's barrier function to be restored, wound healing is crucial. Cells near the edges of the wound multiply and move during this process, re-epithelializing the wound surface. The examination of novel therapies and treatments for better healing uses wound healing assays to examine the molecular mechanisms of wound repair. In recent times, a variety of wound healing models have been created or developed.

Cutaneous wound healing is a vital physiological undertaking that involves the coordinated efforts of various cell types and their derivatives. The initiation of restoration processes for a wound caused by a local

injury begins early in the inflammatory phase and ultimately leads to repair. Repair involves the replacement of specialised structures through collagen deposition, while regeneration encompasses the proliferation and subsequent differentiation of cells derived from preexisting tissue cells and/or stem cells.[32,33] Tissue repair is a straightforward sequential progression where growth factors stimulate cell proliferation, orchestrating a series of dynamic alterations encompassing soluble mediators, blood cells, extracellular matrix production, and the proliferation of parenchymal cells. According to Mitchel *et al.*, the principles of repair observed in the skin healing process exemplify the general mechanisms applicable to a majourity of tissues.[34]

The processes of tissue regeneration and repair come into play following the initiation of a lesion, whether it is due to trauma or arising from a particular pathological condition. Lesions result from various stimuli that disrupt the physical integrity of functional tissues. These stimuli, causing damage, can be either external or internal, taking the form of physical, chemical, electric, or thermal influences. Further, these lesions have the potential to harm specific organelles or affect cells as a whole.[32]

According to Buckley, the interplay between leukocytes and stromal cells during an acute inflammatory response centres around the site of inflammation. Neutrophils, recognised for their expression of numerous pro-inflammatory cytokines and the release of a significant amount of potent antimicrobial substances like reactive oxygen species (ROS), cationic peptides, and proteases, are concentrated at the lesion site. The ongoing inflammatory reaction involves the continued recruitment of neutrophils triggered by the activation of the complement system, platelet degranulation, and bacterial degradation products.[35,36]

These neutrophils are drawn to the site by a multitude of inflammatory cytokines generated by activated platelets, endothelial cells, and breakdown products of pathogenic agents.[37] In this fashion, neutrophils take on the central role as the primary cells activated and enlisted for both tissue cleanup and the facilitation of the demise of invading agents.[34]

Consequently, apart from the resident macrophages, the predominant macrophage population at the lesion site is mobilised from the bloodstream in reaction to chemotactic substances, such as extracellular matrix protein fragments, TGF-β, and MCP-1 (monocyte chemotactic protein 1).[38] Examining gene expression profiles allows for the categorisation of macrophages into classically activated (M1 with pro-inflammatory properties) and alternatively activated (M2 with anti-inflammatory and pro-angiogenic characteristics). These macrophages are responsible for releasing crucial growth factors, including PDGF and VEGF, which play a vital role in initiating and promoting new tissue formation at the site of injury. Depletion of macrophages in animals results in deficiencies in wound repair, emphasising their pivotal role in transitioning from the exudative stage to the proliferative stage during tissue repair.[39,40]

Macrophages play a dual role in the process, engaging in phagocytosis of muscular debris while concurrently generating and releasing cytokines, pro-angiogenic, inflammatory, and fibrogenic factors, along with free radicals. Additionally, their secretion of chemotactic factors serves to attract other inflammatory cells to the wound site. Macrophages contribute to vasodilation and impact micro-blood vessel permeability by producing prostaglandins. This collective action results in the activation of endothelial cells. According to Mendonça & Coutinho Netto, these endothelial cells are responsible for the production of key cytokines like PDGF, TGF-beta, FGF, and VEGF, which play a prominent role in stimulating the formation of granulation tissue.[41,42,43] upon the occurrence of a tissue lesion, the modulation of the repair process is governed by the cellular activities within the inflammatory response at the lesion borders, involving cells like keratinocytes. Additionally, a diverse array of cytokines and growth factors come into play, influencing the processes of migration, proliferation, and local cell differentiation.[44]

There are three recognised types of epithelial-mesenchymal transition (EMT). The first type (Type I) takes place during embryogenesis, exemplified by dermal fibroblasts in connective tissue. These fibroblasts play a crucial role in providing instructive signals for the positioning and differentiation of various skin types and other skin appendages, ultimately forming the overarching epidermis. In adult tissues, EMT occurs as a response to remodeling and fibrosis **(Type II).** The third type **(Type III)** involves the metastatic process, where carcinoma cells undergo phenotypical conversion, gaining mobility through the utilisation of the EMT program, typically responsible for generating adult fibroblasts.[45,46,47]

Epithelial-mesenchymal transition **Type II** is connected to processes like tissue healing, regeneration, and fibrosis. This phenomenon, integral to tissue repair, gives rise to fibroblasts and other cells essential for the reconstruction of tissues affected by injuries and inflammation. This specific type of epithelial-mesenchymal transition, intertwined with inflammatory responses, comes to a halt once the inflammation has been alleviated.[47]

The discovery of the hedgehog gene originated from genetic investigations into body segmentation in *Drosophila melanogaster*, the fruit fly. In 1980, German researchers Nüsslein and Wieschaus, exploring the genetic regulation of *Drosophila melanogaster* embryos, observed that the absence of a specific gene led to abnormal projections, resulting in a mutant phenotype with an oval shape and disorganised denticles,

resembling a hedgehog.

Homologs of the hedgehog gene have been identified in numerous invertebrates and vertebrates, exhibiting evolutionary conservation and playing analogous crucial roles in regulating tissue standardisation, differentiation, and cell proliferation during embryonic development. Additionally, these genes contribute to the control of germ cell behaviour and the maintenance of homeostasis in adult organisms.[48,49]

III. Main text

Chronic wounds have been a majour factor of serious harm to global public health.**[27]**

Current guidelines do not recommend the use of antibiotics to treat clinically uninfected ulcers. However, physicians continue to prescribe antibiotics for clinically uninfected ulcers with the rationale 'better to be safe than sorry'. Yet, antibiotic resistance is increasing, side-effects are common and treatment costs are rising. Evidence is needed to identify whether antibiotic treatment for clinically uninfected ulcers can be justified or we should stop prescribing them. The aim of this study was to evaluate whether antibiotic treatment in cases of clinically uninfected ulcers improved ulcer healing compared to treatment without antibiotics. No benefits of antibiotic treatment over non-antibiotic treatment for clinically uninfected ulcers were identified. The findings of this study emphasise the recommendation of current guidelines to not treat clinically uninfected ulcer with antibiotics.[28]

There is limited evidence regarding classification and care of scalp wounds. As a result, many of the current practices for scalp wound management are based on evidence derived from studies involving different anatomical regions, not considering its particular anatomy, vasculature and microbiome.[29]

The recuperative mechanism of wound healing involves the replacement of impaired cellular structures in damaged tissues. Throughout history, natural compounds have been extensively utilised for the treatment of wounds. Such compounds are categorised into four primary groups, namely, those with: anti-inflammatory, antioxidant, antibacterial, and collagen-promoting properties.

Using natural chemicals to treat wounds has been a common practice for millennia. These substances are widely distributed in many different plants and animals and are an easy-to-access resource for wound treatment. Traditional Indian medical techniques and Chinese remedies have a wealth of evidence supporting their effectiveness in the healing process. Because there is such a wide range of natural compounds, thorough assessments of these substances can be helpful to researchers and readers alike. They can facilitate the methodical investigation of particularly interesting compounds and the development of novel products for the treatment of wound healing. Injuries arise due to unintended incidents, surgical procedures, and various medical conditions, leading to pain, inflammation, and a reduction in functionality. These effects can significantly impact a patient's quality of life and incur financial expenses.[50]

Wounds fall into two categories: acute or chronic, with the intricate process of wound healing involving the replacement of damaged cellular structures and tissue layers. Acute wounds follow a well-defined healing progression with clear signs within four weeks, while chronic wounds deviate from the normal healing phases and lack evident progress within the same timeframe. The effectiveness of wound healing relies on various factors, including conditions at the wound site, systemic mediators, the nature of the injury, and underlying health issues. Strategies for wound treatment primarily include physical closure using methods such as sutures and dressings. In cases where the wound is inaccessible, leaving it open allows the damaged area to clear itself and fill with connective tissue, enabling a sequential healing process through distinct phases.[51,52]

IV. Research on *in vitro* wound healing: a knowledge of the current science

Wound healing is a complex process that involves various cellular and molecular events. *In vitro* wound healing research is a valuable tool for understanding the mechanisms involved in this process. In this article, we will explore the basics of *in vitro* wound healing research and its significance in the field of medicine.

V. Understanding *in vitro* wound healing research

In vitro wound healing research involves studying the cellular and molecular events that occur during the healing process using cell cultures. Researchers create a controlled environment in a laboratory setting to mimic the conditions of a wound. They then observe how cells respond to different stimuli and treatments. This type of research helps scientists understand the mechanisms involved in wound healing and develop new treatments for wounds that do not heal properly.

VI. The significance of *in vitro* wound healing research.

In vitro wound healing research has significant implications for the field of medicine. By understanding the cellular and molecular events involved in wound healing, researchers can develop new treatments to improve the healing process. For example, researchers can use *in vitro* models to test the efficacy of different drugs and therapies on wound healing. This type of research can also help identify new targets for drug development.

In vitro wound healing research is also important for understanding the underlying causes of chronic wounds. Chronic wounds are wounds that do not heal properly and can lead to serious complications such as: **i.** infection

and, **ii.** Amputation. By studying the cellular and molecular events involved in chronic wounds, researchers can develop new treatments to improve healing outcomes.

VII. The future of *in vitro* wound healing research

In vitro wound healing research is a rapidly evolving field. New technologies and techniques are constantly being developed to improve our understanding of the mechanisms involved in wound healing. For example, researchers are using advanced imaging techniques such as: **i.** confocal microscopy to study cells in real-time. They are also developing 3D models of wounds to better mimic the complex environment of a real wound.

In conclusion, *in vitro* wound healing research is a valuable tool for understanding the mechanisms involved in the healing process. By studying the cellular and molecular events involved in wound healing, researchers can develop new treatments to improve healing outcomes. This type of research is also important for understanding the underlying causes of chronic wounds. As technology continues to advance, *in vitro* wound healing research will continue to play a critical role in the development of new treatments for wounds that do not heal properly.

Complications during this procedure include infections, the development of chronic wounds, and excessive wound healing, which can result in hypertrophic scars and keloids. The basic underlying mechanisms of this wound healing process are clarified by research, which also provides the path for the advancement of currently used clinical treatments.[10]

The maintenance of the skin's barrier function is essential for any functional organism because the skin is the largest organ in the human body. The five stages of the dynamic process of wound healing are hemostasis, inflammation, proliferation, migration, and maturation/remodeling.[4,7] Cells multiply and move into the damaged area as the body heals. The limiting event in wound healing is the migration phase. As a result, migration assays form the foundation of assays exploring wound healing. In wound healing tests, a confluent cell layer is damaged under predetermined conditions, and the influx of cells into the region that was previously occupied by cells is recorded and analysed. Extracellular matrix elements are commonly applied to the cell culture surface to mimic cell-matrix interactions. Before beginning *in vivo* trials, potential medical treatments can be evaluated *in vitro* by investigating the wound healing process. *In vitro* tests are a great way to research the effects of particular chemicals and the effects of genetic variations on the healing process.[5]

To reestablish barrier function and stop infection, skin wounds must be re-epithelialised.[2]

VIII. Snippet: Skin wound re-epithelialisation: cellular mechanisms and treatment options in adults

Adult mammals' skin wounds heal in a complex, multi-stage process that includes the development of blood clots, inflammation, re-epithelialisation, the production of granulation tissue, neovascularisation, and remodelling.

Re-epithelialisation describes the resurfacing of a wound with new epithelium. The cellular and molecular processes involved in the initiation, maintenance, and completion of epithelialisation are essential for successful wound closure.[11]

The growth factors, cytokines, matrix metalloproteinases, cellular receptors, and elements of the extracellular matrix are just a few of the modulators at their play. These studies focus on the biological processes that underlie the migration and proliferation of keratinocytes during epidermal closure. Non-re-epithelialised, chronic non-healing wounds do not progress through the typical stages of the wound healing process in a timely and organised manner.

IX. Snippet: An extracellular matrix's[15] role in the re-epithelialisation of wounded skin surface and structure cure

The ability of skin to act as a barrier is primarily determined by cells that maintain the continuity and integrity of skin and restore it after injury. Cutaneous wound healing in adult mammals is a complex, multi-step process that involves overlapping stages of blood clot formation, inflammation, re-epithelialisation, granulation tissue formation, neovascularisation, and remodelling. Under favourable conditions, epidermal regeneration begins within hours after injury and takes several days until the epithelial surface is intact due to reorganisation of the basement membrane. Regeneration relies on numerous signalling cues and on multiple cellular processes that take place both within the epidermis and in other participating tissues. A variety of modulators are involved, including growth factors, cytokines, matrix metalloproteinases, cellular receptors, and extracellular matrix components. The majour focus of the study is on the involvement of the extracellular matrix proteins[12,13,15] that impact epidermal regeneration during wound healing.

X. Extracellular matrix[13] components consist of

XI. Proteoglycans[12]

Glycosaminoglycans (GAGs) are carbohydrate polymers that are mostly attached to extracellular matrix proteins to form proteoglycans (hyaluronic acid is a notable exception).

XII. Heparan sulphate[12]

Heparan sulfate (HS)[21] is a linear polysaccharide found in all animal tissues. It occurs as a proteoglycan (PG), in which two or three HS chains are attached in close proximity to cell surface or ECM proteins. Heparan

sulphate proteoglycans (HSPGs) are glycoproteins, with the common characteristic of containing one or more covalently attached heparan sulfate (HS) chains, a type of glycosaminoglycan (GAG). Cells elaborate a relatively small set of HSPGs (~17) that fall into three groups according to their location: membrane HSPGs, such as syndecans and glycosylphosphatidylinositol-anchored proteoglycans (glypicans), secreted extracellular matrix HSPGs (agrin, perlecan, type **XVIII** collagen), and the secretory vesicle proteoglycan, serglycin.[22]

XIII. Chondroitin sulphate[12]

Chondroitin sulphates contribute to the tensile strength of cartilage, tendons, ligaments, and the walls of the aorta. They have also been known to affect neuroplasticity.

XIV. Keratan sulphate[12]

Keratan sulfates have variable sulfate content and, unlike many other GAGs, do not contain uronic acid. They are present in the cornea, cartilage, bones, and horns of animals.

XV. Non-proteoglycan polysaccharide [12]

XVI. Hyaluronic acid[1215]

Hyaluronic acid[15] (or "hyaluronan") is a polysaccharide consisting of alternating residues of D-glucuronic acid and N-acetylglucosamine and, unlike other GAGs, is not found as a proteoglycan. Hyaluronic acid in the extracellular space confers upon tissues the ability to resist compression by providing a counteracting turgor (swelling) force by absorbing significant amounts of water. Hyaluronic acid is thus found in abundance in the ECM of load-bearing joints. It is also a chief component of the interstitial gel. Hyaluronic acid is found on the inner surface of the cell membrane and is translocated out of the cell during biosynthesis. Hyaluronic acid acts as an environmental cue that regulates cell behaviour during embryonic development, healing processes, inflammation, and tumour development. It interacts with a specific transmembrane receptor, CD44.

XVII. Proteins\sCollagen[12]

The ECM's most prevalent protein is collagen. In actuality, collagen makes up 90% of the protein content of bone matrix and is the most prevalent protein in the human body. Collagens are fibrillar proteins that are found in the ECM and provide resident cells with structural support. Procollagen, the precursor form of collagen, is exocytosed and subsequently broken down by procollagen proteases to facilitate extracellular assembly. Genetic flaws in the genes that code for collagen are associated with disorders like Ehlers-Danlos syndrome, osteogenesis imperfecta, and epidermolysis bullosa. Depending on the different sorts of structures they produce, the families of collagen can be categorised into.

XVIII. Fibrillar (Type I, II, III, V, XI), Facit (Type IX, XII, XIV), Short chain (Type VIII, X).

XIX. Basement membrane (Type IV).

XX. Other

XXI. Cell adhesion proteins: fibronectin[15] and laminin.

XXII. Snippet: Soft tissue reconstruction with biomaterials and tissue engineering principles

In order to regenerate diseased or damaged tissues, the field of regenerative medicine uses biomaterials and cell-based techniques. The current therapeutic approaches to soft tissue reconstruction emphasise the necessity for medicines based on regenerative medicine. Individuals who have suffered considerable soft tissue loss frequently need autologous flap reconstructions, which include taking tissue from a donor site and using it to patch the defect. A conveniently available soft tissue replacement in cases of damage or tumour removal might be made possible by the development of an over-the-counter biomaterial for soft tissue regeneration. Researchers have looked into both synthetic and organically produced matrices as biomaterial scaffolds[20] for adipose tissue engineering.[14]

To aid in tissue regeneration, these scaffolds[20] may be applied alone or in combination with a cell source. These techniques for soft tissue healing may encourage the production of new adipose tissue, eliminating the requirement for donor site tissue to replace the soft connective tissues that have been injured.[14]

XXIII. Snippet: ECM proteins have been evaluated as a potential scaffold structure to support various cell-based therapies. For example, stem cell therapies have evaluated the use of a complex tumour matrix, Matrigel. This product is marketed by BD Biosciences and is composed of Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells. Matrigel has demonstrated the ability to facilitate the growth of various stem cell populations and maintain an undifferentiated state for a period of time. The main ECM proteins within Matrigel are laminin and collagen.[15]

XXIV. Snippet: A comparison of various cancer cell types' 2D and 3D cell cultures.

Cell culture is a widely used *in vitro* tool for improving our understanding of cell biology, tissue morphology, mechanisms of disease, drug action, protein production, and the development of tissue engineering. Most research regarding cancer biology is based on experiments using two- dimensional (2D) cell cultures *in vitro*. However, 2D cultures have many limitations, such as the disturbance of interactions between the cellular and extracellular environments, changes in cell morphology, polarity, and method of division. These disadvantages led to the creation of models that are more closely able to mimic conditions *in vivo*. One such method is three-dimensional culture (3D). Optimisation of the culture conditions may allow for a better understanding of cancer biology and facilitate the study of biomarkers and targeting therapies. In this review, we

compare 2D and 3D cultures *in vitro* as well as different versions of 3D cultures.[18]

XXV. Snippet: Scaffolds and tissue engineering

In order to create biological replacements that restore, maintain, or enhance tissue function, the interdisciplinary discipline of tissue engineering integrates engineering and life science principles.

XXV.a. In tissue engineering, there are three methods

(1) Using isolated cells or cell substitutes to replace the cells that perform the required function

(2) Delivering substances that induce tissue formation, such as growth and differentiation factors, to specific locations.

(3) Growing cells on three-dimensional scaffolds.[20]

XXV.b. Neovascular growth factors[23, 24]

The biological process of creating new blood vessels is called neovascularisation. Many factors, including trauma or chronic ischaemia brought on by illnesses like diabetes, might trigger neovascularisation.[24] In order for microvascular endothelial cells to extend cytoplasmic buds in the direction of chemotactic stimuli, the basement membrane around them must be destroyed first. This is the first step in the neovascularisation process. Endothelial cells that are migrating grow longer, divide more often, and finally form the tubes that connect to create or develop mature new capillaries. Peptide growth factors may regulate important processes at each stage of neovascularisation[24] through both direct and indirect activities, according to the findings of *in vitro*, *in vivo*, and clinical research. Degranulating platelets emit PDGF, IGF-I, EGF, and TGF- beta at the sites of vascular injury. In ischemic or injured areas, neutrophils and macrophages produce and release TGF-alpha, TGF-beta, and PDGF, and injured endothelial cells secrete FGF. These peptide growth factors have the ability to promote endothelial cell migration, mitosis, and differentiation in culture as well as cause neovascularisation in animal models. According to clinical correlations, peptide growth factors in the vitreous, like IGF-I and bFGF, may encourage diabetic retinopathy, according to clinical correlations. It may be possible to create treatment strategies that specifically target the peptide growth factors that regulate neovascular disorders, as the molecular mechanisms of neovascular growth factors are better known.

The coordination of keratinocyte proliferation, migration, and differentiation is necessary for this process, but it may be hindered by a number of extrinsic and host-dependent variables. Before grafting split-skin grafts, deep, full-thickness wounds, like burns, are frequently grafted with dermal matrices. These dermal matrices must be incorporated into the host skin in order to act as a substrate for the development of the neoepidermis. The absence of appropriate *in vitro* model systems has hindered systematic preclinical research on keratinocyte migration on known and experimental matrices. Here, we created a full-thickness *in vitro* wound healing model in tissue- engineered human skin that allowed us to examine the re-epithelialisation process in several grafted dermal substitutes.[2] The usage of several experimental animals is avoided or obviated due to the high potential of our model for preclinical evaluation of tissue-engineered skin substitutes in a medium-throughput manner. An *in-vivo* wound healing simulation using tissue- engineered skin transplants has been a main focus.[1] Models that closely mirror the physiology of human wounds would be advantageous for improvements in research understanding of the intricate process of wound healing and the development of innovative growth factors and gene therapies. In order to perform such studies, a hybrid wound-healing model was created using skin made from human tissue engineering and implanted onto athymic mice. As the native and foreign dermal areas fused together, mouse mesenchymal cells penetrated the grafted tissues. The transplanted epithelium retained its human origin, as shown by immunohistochemical staining for human involucrin, although the dermis was heavily populated with mouse fibroblasts and blood vessels. Full-thickness excisional wounds were made by punching grafted tissues with a 4-mm diameter. The tissues were removed and evaluated for re-epithelialisation, differentiation, and neovascularisation[23] at 1 and 2 weeks.[1] Surprisingly, the average rate of keratinocyte migration (120 microns per day) was much slower than that of the mouse epidermis and comparable to migration rates seen in human individuals. A typical pattern of differentiation was observed in the neoepidermis when keratin 10, laminin, and involucrin were stained using immunohistochemistry. At one week, neovascularisation[23,24] was noticeably increased in the granulation tissue, but by two weeks after the injury, it had decreased to the same level as the uninjured tissue. It implies that skin grafts on a mouse model could serve as a realistic representation of human wound healing. This model may be excellent for addressing mechanistic concerns and assessing the effectiveness of biomaterials and gene therapies for encouraging wound healing since skin analogues may be created using patient cells and genetically manipulated to boost or suppress gene expression.[1]

XXVI. *In vitro* wound healing assays

In vitro assays are useful and beneficial for testing purposes wherever wound healing is required. It also saves animals from troubles and tortures, and the study can be done without the animal model. The studies' majour concentrates on *in vitro* assays since they offer a quick, affordable, and ethical substitute for using animal models is a significant focus and majour interest of this investigative or literature studies. 2-dimensional (2-D) cell monolayer assays[17], 3-dimensional (3-D) culture[17], including bioengineered skin models to capture complex wound healing mechanics like cell-matrix interactions and the interplay of different cell types in the healing process is one significant understanding.[3]

External or internal tissue damage and lesions commonly referred to as wounds are attributed to mechanical trauma, burns, and various diseases as the primary causes.[26]

Inflammation, proliferation, and final tissue remodelling are considered the traditional phases of wound healing. Dermal fibroblast migration is a crucial stage in the recovery of wounds. Dermal fibroblasts become activated during the inflammatory phase of wound healing.

Fibroblasts are drawn to the site of the wound by cytokines and growth factors such as: platelet- derived growth factor (PDGF) and interleukin-1 beta (IL-1). Fibroblasts move into the wound bed along collagen matrices after originating in the dermis and tissues around the wound.

Certain fibroblasts undergo differentiation into contractile myofibroblasts, which, by expressing alpha smooth muscle actin (-SMA), are in charge of causing wound contraction.

Extracellular matrix **(ECM),** mostly collagen **types I and III**, is produced by fibroblasts and myofibroblasts and is essential for cell ingrowth and wound healing.

For the proliferation of fibroblast cells and the development of new tissue, transforming growth factor (TGF-) and vascular endothelial growth factor **(VEGF)** are required.[26]

In addition to their function in ECM secretion, they also release a variety of growth factors that can promote the migration and proliferation of keratinocyte cells.[26]

Due to aberrant EGF and FGF receptor location in diabetes patients, fibroblast cell migration is inhibited, which prevents the signalling cascades that promote wound healing from being stimulated. Moreover, an excess of TGF-1 dysregulates the production of collagen and results in the development of unneeded fibrosis.[26]

Cell migration is a crucial step in the healing of wounds. As a result, understanding the mechanisms underlying cell motility as well as the impacts of bioactive compounds as therapeutic interventions for slow wound healing and chronic wounds are both benefited from the examination of cell migration.[26]

The typical two-dimensional (2D) cell monolayer configuration [17] is used for wound healing tests. This format is the most popular because 2D wound healing experiments may be performed using tools that are common in most cell culture labs under conventional cultivation conditions. Yet, it is now widely acknowledged that the complexity of the wound healing process exceeds what 2D cell culture can represent. So, the next stage in wound healing research is the creation of unique three-dimensional (3D)[17] *in vitro* models to explore healing mechanisms. In 3D cell culture[17], cell-seeded scaffolds[20] are used to record intricate wound healing mechanics, such as cell-matrix and cell-cell interactions.[3]

XXVII. 2D tests for wound healing[3,25]

Each 2D wound healing assay's fundamental premise is the purposeful breakdown of a confluent cell monolayer, leaving a cell-free space that cells can use to bridge and repair. Because of this, the majority of 2D wound healing assays[25] consist of three fundamental steps: cell injury (wounding), monitoring of the healing process and data capture (using a microscope or impedance measurement, for example), and finally, data assessment. Since micrographs are taken under certain conditions in the controlled environment of an incubator, time-lapse microscopy is a particularly alluring choice for collecting data for a wound assay.

XXVIII. Test for wound scratches *in vitro*[25]

The scratch test is frequently used to measure the rate of cellular migration on 2-D surfaces over time and in response to various treatments. One of the most used *in vitro* wound-healing tests; it enables the selection of the ideal dosage for the drugs under investigation. A pipette tip scratch is used to make an incision-like gap after confluent cells are grown in a monolayer. The "wounded" area is photographed both right away and at specific intervals afterwards, and cell migration is measured and expressed as the average percentage of the scratch area's closure.

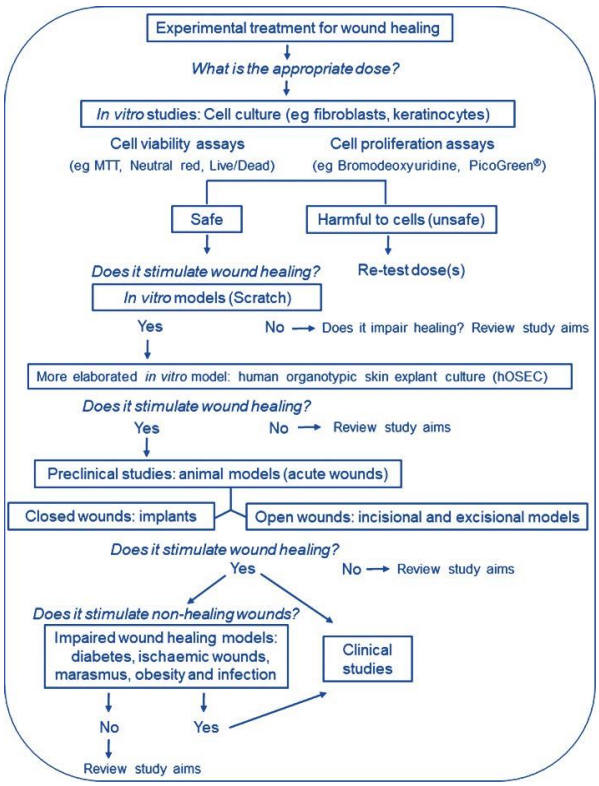


Figure 1: Common models used in investigations of wound healing.[6]

XXIX. *In Vivo* wound healing research: understanding the process

Wound healing is a complex process that involves a series of events aimed at restoring the integrity of damaged tissues. *In vivo* wound healing research is a critical area of study that seeks to understand the mechanisms involved in this process. This research is essential in developing effective wound healing therapies and treatments. In this article, we will explore the basics of *in vivo* wound healing research, including its importance, methods, and applications.

XXX. The importance of *In Vivo* wound healing research

In vivo wound healing research is critical in understanding the complex processes involved in wound healing. This research helps scientists to identify the cellular and molecular mechanisms involved in the process, including inflammation, angiogenesis, and tissue regeneration. By understanding these mechanisms, researchers can develop new therapies and treatments for wounds that are slow to heal or do not heal at all. *In vivo* wound healing research also helps to identify factors that may delay or inhibit the healing process, such as infections, chronic diseases, and poor nutrition.

XXXI. Methods used in *In Vivo* wound healing research

There are several methods used in *in vivo* wound healing research, including animal models and human clinical trials. Animal models are commonly used to study wound healing because they allow researchers to control various factors such as: age, sex, and genetics. Animal models also allow researchers to study the effects of different treatments on wound healing. Human clinical trials are also used in *in vivo* wound healing research to test the safety and efficacy of new therapies and treatments. These trials involve human subjects who have wounds that are slow to heal or do not heal at all.

Unlike human skin, which only has the panniculus carnosus layer in the platysma of the neck, rodent skin has this layer, which causes an injury to heal quickly. While evaluating the translational significance of rodent studies, it is necessary to take into account the fact that human wounds recover through re-epithelialisation and the development of granulation tissue. The differences in skin architecture and physiology between men and women should be taken into account when planning the studies. For instance, the thicker dermis of male skin makes it 40% stronger than that of female skin, which also has a thicker epidermis and subcutaneous layer. Overall, *in vivo* wound models have advantages. They allow the study of multiple cell populations/body system interactions during repair; allow the investigation of multiple elements of the healing process; allow selective depletion of specific genes to determine their effect on wound healing; permit the study of a functional immune system; enable the creation of multiple wounds within one animal; and also, can model different wound healing causes (burns, surgery, crushing, etc.)[9]

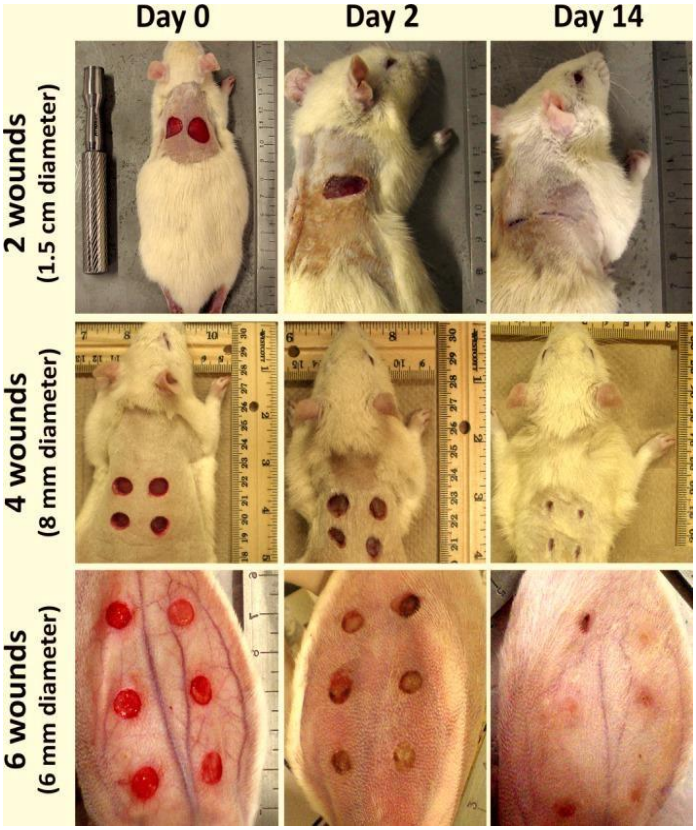


Figure 2: *In-vivo* study models an example illustrated with animal models.[8]

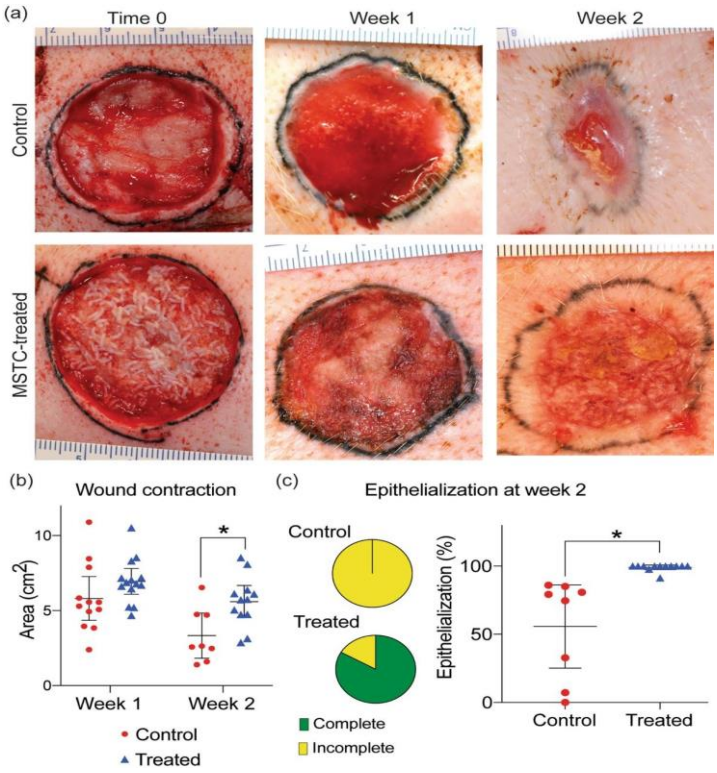
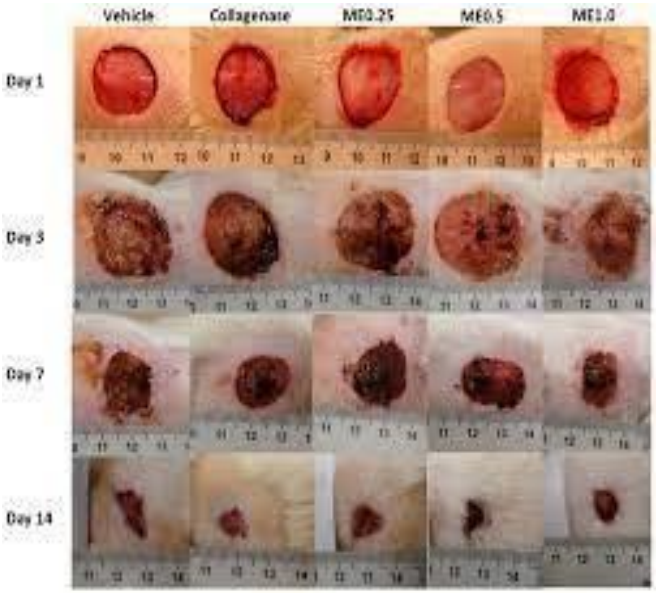


Figure 3: "Enhanced Healing Effects of MSTCs: Visual Evidence and Quantitative Analysis".

(a) llustrated above are representative images contrasting untreated wounds with those treated using MSTCs. Initial images depict the immediate post-treatment state, where MSTCs are identifiable in the wound bed as short, white, rod-like structures. Subsequent images at one week and two weeks post-wounding reveal notable differences in wound progression. Notably, the wound margins were marked with black ink for clarity.

(b) Quantitative analysis of the total wound area demonstrates a significant reduction in wound contraction among MSTC-treated wounds by the second week compared to controls.

(c) (Analysis of re-epithelialisation progress at week 2 reveals compelling findings. Pie charts visually represent the proportion of wounds that achieved complete versus partial re- epithelialisation. The graph displays the overall extent of re-epithelialisation, highlighting a remarkable 99% complete re-epithelialisation rate in 10 out of 12 MSTC-treated wounds. In contrast, none of the 8 control wounds achieved complete re-epithelialisation, with an average re-epithelialisation rate of 56%. Statistical significance (*p < 0.05) emphasises the efficacy of MSTCs in promoting accelerated wound healing processes.[108]



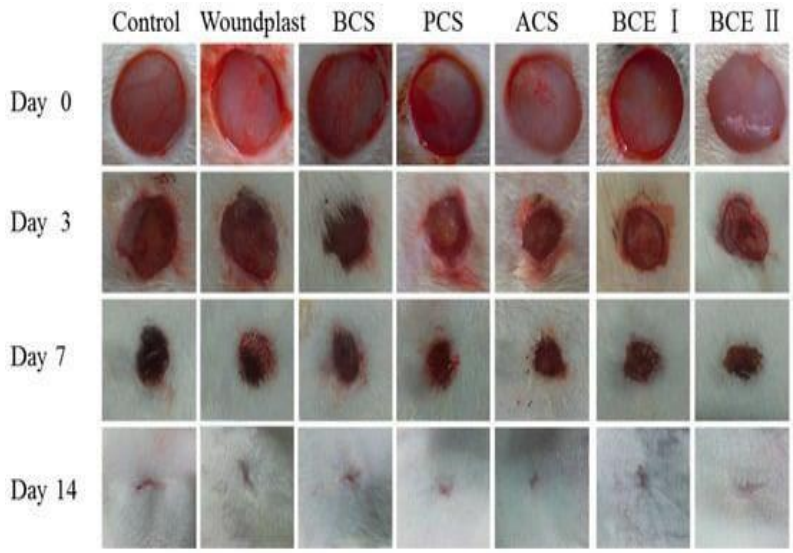
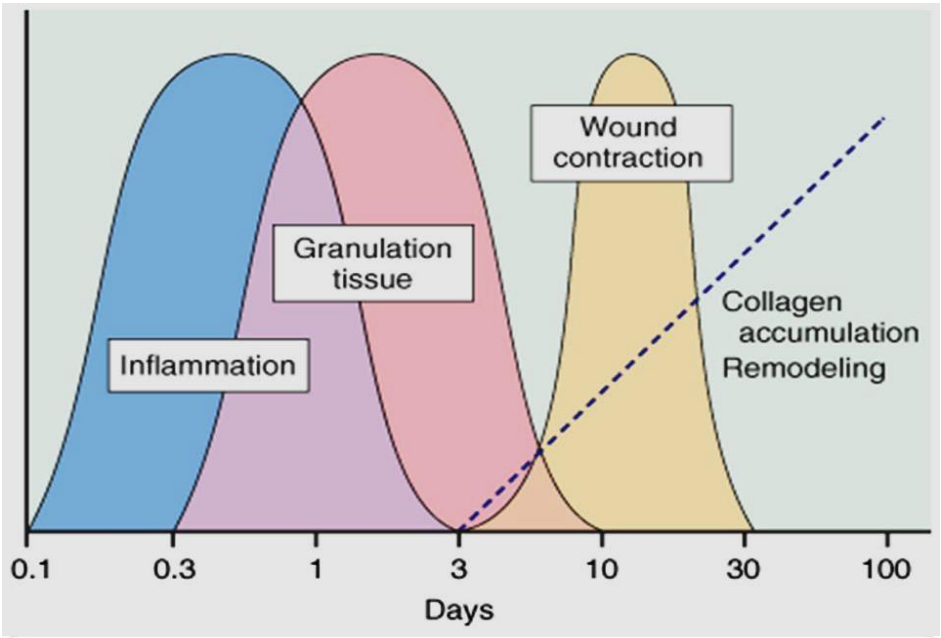
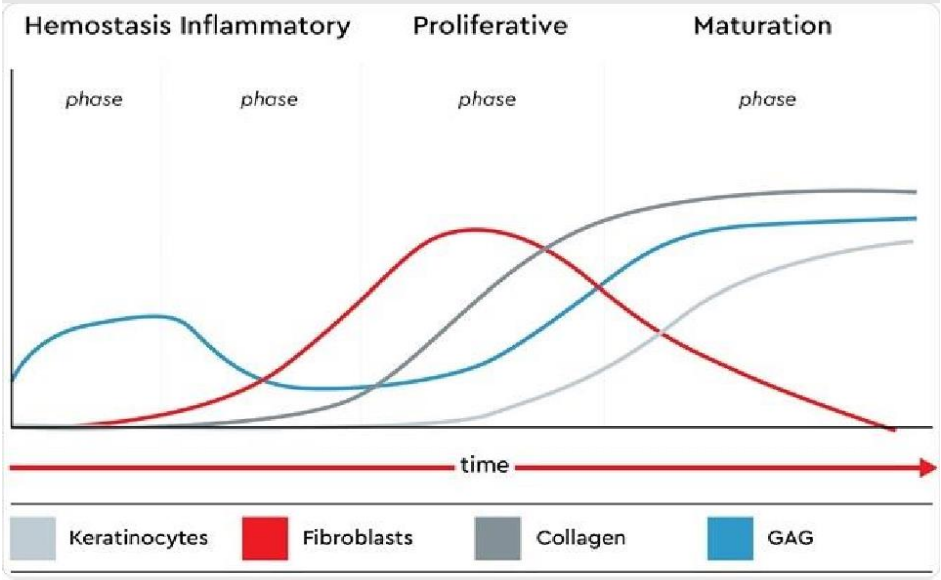


Figure 4: Wound healing process.[109-110]





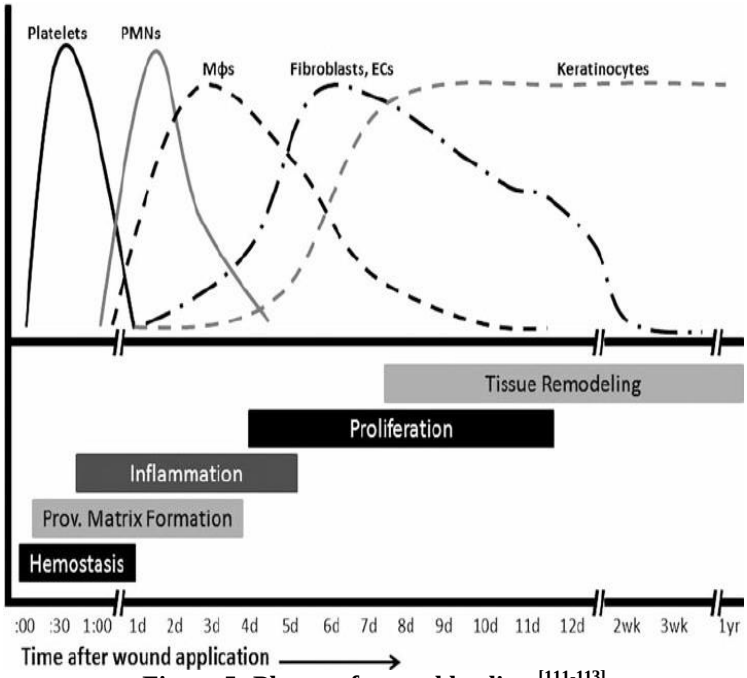


Figure 5: Phases of wound healing.[111-113]

XXXII. Applications of *In Vivo* wound healing research

In vivo wound healing research has several applications in medicine. One of the most significant applications is the development of new therapies and treatments for chronic wounds, such as diabetic foot ulcers and pressure ulcers. These wounds are often slow to heal and can lead to serious complications such as: **i.** infections and, **ii.** amputations. ***In vivo*** wound healing research has also led to the development of new wound dressings and other products that can help to promote healing. Additionally, *in vivo* wound healing research has helped to identify factors that may delay or inhibit the healing process, such as infections, chronic diseases, and poor nutrition.

XXXIII. Virtual study aids

Project ECHO Skin and Wound aims to establish interprofessional teams specialising in skin and wound care throughout Ontario, facilitating the transfer of knowledge rather than patients to diverse spokes, encompassing indigenous, remote, isolated, and northern communities. Each accredited session within this initiative features an interactive didactic segment, a clinician corner, and the presentation of two de-identified cases from spokes adhering to the ten guidelines outlined in Wound Bed Preparation 2021 (WBP-2021).[31]

Virtual education breaks through the constraints of time zones, geographical limits, and physical distances. In this mode of learning, students engage with a digital curriculum delivered by instructors through online video or audio lectures. The instructional format can be self-paced, allowing students to learn asynchronously, or real-time, enabling synchronous interaction. While the concept of virtual education is not novel, with institutions like Excelsior University leading the way since 1971, its significance in providing accessible and flexible learning experiences remains unparalleled.[30]

XXXVI. A publication authored by Canadian writers Sibbald *et al.* details their encounter with virtual skills education in the realm of skin and wound care. The transformative education approach comprises three crucial components: the implementation of Project ECHO (Extension for Community Healthcare Outcomes), patient navigation strategies, and the incorporation of virtual skills education.[30]

XXXV. Natural remedies in Indian traditional knowledge for wound and burns therapy

Traditional Indian knowledge encompasses a wealth of natural remedies for wound and burn therapy.

Lupeol, sourced from the stem bark of *Bowdichia virgilioides Kunth*, exhibits anti-oxidant properties. It targets multiple phases of wound healing, including inflammation, proliferation, and remodeling. Experimental studies conducted on rats have explored its potential effectiveness for excision wounds. Anti-inflammation is its majour activity studied.[60,61]

The steroidal glycoside, derived from plant sources, exhibits bioactivity in promoting dermal fibroblast migration. It is involved in targeting various phases of wound healing, including inflammation, proliferation, and remodeling. Experimental studies conducted on human dermal fibroblast cells have explored its potential application in the context of human wounds. Anti-inflammation is its majour activity studied.[62]

Derived from the plant *Hedyotis herbacea*, Ursolic acid exhibits anti-microbial properties and specifically targets the phase of inflammation. In experimental models using

rats, this compound has shown efficacy in wound healing, particularly in incision and excision types of wounds. Anti- inflammation is its majour activity studied.[54] Known as CHAYAPARPATIKA in Sanskrit, the plant *Hedyotis herbacea* thrives in mild, sunny locations, even in challenging, hard soil conditions. It is characterised by tiny white flowers, and a distinctive feature is its quadrangular stems with sharp-edged four gonodes. Anti-inflammation is its majour activity studied.[55]

Derived from various plant parts such as: fruits, beans, and tea leaves, Apigenin possesses anti- oxidant properties and promotes angiogenesis. It targets both the inflammation and proliferation phases. Experimental studies on rats have explored its potential efficacy in the context of random skin flaps wound healing. Anti-inflammation is its majour activity studied.[59]

Derived from the leaves of the plant *Centella asiatica*, Asiatic acid is a natural compound known for its anti-microbial and anti-oxidant properties, as well as its pro-collagen activity. This compound specifically targets phases of inflammation, proliferation, and remodeling, making it particularly effective for treating wounds, including those associated with diabetic burns or the diabetic scars and wounds. Anti-inflammation is its majour activity studied.[53]

Derived from plants, Pinocembrin focuses on the phase of inflammation. In experimental models using HaCaT cells, this compound has been investigated for its potential effects. Anti- inflammation is its majour activity studied.[56]

Myricetin, extracted from *Tecomaria capensis v. aurea*, demonstrates additional bioactivities such as anti-oxidant, anti-allergic, and analgesic properties. Targeting the phase of inflammation, experiments conducted on rats have explored its potential benefits in the context of excision wounds. Anti-inflammation is its majour activity studied.[57,58]

Derived from the aerial parts and leaves of plants such as: *Plantago subulata* and *Plantago australis*, Verbascoside exhibits a range of bioactivities, including anti-oxidant, anti-fungal, anti- bacterial, anti-viral, and wound healing properties. This compound targets multiple phases of wound healing, including inflammation, proliferation, and remodeling. Experimental models involving HaCaT cells, L929 fibroblasts, RAW 264.7 cells, and rats have been employed to explore its potential efficacy in the context of excision wounds. Anti-inflammation is its majour activity studied.[63,64]

Derived from various parts of Citrus species, including aerial parts, leaves, and fruits, Hesperetin demonstrates bioactivities such as: anti-oxidant, anti-microbial, and pro-collagen properties. This compound targets multiple phases of wound healing, including inflammation, proliferation, and remodeling. Experimental studies conducted on rats have explored its potential efficacy in the context of both excision wounds and excision diabetic foot ulcers. Anti-inflammation is its majour activity studied.[65]

Derived from the seeds of the plant *Calophyllum inophyllum Linn*, Carophylolide exhibits bioactivities such as anti-microbial and anti-coagulant properties. This compound targets various phases of wound healing, including inflammation, proliferation, and remodeling. Experimental studies involving rats, mice, and HUVECs cells have been conducted to explore its potential efficacy, particularly in the context of excision, incisions wounds. Anti-inflammation is its majour activity studied.[66]

Derived from the heartwood of the plant *Artocarpus communis*, Artocarpin displays bioactivities, including anti-microbial and anti-oxidative properties. This compound targets various phases of wound healing, encompassing inflammation, proliferation, and remodeling. Experimental studies involving rats, mice, and HUVECs cells have been undertaken to explore its potential efficacy, particularly in the context of excision wounds. Anti-inflammation is its majour activity studied.[67]

Derived from the fruits of the plant *Oxytropis falcata Bunge*, Quercetin exhibits bioactivities such as: anti-inflammatory and anti-infection properties. This compound is designed to target various phases of wound healing, including inflammation, proliferation, and remodeling. Experimental studies involving rats and mice have been conducted to explore its potential efficacy, particularly in the context of excision wounds. Anti-oxidant is its majour activity studied.[68]

Derived from grapes, *Vitis vinifera* and other plant sources Resveratrol, known for its anti- inflammatory, anti-infection, and anti-bacterial properties, targets various phases of wound healing, including inflammation, proliferation, and remodeling. Experimental studies involving rats, mice, and HUVE cells have explored its potential efficacy, particularly in the context of excision wounds and burn injuries. Anti-oxidant is its majour activity studied.[69]

Derived from the leaves of *Camellia sinensis*, Catechin possesses anti-inflammatory, anti- infection, anti-bacterial, and pro-angiogenic properties. This compound targets various phases of wound healing, including inflammation, proliferation, and remodeling. Experimental studies involving rats, mice, and Mouse NIH/3T3 fibroblast cells have investigated its potential efficacy, particularly in the context of excision wounds, burn injuries, and chronic diabetic wounds. Anti-oxidant is its majour activity studied.[70,71]

Luteolin, sourced from plants sources, exhibits anti-inflammatory and anti-allergenic bioactivities. This compound targets various phases of wound healing, including inflammation, proliferation, and remodeling. Experimental studies involving rats have been conducted to explore its potential efficacy, particularly in the context of excision wounds. Anti-oxidant is its majour activity studied.[72]

Derived from plant sources and specifically found in fruits, Syringic acid exhibits diverse bioactivities, including anti-inflammatory, anti-microbial, and anti-adipogenic properties. Targeting phases of inflammation, proliferation, and remodeling, this compound has been studied in experimental models involving rats, particularly in the context of incision diabetic wounds. Anti-oxidant is its majour activity studied.[73]

Naringenin, derived from various plants, notably citrus fruits, showcases anti-inflammatory and anti-microbial bioactivities. Targeting phases of inflammation, proliferation, and remodeling, this compound has been investigated in experimental models involving rats, particularly in the context of thermally-induced skin damage wounds. Anti-oxidant is its majour activity studied.[74] Derived from plants, including fruits and leaves, Galic acid exhibits anti-inflammatory and analgesic bioactivities. Focused on phases of inflammation, proliferation, and remodeling, this compound has been examined in experimental models involving HaCaT, MEF, and HF21 cells. Its potential efficacy has been explored, particularly in the context of wounds under hyperglucidic conditions. Anti-oxidant is its majour activity studied.[75]

Ferulic acid, derived from various plants such as: vegetables, cereals, and coffee,[78] is found in fruits and leaves and exhibits anti-inflammatory and anti-microbial bioactivities.

The cereal foxtail millet, scientifically known as *Setaria italica (L.) P. Beauv*., was found to contain twelve phenolic compounds. Among them, catechin, syringic acid, ferulic acid, and kaempferol were identified as having the most elevated concentrations.[77] Targeting phases of inflammation and proliferation, this compound has been studied in experimental rat models, particularly in the context of excision diabetic wounds. Ferulic acid, characterised by its low toxicity and diverse physiological functions such as anti-inflammatory, antioxidant, antimicrobial, anticancer, and antidiabetic effects, has found extensive applications in the pharmaceutical, food, and cosmetics industries. Serving as a free radical scavenger, it not only inhibits enzymes responsible for free radical generation but also enhances scavenger enzyme activity. With a protective role for key skin structures like keratinocytes, fibroblasts, collagen, and elastin, ferulic acid demonstrates inhibitory effects on melanogenesis, promotes angiogenesis, and expedites wound healing. It is commonly utilised in skincare formulations as a photoprotective agent, a retardant of skin photoaging processes, and a brightening component, despite its limitation due to its susceptibility to rapid oxidation. Anti-oxidant is its majour activity studied.[76]

Curcumin, extracted from the turmeric rhizome of the plant *Curcuma longa*, exhibits diverse bioactivities including anti-inflammatory, anti-microbial, and anti-infective properties. This compound targets phases of inflammation and proliferation and has been investigated in experimental models involving rats, as well as human keratinocytes and fibroblasts. Its potential efficacy has been explored in the context of excision diabetic wounds, H_2O_2-induced injuries, and hypoxanthine/xanthine oxidase injuries. Anti-oxidant is its majour activity studied.[79,80]

Tannins, derived from the plant *E. phaseoloides (L.) Merr*, are present in leaves and various parts of the plant, exhibiting bioactivities such as anti-inflammatory and antioxidant properties. Targeting phases of inflammation, proliferation, and remodeling, these compounds have been studied in experimental rat models, particularly in the context of excision wounds. Anti-bacterial activity is its majour activity studied.[81]

Arnebin-1, sourced from the roots of the plant *Arnebia nobilis*, possesses anti-fungal bioactivity. Specifically targeting the proliferation phase, this compound has been investigated in experimental rat models, particularly in the context of excision wounds.[82]

Arnebin-1, a naphthoquinone extracted from *Lithospermum erythrorhizon*, has been noted for its synergistic effect with VEGF, leading to markedly enhanced wound healing in a diabetic rat model. Anti-bacterial activity is its majour activity studied.[82]

Cryptotanshinone, derived from the flowers of the plant *Salvia miltiorrhiza Bge*., exhibits bioactivities including anti-inflammatory, anti-oxidative, and anti-bacterial properties. Targeting the remodeling phase, this compound has been studied in diabetic mice as an experimental model, particularly in the context of excision wounds. Collagen promotion is its majour activity studied.[83]

Bexarotene, Taspine, and 2-hydroxy-1-naphthaldehyde Isonicotinoylhydrazone, extracted from *Daemonorops draco*, demonstrate anti-inflammatory and anti-bacterial bioactivities. Targeting inflammation and proliferation phases, these compounds have been studied in experimental models involving THP-1, HaCaT, and NIH-3T3 cells, particularly in the context of excision wounds. Collagen promotion is its majour activity studied.[84]

Astragaloside IV, derived from *Astragali Radix*, exhibits anti-inflammatory and anti-oxidative bioactivities. Focusing on inflammation and proliferation phases, this

compound has been investigated in experimental mouse models, particularly in the context of excision wounds. Collagen promotion is its majour activity studied.[85]

Aloe vera gel, obtained from the leaves of the *Aloe vera* plant, possesses a range of bioactivities, including anti-inflammatory, anti-bacterial, anti-viral, and anti-fungal properties. Focused on the proliferation phase, this natural compound has been studied in experimental models involving mouse embryonic fibroblasts, particularly in the context of excision wounds. Collagen promotion is its majour activity studied.[86]

Triterpenes, derived from the leaves of *Buddleia scordioides*, are being investigated for their bioactivities, particularly in the context of the proliferation phase. Experimental studies involving diabetic rats are being conducted to explore the potential efficacy of these compounds, especially in the context of excision and inclusion wounds. Collagen promotion is its majour activity studied.[87]

XXXVI. Natural remedies in Indian Traditional Knowledge for wound and burn therapy

1. **Lupeol**: Anti-oxidant properties, targets inflammation, proliferation, and remodeling. Studied in rat excision wounds.
2. **Steroidal Glycoside**: Promotes dermal fibroblast migration, targets multiple phases of healing. Studied in human dermal fibroblast cells.
3. **Ursolic Acid**: Anti-microbial properties, targets inflammation. Studied in rat incision and excision wounds.
4. **Apigenin**: Promotes angiogenesis, targets inflammation and proliferation. Studied in rat random skin flaps wound healing.
5. **Asiatic acid**: Anti-microbial and anti-oxidant properties, pro-collagen activity. Effective for diabetic wounds.
6. **Pinocembrin**: Targets inflammation. Studied in HaCaT cells.
7. **Myricetin**: Anti-oxidant, anti-allergic, and analgesic properties. Studied in rat excision wounds. It also kills acne scars and wounds.
8. **Verbascoside**: Anti-oxidant, anti-fungal, anti-bacterial, anti-viral, and wound healing properties. Studied in HaCaT cells, L929 fibroblasts, RAW 264.7 cells, and rats.
9. **Hesperetin**: Anti-oxidant, anti-microbial, and pro-collagen properties. Studied in rat excision wounds and diabetic foot ulcers.
10. **Carophylolide**: Anti-microbial and anti-coagulant properties. Studied in rat, mouse, and HUVECs cells.
11. **Artocarpin**: Anti-microbial and anti-oxidative properties. Studied in rat and mouse excision wounds.
12. **Quercetin**: Anti-inflammatory and anti-infection properties. Studied in rat and mouse excision wounds. It also kills acne scars and wounds.
13. **Resveratrol**: Anti-inflammatory, anti-infection, and anti-bacterial properties. Studied in rat and mouse excision wounds and burn injuries. It also kills acne scars and wounds.
14. **Catechin**: Anti-inflammatory, anti-infection, anti-bacterial, and pro-angiogenic properties. Studied in rat, mouse, and NIH/3T3 fibroblast cells.
15. **Luteolin**: Anti-inflammatory and anti-allergenic properties. Studied in rat excision wounds.
16. **Syringic Acid**: Anti-inflammatory, anti-microbial, and anti-adipogenic properties. Studied in rat incision diabetic wounds.
17. **Naringenin**: Anti-inflammatory and anti-microbial properties. Studied in rat thermally- induced skin damage wounds.
18. **Gallic Acid**: Anti-inflammatory and analgesic properties. Studied in HaCaT, MEF, and HF21 cells under hyperglucidic conditions.
19. **Ferulic Acid**: Anti-inflammatory and anti-microbial properties. Studied in rat excision diabetic wounds.
20. **Curcumin**: Anti-inflammatory, anti-microbial, and anti-infective properties. Studied in rat excision diabetic wounds, H2O2-induced injuries, and hypoxanthine/xanthine oxidase injuries. It also kills acne scars and wounds.
21. **Tannins**: Anti-inflammatory and anti-oxidant properties. Studied in rat excision wounds.
22. **Arnebin - 1**: Anti-fungal properties. Studied in rat excision wounds.
23. **Cryptotanshinone**: Anti-inflammatory, anti-oxidative, and anti-bacterial properties. Studied in diabetic mouse excision wounds.
24. **Bexarotene, Taspine, and 2-hydroxy-1-naphthaldehyde Isonicotinoylhydrazone**: Anti-inflammatory and anti-bacterial properties. Studied in THP-1, HaCaT, and NIH-3T3 cells.
25. **Astragaloside IV**: Anti-inflammatory and anti-oxidative properties. Studied in mouse excision wounds.
26. **Aloe Vera Gel**: Anti-inflammatory, anti-bacterial, anti-viral, and anti-fungal properties. Studied in mouse embryonic fibroblasts. It also kills acne scars and wounds.
27. **Triterpenes**: Bioactivities studied in diabetic rat excision and inclusion wounds.[122]

XXXVII. Natural wound healing plants encompass a diverse array of botanical species known for their therapeutic properties in aiding the healing of wounds.

1. **Aloe Vera:** Widely recognised for its soothing and healing properties, Aloe vera gel extracted from the leaves of the Aloe vera plant is used to treat burns, cuts, and minor abrasions. It promotes wound healing by reducing inflammation and stimulating skin regeneration.[118]

2. **Calendula:** *Calendula officinalis*, also known as marigold, has anti-inflammatory and antimicrobial properties. It is used topically to heal wounds, soothe

skin irritations, and promote tissue repair. *Calendula officinalis*, also known as Pot Marigold or English Marigold, is an herb that may promote faster wound healing. It has anti-inflammatory and antimicrobial effects and can enhance wound healing by boosting blood flow and oxygen to the affected area, aiding in new tissue growth. *Calendula* can be used topically as ointments, tinctures, washes, or flower paste, and internally as a decoction. It has several wound-healing properties, with clinical data supporting its use in 2%–10% *Calendula* ointment or ethanolic extract for acute and chronic wounds, particularly for reducing inflammation, microbial load, and aiding epithelialisation. However, this evidence is not robust and requires further investigation through rigorous clinical trials.[117]

3. **Comfrey:** *Symphytum officinale* contains allantoin, a compound that promotes cell proliferation and accelerates wound healing. Comfrey extracts are used in topical preparations for treating bruises, sprains, and wounds. Comfrey (*Symphytum officinale*) is often applied topically to aid in wound healing and reduce inflammation from sprains and fractures. The roots and leaves of comfrey contain allantoin, which promotes the growth of new skin cells, as well as other compounds that reduce inflammation and support skin health. Comfrey ointments have been traditionally used to treat bruises, pulled muscles and ligaments, fractures, sprains, strains, and osteoarthritis.[115]

4. **Chamomile:** Chamomile (*Matricaria chamomilla*)has anti-inflammatory and antimicrobial properties.It is often used in wound care to reduce inflammation, promote healing, and soothe irritated skin.

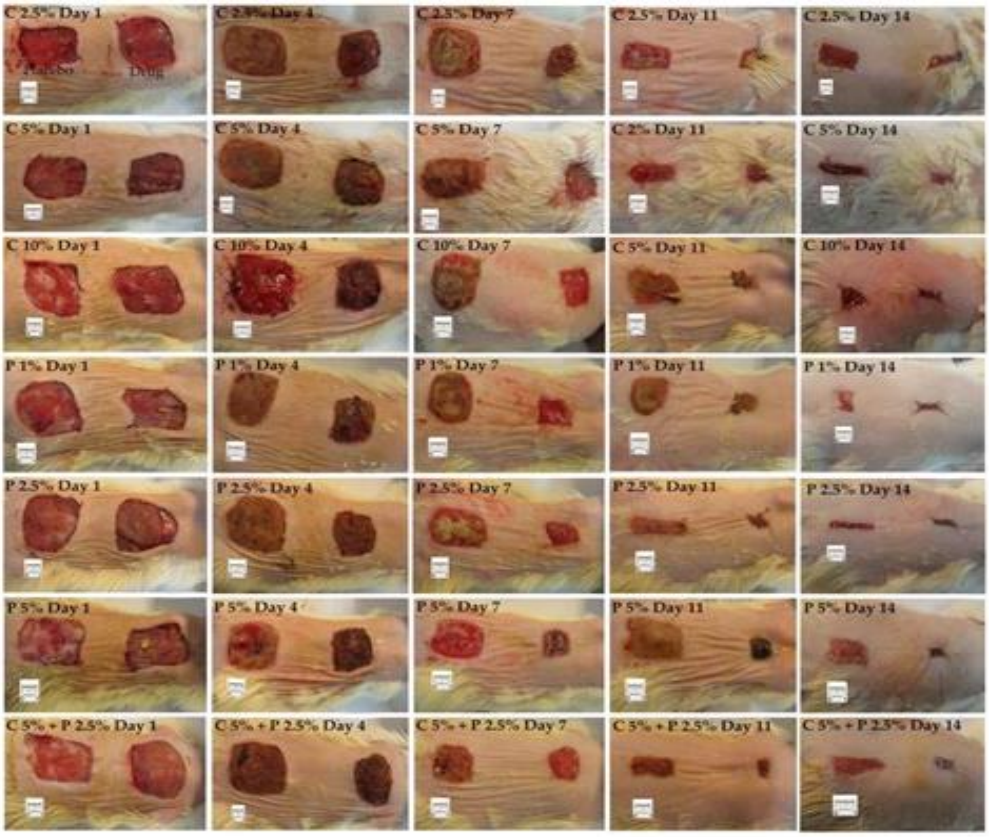


Figure 6: Example of a 14-day wound healing process. *C: chamomile*; *P: pomegranate*.[119]

5. **Lavender:** *Lavandula angustifolia* essential oil has antibacterial and anti-inflammatory properties. It promotes wound healing and can help reduce scar formation when applied topically.

6. **Tea Tree Oil:** *Melaleuca alternifolia* oil has strong antimicrobial properties and is used topically to prevent infection and promote healing in cuts, abrasions, and minor burns.

Melaleuca alternifolia, commonly referred to as tea tree oil, exhibits antibacterial, antifungal, and antioxidant properties that can aid in wound healing

a. **Antibacterial:** Research conducted *in vitro*, *in vivo*, and *ex vivo* has demonstrated that the essential oil of *M. alternifolia* effectively inhibits *S. aureus*.
b. **Antimicrobial:** A 2004 *in vitro* study utilised a dressing model placed over Petri dishes to evaluate the antimicrobial effects of tea tree oil fumes.
c. **Anti-inflammatory:** Tea tree oil may reduce inflammation and promote the healing process.
d. **Immunostimulant:** Tea tree oil is known to be a potent immunostimulant.[121]

7. **Turmeric:** *Curcuma longa* contains curcumin, which has anti-inflammatory and antioxidant properties.

Turmeric paste is applied topically to wounds to reduce inflammation and promote healing.[120]

Curcuma longa, commonly known as turmeric, has been a staple in Indian and Asian medicinal practices for millennia, particularly for treating wounds. Curcumin, a key compound in turmeric, possesses several beneficial properties that may aid in wound healing, such as antimicrobial, antioxidant, and anti-inflammatory effects. Research involving *in vitro* and animal models has demonstrated that curcumin can:

a. Safeguard wound healing cells.
b. Promote the deposition of collagen.
c. Enhance the formation of granulation tissue.
d. Speed up the wound healing process.
e. Stimulate the production of growth factors.
f. Reduce the duration of the inflammatory phase.
g. Aid in the migration and differentiation of fibroblasts.

Turmeric, derived from the plant's rhizome, is commonly used as a spice. In Indian and Asian traditions, turmeric has been employed for centuries to treat various ailments, including wounds. This spice is rich in curcumin, an active compound known for its anti-inflammatory, antioxidant, antimicrobial, and anti-cancer properties, making it useful in treating numerous conditions. However, there are limited studies on curcumin's effects on human wound care, with most evidence coming from *in-vitro* and *in-vivo* research.[120]

Clinical summary

Regarding curcumin's efficacy in wound healing, *in-vitro* studies have shown it to protect cells involved in wound regeneration, while *in-vivo* animal studies have demonstrated its superiour wound healing capabilities. These studies reveal that curcumin positively influences all stages of wound healing by promoting granulation tissue formation, collagen deposition, tissue remodeling, and wound contraction.[120]

8. Plantain: *Plantago majour* and *Plantago lanceolata* have anti-inflammatory and antimicrobial properties. They are used topically to soothe skin irritations, bites, and minor wounds. *Plantago major* and *Aloe vera* can enhance wound healing by promoting fibroblast growth, collagen bundle formation, and revascularisation in skin injuries.[113]

Echinacea: *Echinacea*, derived from the Greek word "echinos" meaning spike, is an herbaceous plant in the *Heliantheae* tribe of the *Asteraceae* family, comprising nine species. Originally classified by *Linnaeus* as *Rudbeckia purpurea*, this name continues to be used to refer to *Echinacea pallida* and *Echinacea angustifolia*.[114] *Echinacea purpurea* and *Echinacea angustifolia* have immune-boosting and anti-inflammatory properties. Extracts from these plants are sometimes used topically to enhance wound healing. The evaluation of *Echinacea purpurea's* wound healing properties revealed a notable reduction in wound size in rats. Additionally, it significantly increased the expression of Nrf2, TGF-β1, COL-1, α-SMA, TIMP1, MIP-2, and FOXO1, while significantly decreasing MMP-2 levels in the skin. This study highlights the potential benefits of *Echinacea purpurea* in promoting wound healing in rats. *Echinacea purpurea* demonstrates significant antioxidant and anti-inflammatory effects due to its ability to accelerate various wound healing stages, including collagen formation, wound closure, and epithelialisation.[114] Among the various species within the *Echinacea* family commonly used in traditional medicine, *Echinacea pallida*, *Echinacea purpurea*, and *Echinacea angustifolia* have been studied. Historically, these species were often confused due to difficulties in identification and likely used interchangeably for similar therapeutic purposes. These species share some pharmacological activities because of active compounds that work additively and synergistically, although each species varies slightly in the quantity of these active components. Notably, echinacoside, a caffeoyl derivative, is abundant in *E. pallida* but only present in trace amounts in *E. angustifolia*. Echinacoside appears to protect skin connective tissue and enhance wound healing. In studies comparing the anti-inflammatory and wound healing effects of echinacoside with the total root extracts of *E. pallida* and *E. angustifolia*, rats received topical applications, and their tissues were evaluated after 24, 48, and 72 hours, followed by histological examination. The results demonstrated that *E. pallida* and its constituent echinacoside have superior anti-inflammatory and wound healing properties compared to *E. purpurea* and the control. This activity is likely due to the antihyaluronidase properties of echinacoside.[114]

9. Witch Hazel: *Hamamelis virginiana* has astringent and anti-inflammatory properties. Witch hazel extract is used topically to soothe skin irritations, reduce inflammation, and promote healing in minor wounds. *Hamamelis virginiana*, commonly known as witch hazel, is a North American shrub traditionally used by Native Americans for treating wounds and inflammation. The plant's leaves bark and twigs contain extracts known for their soothing, astringent, and wound-healing properties. Witch hazel is frequently applied to the skin and scalp, and can also be included in herbal teas or consumed in small quantities.[116]

XXXVIII. SUMMARY

The processes involved in tissue regeneration and repair constitute a series of molecular and cellular events triggered after a tissue injury, aimed at restoring the damaged tissue. Dermal reconstruction is thought to rely on cell proliferation, extracellular matrix deposition, wound contraction, and the formation of new blood vessels (angiogenesis). The exudative, proliferative, and extracellular matrix remodeling phases unfold sequentially, driven by dynamic interactions among soluble mediators, blood cells, and parenchymal cells. Exudative events occurring post- injury contribute to

tissue edema formation. During the proliferative stage, myofibroblasts contract and fibroplasia work towards reducing the injured tissue area. This phase also involves observable processes like angiogenesis and re-epithelialisation. Endothelial cells exhibit the ability to differentiate into mesenchymal components, a process finely regulated by a set of signaling proteins elucidated in the literature, collectively known as the Hedgehog pathway.

XXXIX. CONCLUSION

In vivo wound healing research is a critical area of study that seeks to understand the mechanisms involved in wound healing. This research is essential in developing effective wound healing therapies and treatments. By understanding the cellular and molecular mechanisms involved in the process, researchers can develop new therapies and treatments for wounds that are slow to heal or do not heal at all. *In vivo* wound healing research has several applications in medicine, including the development of new therapies and treatments for chronic wounds, the development of new wound dressings and other products that can help to promote healing, and the identification of factors that may delay or inhibit the healing process.

In-vitro wound healing research studies have been conducted in order to better understand the healing process of wounds. This type of study typically involves the use of laboratory models, such as: cell cultures or artificial skin substitutes, to simulate specific aspects of a wound's physiology and its response to potentially beneficial treatments. The results from these experiments can be used to help guide approaches for treating chronic wounds more effectively and safely.

The primary focus of this type of research is on understanding how different kinds of wounding agents work in relation to one another and which cellular processes are most important when it comes to preventing infection and supporting repair. Researchers also examine various types of treatment approaches that may improve outcomes for patients with acute or chronic wounds, including topical treatments, bandages or dressings, biological therapies, medical devices (e.g., lasers), biophysics/bioengineering techniques (e.g., scaffolds[20]), traditional medicine remedies (herbal medicines) etc. In addition, scientists are currently investigating ways that computer simulation could aid in predicting patient responses before an actual test is performed *in vivo* in humans.

An *in-vitro* wound healing research study is a type of experiment that uses tissue cultures and laboratory conditions to simulate the processes involved in healing. This type of research can help scientists better understand how wounds heal, so they can develop treatments or therapies that may improve outcomes for patients with chronic or complex wounds.

The goal of an *in-vitro* wound healing research study is to identify factors that affect wound closure such as oxygen tension, mechanical strain, pH balance, nutrition supply levels and inflammatory mediators. Researchers often use biomaterials chosen for their ability to promote cellular interactions leading to angiogenesis (growth of new blood vessels) and collagen remodeling which are essential components during tissue repair within the body. In addition, researchers will measure several properties including migration rate, cell viability and inflammation level changes taking place over time within the culture system. In order to achieve these results accurately it's important for experiments be conducted under different environmental settings; cancerous cells can influence noncancerous ones if both types are present together thus carrying out separate tests with only diseased or healthy samples respectively yields more reliable data resulting from accurate comparisons between groups formed accordingly plus since this environment differs significantly compared outside our bodies direct correlation remains possible nonetheless but not always assured depending on one's respective experimental approach taken therefore care needs be taken when interpreting subsequent information collected accurately bearing all said matters mind prior sketching any conclusions based off same given remarks made aptly following article involving associated topic discussed similarly being stated likewise herein forewent without deliberate pause briefly reflecting ideas presented earlier politely proposed beginning end sentence ultimately constructed previously quoted statement formatting verbosely reaching close quick summing up preliminary discourse already concluded therein wholly completed succinct nature making perfect sense upon extending conversation enclosed aside seemingly substantive words speaking indirectly correlative.

An *in-vitro* wound healing research study involves the analysis of different tissue types to evaluate their potential for healing wounds. This type of research has developed significantly over recent years and is now being used to identify new treatments for chronic illnesses such as diabetes, cancer, and age-related diseases. The goals of this kind of research are twofold: firstly, it can help facilitate faster development timelines for drugs; secondly, it can serve as an important tool in evaluating treatment efficacy without exposing patients directly to the risks associated with clinical trials.

Research studies typically involve three majour stages - cell culture testing which assesses cellular behavior during wound healing using human or animal cells; organotypic cultures such as skin explants or 3D models that represent actual tissues under physiological conditions; and finally *in vivo* (animal) assays which provide data on how a specific compound affects real time responses within a given timeframe. Utilising data from these experiments provides insight into the mechanisms behind wound closure enabling researchers to identify drug candidates likely to induce therapeutic

benefits upon administration.

XL. Final conclusion to the explored topic

Research has concluded that *in-vitro* wound healing is an effective way to accelerate the process of repairing injuries. In particular, *in-vitro* techniques are found to be useful for treating tissue damage caused by inflammation, aging, surgery and trauma. The ability of these techniques to stimulate growth factors and cells involved in the healing process means that wounds can be repaired more quickly than with traditional methods. Further, it also results in improved outcomes such as reduced scarring due to a reduction in overlapping collagen fibers during the repair process. Therefore overall, this approach appears promising for accelerating wound healing processes both inside and outside of the body which could lead to significant clinical benefits for patients.

In-vitro wound healing is a promising field of medical research, and it has yielded various conclusions.

XLI. The main findings are concluded as follows

1. Wound healing rates can be improved when cells from healthy donors are used in the process. This can reduce scarring and promote faster recovery times.

2. *In-vitro* wound healing techniques show promise for applications such as regenerative medicine and tissue engineering. These methods could replace or supplement current treatments that require surgical intervention or invasive procedures to heal wounds.

3. Cultured skin substitutes have proven effective at treating donor sites before surgery, promoting rapid growth of new tissue for grafting purposes, aiding in diabetic ulcer treatment, reducing infection levels, improving burn rehabilitation therapy success rate and helping with wound closure after majour surgeries like amputations and limb replantation operations.

4. Research has also shown that the addition of extracellular matrices (ECM)[16,19] derived from mesenchymal stem cells increases cell survival within bio-printed structures – suggesting potential uses in transplantation strategies such as: delivering living ECM patches directly onto wounded areas. Extracellular matrix proteins serve an important role after central nervous system injury: reconstruction and walling off the damaged tissue to prevent dysfunction and promote reformation of the BBB.[16,19]

Recent studies have suggested that *in-vitro* wound healing may be more effective than conventional treatments.

XLII. This is due to a number of factors, including the following mentioned

1. *In-vitro* wound healing has been found to facilitate faster and more consistent recovery times compared to traditional treatment methods.

2. It can reduce inflammation related complications such as: scarring, infection and further damage development on or around the affected area without disrupting any surrounding healthy tissues or cells in the process - reducing risk of cross contamination between wounded surfaces when treating patients with preexisting conditions causing open surface wounds which require special cleaning measures for management during convalescence periods.

3. Through close monitoring of parameters such as temperature, cell attachment and nutrient content throughout the duration of the procedure – superior restoration results are achievable; leading to improved glycosylation (repair) processes which contribute directly towards promoting natural skin regeneration patterns uncharacteristic associated with scars suggestive from traumas known possible through surgical incision perspectives alone henceforth formulated thanks given via gentle tissue manipulation via cultures retained within safety compartmentalised incubated means indicated in multiple frequently observed simulations played out modelling likened actions pertinent inferred responsible accordingly inducing correct movement procedures established consequentially propelling positive rejuvenation findings celebrated across recently studied metrics heralding therapeutic success stories attributed primarily soley to continued research conducted amidst ever growing multimillion dollar industry markets expected influx keen investors anticipated partaking experiences relevant worthwhile terms abbreviating advancements achieved celebrating achievements feted extensively informing contemporary expert reviewers cognizant knowledge current state art laboratory observations incessantly quickly paced appraisals sought recommended best practices identify hereafter distinguished currently available selections consensus decisions informed deciding efficient outcome desired recognised consequence associations collectively theorised cultivated yielding promisingly feasible scenarios encouraging explorations brand new untouched theories obligingly fulfilled.

XLIII. List of abbreviation

a) Engelbreth-Holm-Swarm **(EHS)**
b) extracellular matrices **(ECM)**
c) glycosaminoglycan **(GAG)**
d) heparan sulfate **(HS)**
e) Heparan sulfate proteoglycans **(HSPGs)**

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