FORMULATION AND EVALUATION OF ALLANTOIN LOADED HYDROGEL FOR SKIN REGENERATION AND REJUVENATION

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Abstract

Advancements in dermatology and cosmetics emphasize the significance of skin rejuvenation and regeneration. Hydrogels have emerged as a favorable platform for delivering active agents due to their biocompatibility, hydration potential, and controlled release characteristics. This study focused on creating and evaluating a hydrogel containing allantoin, intended to facilitate skin revitalization and renewal. The hydrogel formulation was meticulously crafted by combining natural and synthetic polymers, optimized to achieve desired viscosity, gel strength, and allantoin-loading capacity. Allantoin, recognized for its skin-calming, hydrating, and regenerative attributes, was successfully integrated into the hydrogel matrix. Comprehensive characterization of the hydrogel encompassed assessments of physicochemical attributes such as pH, rheological performance, drug release kinetics, and mechanical properties. In vitro release profiles exhibited a sustained and controlled release of allantoin from the hydrogel spanning hours, indicating its potential for prolonged topical application. Rheological analysis unveiled the hydrogel’s shear-thinning behavior, a property that augments its ease of application. Mechanical testing ascertained the hydrogel’s mechanical robustness, establishing its suitability for skin contact. Biocompatibility and cytotoxicity evaluations employing human skin fibroblast cells corroborated the hydrogel’s cell-friendly nature and its potential to encourage cellular proliferation. These results imply that the allantoin-infused hydrogel holds promise for stimulating skin regeneration. Furthermore, the hydrogel’s efficacy in retaining skin moisture was validated through tests on a skin-equivalent model. In summary, the allantoin-loaded hydrogel formulation boasts favorable physicochemical attributes, sustained release kinetics, and biocompatibility. This positions it as a viable contender for fostering skin revitalization and renewal. Further validation through in vivo studies is imperative to establish its clinical efficacy and safety. The hydrogel formulation presents a pioneering approach to addressing diverse skin-related concerns and advancing dermatological and skincare...
treatments.

Keywords: hydrogel, allantoin, skin regeneration, rejuvenation, wound healing, hyaluronic acid, biocompatibility, cytotoxicity, fibroblasts, moisture retention.

1. INTRODUCTION

The demand for innovative skin care products that promote skin regeneration and rejuvenation has indeed increased. Allantoin, a natural compound derived from plants, has been widely acknowledged for its moisturizing, soothing, and wound-healing properties. Hydrogels, characterized as 3D structures of hydrophilic polymers, have demonstrated significant promise in terms of effective delivery systems for bioactive compounds due to the fact that of their high-water content and biocompatibility.

1.1 Skin:

1.1.1 Physiology of skin:

There is a growing demand for innovative skin care products that promote skin regeneration and rejuvenation. Allantoin, a natural plant compound, is widely recognized for its moisturizing, soothing and wound-healing properties. Hydrogels, which are three-dimensional networks of hydrophilic polymers, have shown great potential. Hydrogels, consisting of 3D arrangements of hydrophilic polymers, have displayed considerable promise as efficient carriers for bioactive substances. This is attributed to their substantial water content and compatibility with biological systems as effective delivery systems for bioactive compounds due to their high water content and biocompatibility.

The human body's largest and most noticeable organ is the skin. Its primary role is to function as a protective barrier that protects against hot humidity essentially hostile internal environment. In the arid and chilly outdoor surroundings where we reside, it's commonly thought that the skin performs a significant role. Complete resistance to external components positions. In fact, it passes through many substances, including fragrances and colors we may use communicate often. In addition to protection, Undoubtedly, the skin plays a significant role in regulating. The skin maintains physiological temperature and arterial pressure, in addition to serving endocrine roles and hosting numerous sensory receptors. Changes in skin often reflect internal health issues. Hence, nurses play a vital role in observing and tending to patients' skin. Comprehending the skin's structure and physiological functions empowers nurses to assess elements that could trigger changes in how it's made up, looks, or functions. There's a prevailing assertion that healthcare workers often lack sufficient training in addressing skin issues and diseases.

Nurses frequently perform skin assessments as part of their daily patient care routine. During tasks like bathing and dressing, nurses seize frequent opportunities to carefully examine the patient's skin, paying particular attention to skin integrity and pressure points. However, there appears to be a shortage of registered staff to fully meet this need. Executing practical treatments might inadvertently lead to the
oversight of a crucial element of skincare. This highlights the significance of conducting formal assessments and meticulously documenting the condition of the skin. An understanding of the skin’s structure and functions can greatly aid in this process.\(^4\)

### 1.1.2 Structure of the skin:

The skin is segmented into multiple layers, as depicted in Figure 1. The topmost layer, called the epidermis, is mainly composed of keratinocytes. Underneath the epidermis, the dermis includes a basement membrane, also known as the dermo epidermal junction, which securely attaches the epidermis to the dermis. The layer beneath the dermis, referred to as the hypodermis, is primarily comprised of adipose tissue (fat). Those different constituents are further explained below.\(^5\)

![Figure 1. Cross-section through the skin](image)

The epidermis, situated as the topmost part of the skin, is composed of stratified squamous epithelium. This epithelium mainly comprises keratinocytes that undergo sequential phases of specialization. These keratinocytes generate keratin protein and act as the fundamental constituents of the epidermis. Notably, the epidermis lacks blood vessels, rendering it devoid of a conscious perception of pain; however, changes in the epidermis can indicate underlying health issues, enabling caregivers to assess a patient’s condition. The skin is partitioned into two main layers: the epidermis and the dermis. Furthermore, there exists a 3rd layer referred to as the hypodermis, which will be briefly addressed.\(^6\)

The nutrition supply and waste management for the epidermis are entirely dependent on the underlying skin through the basement membrane. The key function of the primary role of the epidermis is to function as a dual physical and biological shield against the external surroundings, obstructing the entry...
of irritants and allergens. At the same time, it safeguards against moisture loss and contributes to the preservation of internal equilibrium. The epidermis comprises layers; most body parts consist of four layers, except for regions with thicker skin like palms, soles, and toes, which have five layers. These layers are as follows:  

Stratum corneum  
Stratum lucidum (present exclusively in areas with thick skin such as palms and soles)  
(Additional layers in regions with thick skin) These layers contribute to the overall structure and function of the epidermis.

1.1.3 The epidermis:
The epidermis is primarily constructed from layered squamous epithelial cells. Epithelial cells, as one of the four main tissue variety in the body... primary tissue types, form barriers between the internal and external environments of the body. As a result, they are not only present in the skin but also line various body tracts such as including the digestive, respiratory, and urogenital tracts. Any part of these systems that encounters mechanical stress or irritation, such as the skin or mucous membranes found in the mouth, nose, and vagina, is enveloped by several tiers of epithelial cells, with the topmost layers being flat. 

Epithelial cells are consistently associated with junctions that connect neighboring cells and control the movement of substances between them. Some cells are so thickly connected that any object attempting to move from one area of the epithelium to the other should pass through these junctions intermediate cells. In contrast, other types of epithelia, like the mucous membranes in the small intestine, have looser connections that enable more movement of substances along the spaces between cells. In the context of the skin, which is an example of dense epithelial tissue, this dense arrangement doesn’t hinder the movement of substances through the skin as one might expect. Cells at the base layer of the epidermis undergo mitosis, and one of the resulting daughter cells remains in the basal layer while the other moves upwards. This process contributes to the renewal and maintenance of the skin. through the layers to the surface. This ascent lasts up to lasting for a span of 28 days and accompanied by notable alterations in cellular composition. which makes to a clear layering of the appearance your epidermis under the microscope. The production of keratin begins spinosum. It granulosum, during which the cells undergo a process of losing their capacity cores and dies. Stratum lucidum and stratum corneum consists of nonliving cells that are full keratin and its surrounding lipids. This flattened outer layer of cells is built up due to the presence of keratin. Keratin is an non soluble protein, which is also the most important representative of human hair and nails and horns and animal hooves Lipids the surrounding cells protect against them water loss and infiltration. the ability to over the scaly layers of the epidermis provide protection opposite mechanical abrasion. Little is known about cell cycle control in the epidermis. Learning is so hard because epidermis is only about 120 micrometers thin and stains numerous cells exhibiting diverse stages of specialization Normal cell cycle times atinocyte is up tp 6
days span, but because it uptake 28 days to reach the daughter cells skin surface and detachment, not the whole base atenocytes are on woke atsimultenously Understanding the regulatory signals behind these processes could have a profound impact on the management of skin conditions like psoriasis, characterized by heightened basal cell activity and prolonged cell transit to the skin surface. Beyond epidermal cells, specifically keratinocytes, the basal layer of the epidermis houses melanocytes. These melanocytes are responsible for generating melanin, a pigment transferred to neighboring keratinocytes, which then shields against the harmful effects of ultraviolet (UV) radiation. In individuals with Caucasian skin, melanin tends to degrade as epidermal cells progress through the layers. Exposure to UV radiation stimulates melanin production, influenced by factors such as radiation and systemic hormones, particularly adrenocorticotrophic hormone (ACTH). Hence, individuals with primary adrenal insufficiency or Addison's disease often experience a tanned appearance. The protective function of melanin against UV radiation becomes compromised. The epidermis is accompanied by several appendages. Hair shafts, composed of epidermal cells, extend beneath the dermis. At the hair's root, there's a cluster of blood vessels. Hair becomes crucial for epithelialization in cases of burns and wounds, where the original epithelial layer is lost. In instances where the injury or burn affects the hair root deeply, the subsequent scar tissue is devoid of hair. Special nerve terminals connect to hair shafts, aiding in detecting subtle touch and movement across the skin's surface. Sebaceous glands, also associated with hair shafts, produce sebum, a lipid secretion. These glands play a role in maintaining the body's internal and external environments. Consequently, sebaceous glands are not only present in the skin but also line the digestive, respiratory, and urogenital tracts. Sections of these systems exposed to mechanical influences or irritation, such as the skin, mucous membranes in the mouth, nose, and vagina, are covered by several layers of flat epithelial cells, with the uppermost layer being flat in shape. Epithelial cells are always found associated in sheets along with the stress joints that occur between them cells and allow different amounts to pass through of cell traffic. Some cells are so closely related that any sub-position to move Substances moving from one side of the epithelium to the other often need to pass through spaces between cells, rather than directly traversing them. In contrast, certain epithelia, like the mucous membranes of the small intestine, have looser connections that allow more substantial movement of substances through the paracellular pathway. The epidermis, forming the skin, exemplifies dense epithelial tissue. Nevertheless, this density doesn't hinder the movement of substances through the skin. Materials can penetrate the skin as typically anticipated. This progression takes around 28 days and results in significant changes in cellular composition, creating distinct layering visible when examining the epidermis under a microscope. The production of keratin initiates in the spinosum layer and continues through the granulosum layer. In this layer, cells lose their nuclei and eventually die. The stratum lucidum and stratum corneum comprise deceased cells abundant in keratin, encircled by lipids. This outer layer of flattened cells accumulates due to the presence of keratin. Keratin, an insoluble protein, is also a prominent constituent in human hair, nails,
and nails. Animal hooves, and horns. The surrounding lipids protect against water loss and infiltration. The scaly layers of the epidermis provide defense against mechanical abrasion. The control of the cell cycle in the epidermis remains a topic with limited knowledge due to challenges in studying it. The epidermis, being around 120 micrometers thick, contains numerous cells in varying stages of differentiation. While the normal cell cycle time for keratinocytes the normal cell cycle time for keratinocytes is approximately six days, but it requires 28 days for daughter cells to migrate to the skin’s surface and be shed. Consequently, indeed, basal keratinocytes do not all remain active at the simultaneously. If the signals governing this process were understood, it could significantly impact skin care and conditions such as psoriasis, which involves heightened basal cell activity and prolonged cell transport time to the skin surface.

Apart Within the basal layer of the epidermis, alongside keratinocytes, reside melanocytes that are responsible for generating melanin. This pigment is then transferred to nearby keratinocytes, offering protection against the harmful effects of ultraviolet (UV) radiation. Notably, individuals with Caucasian skin typically experience a breakdown of melanin as epidermal cells progress upward through the skin layers. Exposure to UV radiation prompts an increase in melanin production, a process influenced by factors like radiation and hormones, especially adrenocorticotrophic hormone (ACTH). This phenomenon accounts for why individuals with primary adrenal insufficiency or Addison’s disease often display a tanned appearance. The protective role of melanin against UV radiation is especially pertinent in this context.

The epidermis also includes various accessories. Hair shafts, originating from layers of epidermal cells projecting into the dermis, are connected to blood vessels at their roots. Sebaceous glands related to hair shafts produce sebum, which is a secretion rich in lipids. These glands are significant in maintaining body’s internal and external environments. They are neither found in skin but also line digestive, respiratory, and urogenital pathways. In regions exposed to mechanical influences and irritation, such as the skin and mucous membranes in mouth, nose, and vagina, have so many layers of flat epithelial cells provide coverage. These epithelial cells are interconnected and permit the passage of different amounts of cellular traffic through them. Some closely associated cells require substances to pass through the spaces between them, while other epithelia, like the mucous membranes of the small intestine, give permission to most movement of products along the same cellular pathway. Despite the dense structure of the epidermis, substances can still pass through, and its characteristics enable it to serve as a protective barrier. The Stratum lucidum and stratum corneum consist of deceased cells filled with keratin and rounded by lipids. This flattened outer cell layer accumulates due to the presence of keratin. Keratin, a water-insoluble protein, is notably significant in human hair, nails, as well as in animal horns and hooves. Surrounding lipids offer shield opposite of water whisper and infiltration. Scaly layers of epidermis are effective at safeguarding against mechanical abrasion. The region’s most active in sebum production are the head, neck, hair follicles, back, and chest. Sebum, an oily secretion, Sebum production is regulated by hormones, especially androgenic hormones, thyroid hormones, and growth.
hormones, which can contribute to the development of acne. Eccrine sweat glands are distributed in the skin, with their ducts traversing the epidermis to release sweat onto the skin's surface. The process of sweat production involves active sodium secretion within the sweat gland, under the influence of the sympathetic nervous system. Sodium attracts water into the gland, and along with potassium, chloride, and urea, these substances are also excreted through sweat. The primary purpose of sweating is to aid in the regulation of body temperature. The evaporation of water from the skin's surface can lead to about 15 percent of heat loss at room temperature. Sweating becomes particularly vital for heat dissipation when external temperatures increase, although its effectiveness decreases in humid environments. In extreme conditions, up to a liter of liquid per hour can be lost through sweat, potentially resulting in significant imbalances in fluid and electrolytes.

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1.1.4 The Dermis:
In the dermis, while epidermal cells are numerous and closely interconnected, they are separated from one another by a complicated network of outer cellular substance. Fibroblasts are the predominant cell species in this layer of the skin, it also contains immune and inflammatory cells, nervous tissue, and blood vessels. Collagen, elastin, and other outer cellular fibers are primary component of dermis, imparting strength and flexibility to the skin. Collagen, the most abundant protein in the body, holds significant importance. Inside of dermis, it forms a meticulously structures meshwork, providing resilience and resistance to stretching. This intricate structure resembles genuine leather and contributes to the skin’s strength. However, during wound healing, this organized structure can break down, resulting in scar tissue that is inherently weaker than healthy skin. Elastin is another structural protein present in the dermis, offering elasticity to the skin. As a person ages, the diminished production of collagen and elastin, combined with the impact of ultraviolet radiation, results in the creation of wrinkles and drooping skin. The areas that exhibit the highest level of sebum production are the hair follicles situated on the head, neck, back, and chest.
stimulates sebum production under the influence of hormones, especially androgenic hormones (as well as thyroid and growth hormones) and this can cause acne. Eccrine sweat glands are found in the skin, whose channels, however, sweat released through the epidermis onto the skin’s surface is a product of active sodium secretion within sweat glands. This secretion is stimulated by the sympathetic nervous system. Sodium attracts water, while potassium, chloride, and urea are also excreted. The primary purpose of sweat is to cooperate in maintaining body temperature. Process of water vaporization from the skin’s surface contributes to approximately 15 percent of heat loss at room temperature. Sweating gains greater significance in facilitating heat dissipation when external temperatures rise, although its effectiveness is reduced in humid conditions. In extreme circumstances, up to a liter of liquid per hour can be lost through sweating. Rapid fluid loss like this can lead to potentially dangerous imbalances. In the dermis, epidermal cells, although numerous and closely associated, are surrounded by a complex extracellular matrix. While fibroblasts are the primary cell type in the dermis, it also contains immune and inflammatory cells, nervous tissue, and blood vessels. Collagen, elastin, and other extracellular fibers are key components of dermis, providing skin with power and streachability. Collagen, most prevalent protein in the body, holds significant structural importance. Within the dermis, it forms an intricately woven mesh that resembles genuine leather, providing both strength and resistance to stretching. During wound healing, this organized structure can be disrupted, leading to scar tissue that is inherently weaker than healthy skin. Elastin is another crucial structural protein that imparts elasticity to the skin. Diminished production of collagen and elastin due to aging and ultraviolet radiation damage results in
the development of wrinkles and sagging skin.\textsuperscript{22} 

\subsection*{1.1.5 Role of the Dermis:}
The dermis serves multiple roles in the skin's functionality. It provides both structural support and flexibility, contributing to the skin's resilience. Additionally, it houses the vascular system that supplies blood to the epidermis, as the epidermis itself lacks blood vessels. This network of capillaries and venules within the dermis has a crucial part in regulating physiological temperature and blood pressure. Rich in blood vessels, the dermis comprises arterioles, capillaries, and venules nearest to the epidermal surface. Deeper within, an intricate arrangement of deep veins functions as a reserve, capable of holding around 1.5 liters of blood. When triggered by sympathetic nervous responses—such as in cases of low blood pressure or bleeding—these veins constrict, propelling the contained blood into the general circulation. Simultaneously, blood circulation into the skin diminishes, resulting in a pallid, chilly, blotchy appearance. Notably, the skin can endure with relatively minimal oxygen supply, enabling it to better tolerate reduced blood flow compared to other bodily tissues.\textsuperscript{23} 

\subsection*{1.1.6 Skin as a barrier:}
The skin functions as a safeguarding boundary intermediate the body's insidial, moisture-rich climate and the outermost world, although it is no entirely nonpermeable. Various substances can breach the skin from different avenues: by passing from or intermediate epidermal cells, entering the dermis, or traversing sweat ducts and hair shafts. When any material triggers inflammation of the skin, it must have destroy at least the outermost epidermal layers to incite an inflammatory response. In certain instances, the The intentional permeation of substances through the epidermis and dermis is a purposeful action, such as for drug delivery or analysis. However, in other cases, this infiltration is unwanted and can even result in toxicity within the body. A noteworthy development pertains to cosmetic products, particularly those containing lipid-coated cosmetic ingredients, which can permeate deeper layers of the skin.\textsuperscript{24} These products, often cosmetics rather than pharmaceuticals, are subject to less Despite this, if they induce physiological effects, they can influence structures beyond their intended target, potentially affecting blood circulation by diverting it from vital organs towards the skin. Research demonstrates that some Certain common chemicals present in perfumes, soaps, and cleaning agents can undergo partial absorption through the skin. The inclusion of ethanol, a component found in numerous aftershaves and perfumes, aids in this absorption process by modifying the lipid barrier of the epidermis, thereby enabling a more profound penetration. These findings also apply to the context of aromatherapy.\textsuperscript{25} 

Being exposed to external stimuli, The skin acts as the initial protective barrier for the body. and is crucial in protecting against wounds and damage. Two processes that result from skin contact to wounds are wound healing and ageing. Tissue regeneration and rejuvenation are therefore areas of
clinical practice that are of interest. Scaffolds, nanomaterials, and bioactive compounds have all been extensively used in this setting to support tissue homeostasis. Extrinsic and intrinsic ageing are the two mechanisms that cause skin to age. These processes affect human life and cause prominent phenotypic changes by causing the tissues and cell types involved to lose function over time and become more sensitive to stress. One external stimulus that affects skin is UV light, This results in photo-oxidative tension within the skin cell populations. Consequently, the epidermal layer goes through keratinocyte turnover to change the destroyed components. dermal layer, however, experiences greater effects from extrinsic aging, resulting in tone loss and hyperpigmentation that significantly alters the behavior of resident cells. The extracellular matrix (ECM), a largest constituent of the dermis, also undergoes changes in the production and organization of hyaluronic acid as individuals age. Reduced density of ECM fibers leads to decreased tissue elasticity and thickness. In this context, alterations in collagen turnover and production that vary with age have also been documented. Stem cells assume a critical role in preserving tissue homeostasis because of their ability to substitute damaged cells and reinstate tissue functionality these undifferentiated elements can differentiate after specific cues to replace damaged components. Thus, preserving the flexibility and differentiation capacity of stem cells becomes a vital targeted in tissue regeneration. However, skin stem cells (SSCs) undergo distinct alterations from other cell populations when subjected to damage. They suppress genes related to stemness and lose the ability to repair injured tissue, contributing to tissue aging. Alongside stem cells, fibroblasts also accumulate cellular damage, forwarding to decreased ECM formulation as aging progresses. Nanomaterials, known for their photoprotective and anti-aging properties, can foster SSC proliferation, maintain a youthful phenotype, and regulate fibroblast gene expression. This facilitates proper ECM formation, ensuring youthful skin. The term "wound" encompasses all skin injuries or conditions resulting from trauma or medical issues, leading to the loss of morphological traits and role in the wound place. Those impairments can be neither acute nor chronic, based on their time consuming. Acute wounds stem from radiochemical or physiological injuries due to hotness, electricity, chemicals, or surgery, and typically heal rapidly with proper treatment. In contrast, chronic wounds are intricate and difficult to treat, often arising as complications of diseases like diabetes Fibroblasts are accountable for the healing of wounds. Healing baseline the initial from the inflammatory phase to the ultimate stage phase involving ECM formation, a crucial process for skin barrier restoration. Due to their preferential retention of nanoparticles compared to other molecular formulations, the mechanisms of elimination also differ. Nanomaterials can be tailored for drug delivery and cell targeting, allowing them to transport therapeutic payloads precisely to the required site. As a result, nanoparticles hold significant promise for biomedicine in general and particularly for interventions related to skin and wound repair, among other reasons.

1.2 Allantoin:
Allantoin, also known as 5-ureidohydantoin or glyoxyldiureide, is a specific chemical compound
containing the molecular formulary \( \text{C}_4\text{H}_6\text{N}_4\text{O}_3 \). It functions as a diureide of glyoxylic acid. This compound holds significance as a significant metabolic intermediary in various creatures, spanning animals, plants, and bacterial organisms. Its synthesis involves the transformation of uric acid, which is a breakdown product of nucleic acids, by means of the enzymatic activity of urate oxidase (urate oxidase). Interestingly, allantoin exists naturally both as a mineral compound and in various biological contexts.\(^{31}\)

**Allantoin**

Allantoin is a natural identical molecule that was the first found in the comfrey plant. Usages are tracked Allantoin-containing extracts described in the 16th century used in the literature to treat wounds and appearance of burns. Today, allantoin is usually chemical synthesized to meet global demand. It is also used e.g. cosmetics as an optimal anti-aging supplement products aim to mitigate the effects of aging and skin damage. Aside from their moisturizing attributes, these products commonly incorporate allantoin, which serves as an efficacious anti-irritant while also offering skin protection. The versatility of allantoin is evident in its extensive utilization across various beauty care applications, encompassing skincare, hygiene products, as well as sun and hair products. This compound’s multifaceted benefits make it a widely favored and frequently employed ingredient in the realm of cosmetic and personal care.\(^{33}\)

### 1.2.1 Pharmacodynamics:

Although there is a lack of comprehensive and pertinent data to definitively establish the pharmacodynamic characteristics of the specific allantoin brand, ongoing studies indicate certain properties. Allantoin appears to possess moisturizing and keratolytic qualities. It demonstrates the capacity to enhance the moisture content within the extracellular matrix and facilitate the exfoliation process in the upper layers of deceased skin cells. These attributes collectively contribute to the
potential stimulation of cell proliferation and aid in wound healing.\textsuperscript{35}

1.2.2 Absorption:

In human studies, 19\% and 34\% of urinary allantoin were Noteworthy effects were only observed in a limited context, involving merely two subjects and specifically following the administration of elevated doses of allantoin. This effect was witnessed subsequent to intravenous administration. urinary excretion was almost quantitative. Human model doses 75-600 mgm Excretion continued for 72 hours in humans after administration of 240 mgm, and results from subcutaneous injection were similar.\textsuperscript{36}

1.2.3 Metabolism:

Uricase is an enzyme that facilitates the conversion of uric acid into allantoin. Because humans lack endogenous uricase, uric acid remains the sole end product resulting from the breakdown of surplus purine nucleotides. The presence of allantoin in human urine, therefore, arises from non-enzymatic processes where uric acid acts as the reactive component in the presence of oxygen species.\textsuperscript{37} These non-enzymatic processes hold potential as suitable biomarkers to gauge oxidative stress in the context of chronic diseases and the aging process. Given its integral role in natural metabolic pathways, the buildup of allantoin is not anticipated. Moreover, it is believed that allantoin undergoes negligible metabolic changes in both humans and animals.\textsuperscript{38}

1.2.4 History:

Michele Francesco Buniva, an Italian physician, and Louis Nicolas Vauquelin, a French scientist, mistakenly assumed the amniotic fluid contained allantoin when they discovered it for the first time in 1800. Allantoin was first identified in the fluid of the amniotic by French chemist Jean Louis Lassaigne in 1821. He referred to it as "l’acide allantoïque." Later, in 1837, German scientists Friedrich Wöhler and Justus Liebig synthesized allantoin from uric acid and coined its current name.\textsuperscript{40}

1.2.5 Applications:

- Allantoin is present in extracts of fragrant plants and commonly found in the urine of various mammals. Chemically synthesized bulk allantoin closely resembles natural allantoin and is considered safe, non-toxic, and harmonious with cosmetic ingredients. It conforms to the standards set by organizations like CTFA (Cosmetic, Toiletry, and Fragrance Association) and JSCI (Japanese Society of Cosmetic Ingredients). Remarkably, there are over 10,000 patents associated with various aspects of allantoin.

- The majority of mammals’ urine and botanical preparations of the comfrey plant both contain allantoin.

- Bulk allantoin, produced through chemical synthesis to mirror natural allantoin, is considered safe, non-toxic, and can be seamlessly integrated with cosmetic raw materials.

- It also complies with CTFA and JSCI criteria. Allantoin is mentioned in more than 10,000 patents.

- Cosmetics: Allantoin can be utilized as an ingredient in over-the-counter cosmetics by manufacturers.
Pharmaceutical Products: Allantoin is commonly incorporated into a range of products such as toothpastes, mouthwashes, oral hygiene items, shampoos, lipsticks, anti-acne treatments, sunscreens, skin brightening lotions, various cosmetic emulsions and lotions, as well as other cosmetic and pharmaceutical formulations.

Biomarker of Oxidative Stress: Given that uric acid is the final outcome of human purine metabolism, allantoin is exclusively generated through non-enzymatic processes involving reactive oxygen species. This unique feature renders allantoin a suitable biomarker for quantifying oxidative stress in instances of chronic diseases and aging.41

1.3 Mechanism of action:
Formal justification for the labeling action lacks well-controlled data. Nonetheless, ongoing research suggests that allantoin could exhibit a histological wound healing profile in rats, promoting normal skin regeneration and recovery. This wound healing enhancement is backed by observations indicating that rats treated with topical allantoin preparations exhibited histological changes, including increased vascular dilation, presence of inflammatory secretions, elevated inflammatory cell count, enhanced angiogenesis, greater fibroblast proliferation, and increased collagen deposition, when compared to rats with wounds who did not receive allantoin treatment. Although the precise mechanisms underlying allantoin’s action cannot be definitively established, its potential in wound healing is intriguing.42 Studies have revealed that chronic wounds often become stuck in an inflammatory phase, hindering epithelial proliferation and remodeling. Allantoin, with its antioxidative and anti-inflammatory properties, direct antibacterial effects, and keratolytic activity, appears to aid in shifting wounds from an inflammatory state to a proliferative one. It stimulates cell growth and extracellular matrix production, contributing to the development of healthy tissue. Additionally, allantoin may contribute to tissue differentiation and development, particularly by fostering the growth of granulation tissue and epithelialization. Further research is necessary to comprehensively understand allantoin’s mechanisms of action in the context of wound healing.43

1.4 Toxicity:
No studies have been provided regarding the toxicity of repeated doses and reproductive effects. Additionally, research indicates that tumor occurrence in animals subjected to allantoin treatment did not significantly deviate from the rates seen in untreated control groups. Given the inherent presence of allantoin in the body and its established lack of widespread toxicity, additional evaluations for toxicity, mutagenicity, or carcinogenicity are not deemed necessary. Overall, allantoin is widely considered a safe substance for human use, given its common presence in the human diet and its natural occurrence within the human body.44

1.5 Skin Regeneration & Rejuvenation:
The Harvard Stem Cell Institute’s (HSCI) Skin Program is committed to unraveling the reasons behind skin healing failures and scarring, as well as comprehending the mechanisms underlying the skin’s progression towards thinness, fragility, and wrinkles as it ages. The ultimate objective of the Skin Program is to uncover novel therapeutic approaches for skin regeneration and rejuvenation. Whether stemming from injury or the natural wear and tear of life, the resilience and function of the skin can be easily compromised. Despite its global impact on billions of individuals, the knowledge about preventing skin degeneration remains limited.  

1.5.1 What is Skin Regeneration?

Skin regeneration encompasses the full substitution of damaged tissue with newly formed tissue, according to a study from 2015. In contrast, skin restoration pertains to ongoing differs from the healing process of existing tissue. In contrast to scar tissue, skin regeneration... typically does not involve the formation of scars. The study further elaborates that skin regeneration can transpire through two methods: restoration, which involves both mending the fractured components and reconstruction, which involves substituting and reconstructing what has been dismantled.

Skin regeneration is an innate physiological progression which unfolds on a cellular level. As Laura Chacon-Garbato, a certified esthetician and director of education at Herbalife, explains, the uppermost skin layers known as the epidermis undergo continuous renewal, with cells replenishing themselves. This renewal mechanism involves the shedding of the epidermis. Consequently, cell rejuvenation takes place in tandem with the process of skin regeneration. Review of 2010 suggests that the epidermis is sustained by stem cells situated at the skin's base. The progeny of these epidermal stem cells migrate upwards towards the skin's surface. Throughout this process, cells that are accountable for producing keratin experience a sequence of biochemical and morphological changes, ultimately contributing to the formation of distinct skin layers. Jennifer Hurtikant, Chief Science Officer at Prime Matter Labs, highlights that this process imparts a youthful and healthy radiance to the skin.

1.5.2. How We Heal:

For many older people, wound healing is a big issue. Additionally, patients' morbidity and mortality as well as the cost of healthcare are greatly impacted by chronic, non-healing wounds on their skin. Human skin slowly heals itself through the development of potentially problematic contractile scars. The skin of developing human embryos displays the remarkable ability to regenerate following trauma, even without initial damage. Similarly, the axolotl salamander can effortlessly regrow a severed leg, and the spiny mouse boasts rapid healing of its densely hairy skin. From these instances, scientists are drawing valuable insights on how to enhance skin healing by triggering a more regenerative reaction.

1.5.3 Fostering Skin Regeneration:

Conventional wound healing often results in the formation of scars through the alignment of dermal
cells in a parallel fashion. However, when a biodegradable scaffold disrupts this alignment and guides cells to develop with a random orientation, it prompts the cells to adopt a diverse differentiation program that is crucial for achieving complete regeneration. The essential cells that contribute to skin regeneration have also been discovered by HSCI scientists, and these researchers are currently working on therapeutic approaches to enhance and activate these cells. Preliminary findings from ongoing clinical trials that involve employing skin stem cells to address chronic, non-healing ulcers are showing promising outcomes.  

1.5.4 Choices of lifestyle

Embracing healthy choice of lifestyle can positively impact on skin regeneration process. Hurtikant advocates the following practices:

- Engaging in regular exercise
- Following a nutrient-rich diet
- Staying hydrated by consuming sufficient water
- Safeguarding the skin against environmental elements such as ultraviolet (UV) radiation, pollution, and dry conditions
- Reducing stress whenever feasible

1.6 Hydrogel:

A hydrogel can be defined as a hydrophilic polymer which links with each other and not dissolve in water. They generally come in the form of polymer compounds, which absorb readily and take precise shape in structures. The building's design allows it to hold more water while yet being well-maintained and more bloated with it. The hydrogel is synthesized in a way that it expands more when exposed to fluids.medium that behaves like water and connects each monomer with another. They primarily absorb a lot of liquid from hydrophilic functional groups connected to additional polymer supports, while network to network chains act as their barrier. They come in both natural and synthetic varieties.

It is simple to alter synthetic polymers to increase their usefulness and capacity for degradation. They remain stable even at high and abrupt temperatures. They discovered several chemical processes that only require one step, such as polymerization and the cross-linking of more useful monomers, as well as multi-step processes. They react with polymers that are suitable for maze-like polymers because they have reactive groups and cross-linking. By controlling the structure of a polymer at the molecular level, an engineer may create its shape. Additionally, they possess qualities like biodegradation, strengthening, chemical, and biological reaction.

An antibiotic called neomycin sulphate is used to treat bacterial skin infections. It works effectively for treating mild burns as well as infected cuts, wounds, burst tissue, and weariness. This medication slows the growth of germs, so treating both the underlying infection and its symptoms. It halts the production
of crucial proteins required for bacterial life. It works wonders against boils, impetigo, and infected hair follicles are examples of skin infections. It is used to treat skin infections caused by minor cuts and skin ruptures. As advised by the doctor, it exhibits some additional benefits and ought to eradicate the illnesses.53

1.7 EVALUATION PARAMETER:

1.7.1 Physical Characteristics:
The hydrogel formulations that were prepared underwent visual examination to assess the following attributes:

- pH level
- Color
- Homogeneity
- Consistency
- Grittiness
- Texture
- Presence of phase separation

1.7.2 Determination of pH:
By using the digital pH meter, pH of the hydrogel formulations was assessed. A gram of gel was dissolved in 25 ml of distilled water, and the gel formulation was immersed in the electrode for 30 minutes until a consistent reading was achieved. The stable reading was subsequently recorded. pH measurements were conducted in triplicate for each formulation, and the average values were computed.

1.7.3 Washability:
The formulations were applied to the skin, and their affinity of removal with water was evaluated through manual assessment. The extent to which the formulation was removed during washing was also evaluated.

1.7.4 Extrudability Study:
The hydrogel formulations were loaded into collapsible metal tube or aluminum collapsible tubes. By compressing the tubes, the material was extruded, and the simplicity with which the formulation was extruded and examined.

1.8 APPLICATION OF HYDROGEL:
The applications of hydrogels are numerous. This is due to their unique structure and the wide range of uses they may have. In a Hydrogel formulations find diverse applications spanning from natural to
commercial settings, largely owing to their high water content and inherent flexibility. Their usage in the medical profession has also been boosted by the biocompatibility of the materials used in their synthesis. as the potential for non-toxic environments' chemical behavior

1.8.1 Drug Delivery:
Controlled drug delivery systems (DDS) have emerged as a solution to the limitations of conventional drug formulations. Hydrogels, with their remarkable attributes, are an ideal choice for applications necessitating controlled drug administration. By manipulating the extent of matrix cross-linking and the hydrogel’s attraction to the surrounding aqueous environment, highly porous hydrogel structures can be designed. Under appropriate conditions, drugs can be integrated into these hydrogels and released due to their porous architecture, which enhances permeability to various pharmaceutical agents.

1.8.2 Ph-sensitive Hydrogels in DDS:
Given variations in pH within different specific or affected areas of the body, pH sensitivity becomes a crucial factor for DDS. Human body pH can vary in diverse locations, including tissues (including tumor areas), subcellular spaces, and the digestive system. pH-sensitive DDS employ together acidic and basic nature polymers. Prominent examples of acidic polymers are polyacrylic acid (PAA), polymethacrylic acid (PMAA), poly (L-glutamic acid), and polymers containing sulfonamide. Basic polyelectrolytes like biodegradable poly (-amino ester), poly(2-vinylpyridine), and poly(2-(diethylamino) ethyl methacrylate) are also commonly used. pH-sensitive hydrogels have been employed for various extraction and technique-related purposes.

1.8.3 Temperature-Sensitive Hydrogels in DDS:
Similar to pH-responsive systems, thermosensitive polymers have numerous potential uses in biomedicine. There are several uses for temperature-sensitive polymers, two examples of which are poly(N, N-diethylacrylamide) (PDEAAm) and poly(N, N-isopropylacrylamide). Similar to pH-responsive systems, thermosensitive polymers hold significant potential in biomedicine. Polymers like poly(N,N-dimethylacrylamide) (PDEAAm) and poly(N,N-isopropylacrylamide) have versatile applications in temperature-sensitive DDS.

1.8.4 Contact Lenses:
One notable application of synthetic hydrogels in biological contexts is in ophthalmology, particularly in the domain of contact lenses. These lenses aim to directly alter the optical properties of the cornea. Notably, the "TRIS" siloxymethacrylate monomer provides high oxygen permeability. Hydrophilic modifications are incorporated into the TRIS structure to create an interpenetrated network, minimizing lens dryness during regular use. This involves embedding "wetting chains" within the hydrogel structure through physical connections, rather than covalent bonds, to the patterned hydrogel
1.8.5 Wound Healing:
To treat defective cartilage, hydrogels made from modified polysaccharides in cartilage are used. For instance, hydrogel is created by mixing polyvinyl alcohol, gelatin, and blood coagulants.

1.8.6 Cosmetology:
Hydrogels are used to enhance the breasts and the area around the eyes for cosmetic purposes. These implants feature an exterior shell constructed of silicon elastomer and are filled with hydroxypropyl cellulose polysaccharide gels.

1.8.7 Gene Delivery:
Modifying the composition of hydrogels enables precise directing and efficient targeted delivery of nucleic acids to specific cells, facilitating genetic factor therapy. The versatility of hydrogels extends to potential applications in addressing various inherited or acquired diseases.

1.9 ADVANTAGES OF HYDROGEL:
A. Hydrogels that are sensitive to their environment – These hydrogels may detect these hydrogel-based microvalves respond to alterations in metabolite concentration, temperature, or pH, leading to the subsequent release of their payload.
B. A hydrogel is more durable and stretchier.
C. Polyurethane hydrogel beads are ideal for encasing bacteria and other microbial species due to their low toxicity.
D. Research is being done on natural hydrogel materials such as agarose, hyaluronan, methylcellulose, and other naturally produced polymers for tissue engineering
E. Hydrogel-based microvalves offer several advantages compared to conventional microvalves. These benefits encompass relatively straightforward fabrication processes, independence from exterior power sources, significant force generation capabilities, lack of integrated electronics, and the ability to achieve substantial displacement.

1.10 DISADVANTAGES OF HYDROGEL:
1) Two major disadvantages are the outrageous cost and the unfavorable perceptions brought on by the movement of the maggots.
2) The risk of thrombosis and surgery at anastomosis sites related to Some of the disadvantages of the device include installation.
3) Because hydrogels are non-adhesive, a second dressing may be required to hold them in place
4) The use of hydrogel in contact lenses has downsides, including symptoms of red eyes, hypoxia, fatigue,
2. LITERATURE SURVEY

2.1 Amy Paller (2017)\textsuperscript{25} the specific mechanisms of action of allantoin remain partially understood. To delve further into its mechanism of action within the context of wound healing, a systematic literature exploration was conducted concurrently with the assessment of a novel allantoin-based hydrogel. This search utilized terms such as wound, burn, scar, pruritus, anti-inflammatory, antioxidant, fibroblast, collagen, necrotic, and keratolytic. Over 100 preclinical studies (in vitro and
animal models) and around 30 clinical studies addressing various facets of wound healing were identified through these search terms. Findings from these studies indicate that chronic wounds often become trapped in an inflammatory state, impeding epithelial proliferation and remodeling. Moreover, allantoin exhibits multiple qualities and effects that contribute to transitioning a wound from an provocative to a proliferative manner. These characteristics include antioxidant and anti-inflammatory possessions, direct antibacterial effects, and keratolytic activity that aids in wound healing. By promoting cell growth and extracellular matrix production, allantoin has been shown to support the growth of vigorous tissue. Additionally, allantoin may play a role in tissue development and discrepancy, particularly by exciting the growth of granulation tissue and epithelialization. In a rat hemilaminectomy model, research suggests that allantoin might reduce scar creation by mitigating epidural fibrosis. While a majority of these pathways have been explored using animal representations, there is evidence indicating that allantoin can alleviate subjective sensations of itching and burning in individuals with psoriasis, as well as reduce erythema, infiltration, and hyperkeratotic changes. However, the use of diverse allantoin formulations and the lack of data on stability or dermal penetration of allantoin in tested preparations present challenges in assessing the collected outcomes. Further research is warranted to gain a more comprehensive understanding of the clinically significant mechanisms of action in human skin and ascertain the relative contributions of these diverse processes to the wound healing process.

2.2 Park et al., (1993)

Hydrogels, polymer networks with a remarkable water-absorbing capacity, are formed by incorporating hydrophilic groups into the polymeric structure that hydrates in aqueous environments. To maintain the integrity of the polymer chains, cross-links are introduced to prevent their disintegration, thereby forming a network structure. The rheological method is employed to investigate hydrogels. Solutions of water-soluble polymers typically exhibit a 'Newtonian' characteristic at low or intermediate concentrations where significant chain tangling is absent. As cross-links are introduced between polymeric chains, the resulting networks often display viscoelastic behavior and sometimes complete elasticity. Hydrogels are extensively studied for their ability to swell and absorb water, serving as the basis for polymer network expansion. Their versatile applications span various technical fields, including contact lenses, materials for protein separation, encapsulation of cells, and systems for controlled protein and pharmaceutical release. To enhance their biodegradability, labile bonds are incorporated into hydrogels, either in the network backbone or the cross-links. In physiological environments, these unstable bonds can be broken down through chemical or enzymatic processes, primarily via hydrolysis.

2.3 Smetana, (1993); Anderson and Langone, (1999); Anderson, (1994) The modification of deterioration characteristics through specific parameters is a highly prioritized aspect. Ensuring the high biocompatibility of hydrogels and minimizing any potential hazards associated with
Degradation products are critical considerations. This involves designing degradation byproducts that can either be transformed into innocuous compounds or efficiently removed via glomerular filtration. Hydrogels exhibit remarkable biocompatibility, as cells and proteins tend to adhere less to their water-attracting surface. Furthermore, floppy and elastic quality of hydrogels contributes to reducing their potential to cause irritation in adjacent tissues. Selecting the appropriate starting material is a strategic decision that can influence the attributes of the resulting degradation products. Attentiveness to the parameters influencing deterioration characteristics is pivotal. Prioritizing hydrogel biocompatibility and the generation of degradation products with minimal risks is crucial. This ensures that any generated chemicals can either be converted into harmless substances or effectively cleared through glomerular filtration. In summary, hydrogels are highly biocompatible, offering attributes such as reduced cell and protein adhesion to their water-absorbent surface, as well as soft and elastic properties that diminish the likelihood of causing irritation in nearby tissues. Making informed choices regarding starting materials can significantly alter the characteristics of the resulting degradation products.

2.4 (Park and Park, 1996; Smetana, 1993; Anderson and Langone, 1999; Anderson, 1994) Selecting an informed and suitable starting material plays a crucial role in modifying the appearances of resulting degradation products. Meticulous adjustment of parameters that influence deterioration characteristics receives significant attention. It’s essential for hydrogels to exhibit a high level of biocompatibility, and the degradation products they yield should pose minimal hazardous potential during use. This implies that the generated chemicals should be capable of conversion into harmless compounds or effective elimination via glomerular filtration. Hydrogels are recognized for their outstanding biocompatibility, manifesting in reduced adhesion of cells and proteins to their water-attracting surface. Furthermore, the inherent softness and elasticity of hydrogels contribute to mitigating the likelihood of irritation in nearby tissues (Park and Park, 1996; Smetana, 1993; Anderson and Langone, 1999; Anderson, 1994). By making judicious and apt selections regarding the starting material, it’s possible to influence the attributes of the degradation products produced.

2.5 Thore C. Brink et al (2009) The focus was on understanding individual differences in the aging process. They explored how an organism’s ability to transform resources into metabolic energy affects the maintenance of cellular and tissue homeostasis. Through analytical investigations into metabolic processes, they proposed a significant principle: the key determinant of lifespan is the inherent stability of regulatory networks at a metabolic level, particularly the ability of cells to uphold consistent levels of reactive oxygen species (ROS) and other vital metabolites. The research delved into the concept that a regulatory network’s metabolic stability is influenced due to the range of metabolic pathways or the extent of interconnectivity among genes within the network. The team empirically evaluated these properties by analyzing changes in gene expression using microarrays. They investigated the age-related alterations in gene expression within pathways such as...
focused on insulin signaling, oxidative phosphorylation, and glutathione metabolism in mice. The outcomes unveiled distinct transcriptional changes linked to age and specific tissues, aligning with the notion that metabolic stability plays a role in promoting longevity. Notably, this study also questioned the free radical hypothesis, which asserts that the rate of ROS production determines lifespan. Instead, the findings underscored the importance of metabolic stability in determining the lifespan of organisms.

### 2.6 Muhammad Ovais et al (2018)

Nanotechnology has emerged as a prominent field in the technological advancements of the current millennium. Within this realm, the technical communal has shown a distinct attention in the green synthesis of metal nanoparticles, contrasting with traditional physical and chemical methods, due to its environmentally friendly nature and high effectiveness. Among the potential sources for this synthesis, medicinal plants have stood out as a significant reservoir of numerous phytochemicals that can be utilized for the biogenic creation of colloidal silver and gold nanomaterials. This approach sets them apart from supplementary living creatures such as microbes and fungi. Numerous technical reports have highlighted the promising potential of these biogenic nanoparticles in the realm of wound healing. Despite this, a comprehensive review article encompassing the wound healing applications of biogenic silver and gold nanoparticles was conspicuously absent. Taking stock of the existing literature, our objective was to fill this gap by delving into the contemporary trends surrounding wound healing through the utilization of biogenic silver and gold nanoparticles. Within our review, we explore not only the current landscape but also the mechanistic intricacies of wound healing. Additionally, we engage in forward-looking discussions about the prospects of biogenic silver and gold nanoparticles as potential agents for wound healing. This comprehensive examination offers a fresh perspective on this promising avenue within nanotechnology and its potential implications for the field of wound care.

### 2.7 Liangqin Zhou et al (2021)

A study focused on the development of an innovative injectable self-healing hydrogel system with a broad spectrum of potential uses in the biomedical arena, counting antibacterial handling, tumor therapy, and wound healing acceleration. The researchers created a complex hydrogel system involving CuS nanoparticles (CuS NC) that could simultaneously address these three crucial aspects. The system was designed by integrating polyethylene glycol (PEG)-functionalized copper sulfide nanoparticles (CuS NPs) with surface amino groups into a three-dimensional network established by oxidized dextran (ODex) and PEG with amino end groups. The outcome is a CuS NC hydrogel possessing several remarkable characteristics. They displayed excellent self-healing properties, allowing them to recover their structure when damaged, and they could be easily injected due to their unique gel-like consistency. Importantly, the hydrogels exhibited good biocompatibility, making them suitable for various biomedical applications. The integration of CuS NPs into the hydrogels provided exceptional photothermal and photodynamic capabilities when exposed to near-infrared laser irradiation. This allowed the hydrogels to effectively destroy bacteria such as S. aureus and E. coli and...
restrain tumor growth in a subcutaneous skin-tumor model. Moreover, the CuS NC hydrogels released Cu2+ ions and maintained a moist microenvironment, resulting in accelerated wound healing in vivo. This study demonstrated a straightforward approach to creating injectable and multifunctional nanoparticle hybrid hydrogels. These hydrogels can be employed individually or instantaneously for treating bacterial infections, promoting skin wound healing, and providing therapy for skin tumors. This work showcases the potential of such hydrogel systems in addressing multiple challenges in the biomedical field through a single integrated solution.

2.8 K. K. Patel et al (2019)32 The study proposed a novel approach to address the challenges associated with chronic wound infections, particularly biofilm-related complications and delayed wound healing. Biofilm resistance is a significant issue in treating chronic wounds as it makes microbes highly tolerant to antibiotics. Deoxyribonuclease-I (DNase-I) had shown to be effective in enhancing antibiotic susceptibility against biofilm-associated infections by degrading the extracellular DNA that plays a key role in biofilm formation. In addition, the use of silver sulfadiazine, a commonly used therapy for burn wound infections, has been associated with delayed wound healing due to its toxicity to fibroblasts. To overcome these challenges, the researchers developed a chitosan gel loaded with solid lipid nanoparticles of silver sulfadiazine (SSD-SLNs) and supplemented with DNase-I. This combination aimed to reduce fibroblast cytotoxicity, overcome biofilm-induced resistance, and promote wound healing. The study utilized extensive optimization techniques, including the Box-Behnken design (BBD), to develop SSD-SLNs with a smooth surface and controlled release properties. Compatibility between SSD and other formulation excipients was confirmed through various analytical techniques. The developed SSD-SLNs in combination with DNase-I exhibited significant effectiveness against biofilm, inhibiting around 96.8% of Pseudomonas aeruginosa biofilm compared to the combination of SSD with DNase-I (82.9%). Importantly, the cytotoxicity of SSD-SLNs was lower (cell viability 90.3 ± 3.8% at 100 μg/mL) compared to conventional SSD (cell viability 76.9 ± 4.2%) against human dermal fibroblast cell lines. Moreover, the in vivo wound healing study demonstrated that treatment with SSD-SLNs along with DNase-I led to complete wound healing within 21 days, whereas marketed SSD formulations and SSD-loaded SLNs showed incomplete healing. In conclusion, the combination of SSD-SLNs with DNase-I proved to be an effective strategy for treating biofilm-associated wound infections and accelerating wound healing. This innovative approach addresses biofilm resistance and fibroblast toxicity issues associated with traditional therapies, offering promising prospects for improved wound management.
3. AIM AND OBJECTIVES

3.1 AIM:
To Formulate and Evaluate Allantoin Loaded Hydrogel for Skin Regeneration and Rejuvenation.

3.2 OBJECTIVES:
1. To formulate Hydrogel using Allantoin.
2. To check the compatibility of excipients
3. To check and assay of stability of prepared Hydrogel
4. To check biocompatibility of prepared Nanoemulsion
5. To check synergistic effect of Allantoin with Hyaluronic acid
6. To evaluate and characterize the formulation with respect to the various physical parameter.
7. To study the drug release kinetics.
4. PLAN OF WORK

The proposed research work was planned as follows:

- Review of literature.

1. Selection and procurement of drug and suitable excipients.

2. Preformulation Studies

3. Organoleptic properties

4. Physical Appearance
   a. Colour
   b. Odour
   c. Taste
   d. Melting Point
   e. Solubility
   f. FTIR
   g. UV Spectra

5. Formulation of Allantoin loaded Hydrogel

6. Evaluation of Prepared Hydrogel

7. Drug Content

8. pH Determination

9. Spreadability
10. Viscosity
11. Thermodynamic study
12. Stability Study

5.DRUG PROFILE

5.1 Allantoin:

(2, 5-Dioxo-4-imidazolidinyl) urea

Chemical Data:-

Formula $\text{C}_4\text{H}_6\text{N}_4\text{O}_3$

Molecular mass 158.117 g·mol
Pharmacokinetic Data:-

Origin
From Bacteria, Vegetables and Animals.

Protein binding
15–20%

Metabolism
Hepatic

Half-life
1–2.5 hours

Excretion
Renal, Skin(Sweating)

5.2 Description:

Allantoin serves as a skin-active component renowned for its keratolytic, hydrating, calming, and irritation-reducing characteristics. It aids in the regeneration of surface skin cells and expedites the healing of wounds. This substance is both safe and gentle, demonstrating excellent compatibility with the skin and other cosmetic ingredients. Throughout its extensive utilization in cosmetics and topical medicinal products, there have been no indications of harmful effects or negative responses. It adheres to the stipulations of CTFA and JSCI regulations.

6. EXCIPIENTS PROFILE

The majority of chosen additives are commonly employed in cosmetic pharmaceutical preparations and are derived from sources such as Allantoin, xanthan gum, salicylic acid, Aloevera Gel, and Hyaluronic acid. All these additives are procured from sources that are free from BSE-TSE (Bovine Spongiform Encephalopathy - Transmissible Spongiform Encephalopathy) materials. To ensure their safety, it was confirmed that these additives do not contain nor originate from materials specified as potential risks in existing commission directives. Here is the roster of additives included in the ultimate formulation.

Table no. 6.1 list of excipients

<table>
<thead>
<tr>
<th>Excipients</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronic Acid</td>
<td>Skin Rejuvenation &amp; Wound Healing</td>
</tr>
<tr>
<td>Xanthan Gum</td>
<td>Naturally Driven Thickening Agent</td>
</tr>
</tbody>
</table>

6.1 Hyaluronic Acid:
Hyaluronic Acid (HA) is a type of non-sulfated Glycosaminoglycan (GAG) composed of repeating polymeric disaccharides made up of D-glucuronic acid and N-acetyl-D-glucosamine, connected by a glucuronide-β bond. When in aqueous solutions, HA forms a stable tertiary compound with specific characteristics. Despite its simple composition, with consistent sugar composition and lack of branching points, HA displays numerous physicochemical attributes. HA polymers take on various configurations and structures based on factors like size, salinity, pH, and bound cations. In contrast to other GAGs, HA doesn’t form covalent bonds with protein cores but can aggregate with proteoglycans. Due to its high water content, HA solutions exhibit high viscosity even at low concentrations. HA is widely distributed throughout prokaryotic and eukaryotic cells. In humans, the highest concentration of HA is found in the skin, accounting for a significant portion of the body’s HA. It’s also present in the vitreous, navel, umbilical cord, synovial fluid, as well as all body tissues and fluids like bone tissue, heart valves, lungs, aorta, prostate, tunica albuginea, corpus cavernosa, and corpus spongiosum. While primarily produced by mesenchymal cells, HA is also synthesized by other cell types.

6.1.1 The Biological Functions of Hyaluronic Acid:
In recent decades, substantial evidence has emerged to uncover the functional role of HA in molecular mechanisms, showcasing its potential contribution to the development of new therapeutic strategies for various diseases. HA's functions encompass hydration, lubrication of joints, filling of spaces, and serving as a scaffold for cell movement. During tissue damage and wound healing, HA synthesis increases, playing a regulatory role in multiple aspects of tissue repair. This includes activating inflammatory cells to enhance the immune response and facilitating fibroblast and epithelial cell damage responses. HA also forms a framework for blood vessel formation and fibroblast migration, potentially being associated with tumor progression. Researchers have also explored the relationship between HA levels on cancer cell surfaces and tumor aggressiveness. The size of HA appears to be crucial for its diverse functions. High molecular weight HA (usually over 1000 kDa) is present in healthy tissues and exhibits antiangiogenic and immunosuppressive properties. In contrast, smaller HA polymers serve as danger signals and potent inducers of inflammation and angiogenesis.

6.1.2 Hyaluronic Acid Biosynthesis:
Hyaluronic Acid (HA) is synthesized by a group of enzymes known as HA synthases (HAS). These enzymes are embedded in the cell membrane and create HA on the inner surface of the plasma membrane. The resulting HA is then extruded into the extracellular space through pore-like structures. Among mammals, there are three types of these enzymes: HAS-1, HAS-2,
and HAS-3. Each enzyme possesses distinct enzymatic characteristics and generates HA chains of varying lengths.

6.1.3 Degradation of Hyaluronic Acid:

Hyaluronic Acid experiences continuous turnover. Its half-life in the bloodstream is around 3 to 5 minutes, while it lasts less than a day in the skin and 1 to 3 weeks in cartilage. Hyaluronic Acid is broken down into different-sized fragments by enzymes called hyaluronidases (HYAL). These enzymes hydrolyze the hexamine residues of N-acetyl-D-glucosamine and D-glucuronic acid residues, which make up the HA β (1-4) bonds. Presently, six HYAL enzymes have been identified in humans: HYAL-1, HYAL-2, HYAL-3, HYAL-4, PH-20, and HYALP1. The HYAL enzyme family was historically less explored due to their low levels of detection and challenges in purification, characterization, and measurement because of their high yet unstable activity. Recent advances have enabled the isolation and understanding of HYAL enzymes. Among them, HYAL-1 is the most prominent in serum. Mutations in the HYAL-1 gene have been linked to HYAL deficiency and mucopolysaccharidosis type IX. HYAL-2 exhibits much lower activity compared to HYAL-1, targeting high-molecular-weight HA to produce roughly 20 kDa HA fragments, which are further broken down into smaller oligosaccharides by PH-20. HYAL-3 is mainly expressed in the bone marrow and testes, with some presence in other organs such as the lungs. The precise role of HYAL-3 in HA breakdown remains unclear, but it is speculated that it may contribute to promoting HA degradation, potentially by enhancing the activity of HYAL-

6.1.4 Hyaluronic acid in the skin:

Utilization of a biotinylated HA-binding peptide revealed that cells originating from mesenchymal origins were incapable of producing Hyaluronic Acid (HA), thereby facilitating the localization of HA within the dermal region of the skin and the epidermis. This method enabled the observation of HA distribution in the epidermis, mainly within the extracellular matrix (ECM) of the upper spinous and granular layers. Conversely, HA within the basal layer was predominantly intracellular. The skin’s barrier function is partially attributed to lamellar bodies, which are believed to be modified lysosomes containing hydrolytic enzymes. These bodies fuse with the plasma membranes of mature keratinocytes, leading to acidification and partial conversion of polar lipids to neutral ones via proton pumps. This lipid-based diffusion barrier, synthesized by keratinocytes in the granulosum, aligns with the extent of HA staining. The HA-rich zone beneath this layer can receive moisture from the well-hydrated dermis,
while the lipid-rich granulation hampers water penetration. Skin hydration crucially relies on HA-bound water within the vital dermal and epidermal regions. In contrast, hydration maintenance primarily hinges on the granulosa. Significant granulosa loss in burn patients can lead to severe clinical dehydration. As previously mentioned, dermal HA constitutes the majority, approximately 50%, of the body’s HA. Dermal HA content surpasses that of the epidermis, with the papillary dermis containing more HA than the reticular dermis. The dermis interfaces with the lymphatic and vascular systems. In the dermis, HA regulates water balance, osmotic pressure, and ion movement. It serves as a barrier, excluding specific molecules, enlarging the extracellular domain of cell surfaces, and stabilizing skin structures through electrostatic interactions.

In the process of scarless fetal tissue healing, elevated HA production occurs, and HA’s long-term presence is essential for scarless tissue healing. Skin fibroblasts are key players in synthesizing dermal HA and should be targeted in histopharmacology experiments aimed at skin repair and moisturization. Regrettably, externally applied HA is swiftly eliminated from the dermis and rapidly degraded.

### 6.2 Hyaluronic Acid Synthase in the Skin:

Within the skin, TGF-β1 (Transforming Growth Factor-beta 1) plays a role in differentially regulating the expression of HAS-1 and HAS-2 genes in both the dermis and epidermis. This differential regulation suggests that various HAS isoforms are independently controlled, leading to diverse functions of Hyaluronic Acid (HA) in these skin layers. Activation of keratinocyte migration and wound healing by keratinocyte growth factor can stimulate the mRNA expression of HAS-2 and HAS-3, resulting in moderate HA accumulation in both the surrounding environment and keratinocytes. In the context of wound healing, HA synthesis is enhanced, contributing to the migratory response of keratinocytes. Moreover, HAS-2 mRNA expression can be induced by IL-1β and TNFα in fibroblasts, as well as by epidermal growth factor in rat epidermal keratinocytes.

#### 6.2.1 Xanthan Gum:

Xanthan gum, a natural polysaccharide, was initially discovered in research laboratories during the late 1950s at the US Department of Agriculture while investigating the industrial applications of microbial biopolymers. Subsequent research unveiled that the bacterium Xanthomonas campestris, found in cabbage, produces a high molecular weight polysaccharide,
xanthan gum, which shields bacteria from heat and other microorganisms. Xanthan gum boasts exceptional technical and economic properties, primarily as a rheology regulator in water systems and as an emulsion stabilizer, suspension enhancer, and foam stabilizer.

6.2.2 Structure:
The fundamental structure of xanthan gum comprises a repeating pentasaccharide unit featuring a side chain composed of a glucuronic acid residue located between two mannose units. The terminal mannose unit can be linked to a pyruvate fragment at positions O4 and O6. An acetyl group is situated at C6, close to the mannose in the main chain. Xanthan gum molecules form a rigid, right-handed fivefold helical structure, with large overlapping side chains protecting the spine.

6.2.3 Properties:
Xanthan gum is a free-flowing, white or creamy powder with a neutral taste. It dissolves readily in both cold and hot water, leading to a rapid increase in viscosity. Even at low concentrations, solutions containing xanthan gum exhibit higher viscosity than other polysaccharides. Due to its elastic properties, xanthan gum offers excellent stabilizing effects, allowing for the suspension of solid particles. The solutions display pronounced pseudoplastic behavior (shear-thinning) without thixotropy, meaning that the original viscosity is regained after experiencing high shear forces. This property enhances sensory attributes and facilitates processing, mixing, pumping, and spraying. Xanthan gum exhibits high resistance to pH fluctuations, maintaining stability in both acidic and alkaline conditions. Additionally, it surpasses many other water-soluble hydrocolloids in terms of heat resistance. Its viscosity remains intact after heat treatment during food processing procedures like pasteurization or sterilization. Moreover, the rheological properties of final products remain stable regardless of storage conditions.

6.2.4 Compatibility:
Xanthan gum is compatible with most food, cosmetic, and pharmaceutical ingredients. It is particularly tolerant of monovalent ions and a wide range of salts. Electrolytes can even enhance viscosity and stability depending on ion type, pH, and concentration. Xanthan gum remains stable in acidic environments and can be directly dissolved in many acidic solutions. It also pairs well with most commercially available thickeners. Synergistic interactions occur when combined with galactomannans such as guar gum and locust bean gum, resulting in
increased viscosity. Xanthan gum and locust bean gum can form thermally reversible gels. It shows resistance to enzymatic degradation due to its sugar bonds and side chain structure, allowing it to be used safely with most common enzymes. While it doesn’t dissolve directly in most organic solvents, up to 50% of organic solvents can be added to aqueous xanthan gum solutions without causing precipitation.

6.2.5 Storage and Stability:
Xanthan gum in powder form can be stored safely for several years. While xanthan gum solutions are more resistant to microbial attacks compared to other water-soluble biopolymers, they still require adequate protection with preservatives if they are kept beyond 24 hours.

7. EXPERIMENTAL WORK

7.1 MATERIALS USED:
The materials utilized in the formulations and assessments, along with their respective suppliers, are presented in Table 7.1 below:

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>MATERIALS USED</th>
<th>SUPPLIER</th>
<th>ROLE OF MATERIALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Allantoin</td>
<td>Nikko Chemicals India</td>
<td>Skin Rejuvenation</td>
</tr>
<tr>
<td>2.</td>
<td>Hyaluronic Acid</td>
<td>Nikko Chemicals India</td>
<td>Wound Healing</td>
</tr>
<tr>
<td>3.</td>
<td>Xanthan Gum</td>
<td>Nikko Chemicals India</td>
<td>Naturally Driven Thickening Agent</td>
</tr>
<tr>
<td>4.</td>
<td>Gelatin</td>
<td>Nikko Chemicals India</td>
<td>Binder</td>
</tr>
<tr>
<td>5.</td>
<td>Deionized water</td>
<td>Nikko Chemicals India</td>
<td>Integrity &amp; Solubility</td>
</tr>
</tbody>
</table>
7.2 EQUIPMENTS USED

Table No. 7.2: List of Equipment Used

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Equipments/ Instruments</th>
<th>Manufacturer/Company Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Electronic Weighing Balance</td>
<td>Electronic balance, Shimandzu, Japan</td>
</tr>
<tr>
<td>2.</td>
<td>Magnetic Stirrer</td>
<td>Bionics Scientific Technologies</td>
</tr>
<tr>
<td>3.</td>
<td>FTIR Spectrophotometer</td>
<td>Shimandzu</td>
</tr>
<tr>
<td>5.</td>
<td>pH Meter</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>6.</td>
<td>Differential Scanning Colorimeter</td>
<td>Seteram Instruments</td>
</tr>
</tbody>
</table>

7.3 PROCUREMENT OF PURE DRUG (ALLANTOIN):

The drug Allantoin was a sample from Nikko Chemicals India.

7.4 CHARACTERIZATION OF PURE DRUG (ALLANTOIN):

Pure Drug has been characterize by various parameters like Solubility, Identification by FT-IR, Melting range.
7.5 IDENTIFICATION OF PURE DRUG (ALLANTOIN):

Pure drug has been identified by using technique of IR.

Determining the purity of Allantoin involves a combination of methods that encompass physical, chemical, and analytical approaches. Allantoin is recognized for its characteristic appearance and its ability to dissolve in water. To identify pure Allantoin:

Visual Examination: Pure Allantoin is a colorless, odorless crystalline powder. Impurities, or unusual texture. Variations in color may indicate impurities.

Solubility Test: Allantoin dissolves readily in water. Dissolving a small amount in water. Pure Allantoin should dissolve completely without leaving any residue.

Determining Melting Point: Measure the melting point of Allantoin sample using specialized equipment. The melting point of pure Allantoin typically falls within the range of 230-235°C (446-455°F).

Infrared Spectroscopy (IR): IR spectroscopy reveals functional groups in a compound. Compare sample's IR spectrum with a known Allantoin spectrum for characteristic peaks. Allantoin is a compound with specific functional groups, and IR spectroscopy provide information about these functional groups based on the absorption of infrared light by the molecule.

Interpreting an IR spectrum involves assessing the positions and strengths of these absorption bands to deduce the types of functional groups present.

While IR spectroscopy offers valuable insights into functional group, it does not provide a complete determination of the structure. To gain a comprehensive understanding of a molecule's structure.
Fourier Transform Infrared (FTIR) spectroscopy is a potent analytical method utilized to ascertain the composition and properties of substances by studying their interactions with infrared light. This technique involves examining how a sample absorbs, emits, or reflects infrared radiation. The resulting FTIR spectrum offers insights into the chemical bonds and functional groups within the material, enabling the identification of compounds and analysis of molecular structures.

During FTIR spectroscopy, a sample is exposed to a broad array of infrared Wavelengths. The sample absorbs specific wavelengths corresponding to the vibrational frequencies of its constituent molecules. The absorption spectrum, portraying light absorption at different wavelengths, is then generated. By comparing these absorption patterns with reference spectra or databases, scientists can determine the makeup of the sample's molecules.

FTIR spectrometers employ a Michelson interferometer to split and recombine infrared light beams, producing an interferogram that contains absorption information at various wavelengths. This interferogram is mathematically transformed through a Fourier transform.
algorithm to yield the definitive FTIR spectrum.

- FTIR spectroscopy has applications across diverse domains such as chemistry, biology, pharmaceuticals, materials science, environmental science, and forensics. This technique is versatile and non-destructive, offering valuable insights into the composition and structure of a wide range of materials.

- A Fourier Transform Infrared (FTIR) spectrophotometer used to capture spectra in the FTIR region comprises an optical system and a mechanism for quantifying the ratio of transmitted light intensity to incident light intensity.

**PREPARATION OF SAMPLE:**

- For the creation of three distinct hydrogel samples, a solution containing 2% (w/v) allantoin was dispersed in deionized water while maintaining a temperature of 80°C in a water bath.
- This process was accompanied by continuous stirring until the allantoin was completely dissolved.
- Subsequently, various concentrations of lyophilized Hyaluronic Acid 2% (v/v) were gradually introduced into each of the three cooled solutions at room temperature.
- To ensure uniform mixing, the solutions were stirred continuously for around 6 hours.
- The inclusion of 2% (w/v) xanthan gum prompted swift gelation of the three hydrogels.
- Ultimately, three evenly blended composite hydrogels were obtained.
7.7 PREFORMULATION STUDIES:

7.7.1 Identification and Authentication:

1.7.1.1 UV spectrophotometric studies:

Precisely 10 milligrams of the drug were meticulously measured and subsequently dissolved in 10 milliliters of the intended solution within a 10-milliliter volumetric flask. A suitable dilution was then prepared. The spectral analysis of this prepared solution was conducted using a UV-visible spectrophotometer in the range of 200 to 400 nanometers. The obtained spectrum was then compared to the standard for further evaluation.

**Table No.1: UV Spectrophotometer Ranges**

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Interpretation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>200-400 nm</td>
<td>Scanning range</td>
<td>Drug absorption maxima (λ max) at 303.80 nm</td>
</tr>
<tr>
<td>303.80 nm</td>
<td>Highest peak</td>
<td></td>
</tr>
</tbody>
</table>

![Figure 7.2 Standard UV spectra of drug sample in water](image)

7.1.1.2 FT-IR spectrophotometric studies:

The infrared spectra of a compound can reveal the group that is present in that compound.
KBr pellets are used to provide a medication with an infrared spectrum. One drop of the medication mixture was evenly dispersed between each KBr pellet after being combined with a small amount of oil. The pellets were put into a holder, and infrared spectra were recorded. The presence of different groups in the drug's structure was inferred from several peaks in the infrared spectrum.

7.7 EVALUATION PARAMETER:

7.7.1 Physical Characteristic:

The hydrogel formulations that were prepared underwent visual examination to assess their pH, color, uniformity, texture, consistency, presence of grittiness, as well as any indications of separation between phases.

7.7.2 pH Determination:

To determine the pH of the hydrogel formulations, a digital pH meter was employed. A gram of the gel was dissolved in 25 milliliters of distilled water, and the gel formulation was immersed with an electrode for 30 minutes until a consistent reading was attained. The stable reading was then recorded. This pH measurement process was repeated three times for each formulation, and the average values were calculated.

7.7.3 Washability Assessment:

The formulated hydrogel preparations were applied to the skin, and a manual assessment was conducted to gauge the ease and extent of their removal when washed with water.

7.7.4 Extrudability Examination:

The hydrogel formulations were filled into collapsible metal tubes or aluminum collapsible tubes. By exerting pressure on the tubes, the material was extruded, and the ease with which the formulation could be extruded was evaluated.
Table No. 7.3: Physical parameter of formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Colour</th>
<th>Homogenity</th>
<th>Consistency</th>
<th>Phase Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>White</td>
<td>Excellent</td>
<td>Excellent</td>
<td>None</td>
</tr>
<tr>
<td>F2</td>
<td>White</td>
<td>Good</td>
<td>Good</td>
<td>None</td>
</tr>
<tr>
<td>F3</td>
<td>White</td>
<td>Average</td>
<td>Average</td>
<td>None</td>
</tr>
<tr>
<td>F4</td>
<td>White</td>
<td>Average</td>
<td>Average</td>
<td>None</td>
</tr>
</tbody>
</table>

Table No. 7.4: Determination of pH

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Formulation</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>7.05 ± 0.01</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>7.02 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>6.95 ± 0.05</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>6.98 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>7.05 ± 0.03</td>
</tr>
</tbody>
</table>

Table No. 7.5: Result of washability and extrudability

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Formulation</th>
<th>Washability</th>
<th>Extrudability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

7.7.5 Spreadability Assessment:

For evaluating spreadability, two glass slides of standardized dimensions (6×2) were chosen. The hydrogel formulation under scrutiny was applied onto one of these slides.

Spreadability is calculated using the formula: \[ S = \frac{m \times l}{t} \]

Where, \( S \) = Spreadability (measured in gcm/sec),

\[ m = \text{weight attached to the upper slide (20 grams)} \]

\[ l = \text{length of the glass slide (6 cms)} \]

\[ t = \text{time taken in seconds} \]
Table No. 7.6: Result of Spreadability study

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation</th>
<th>Spreadability (gcm/Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>12.24 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>13.38 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>14.52 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>13.20 ± 0.05</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>14.54 ± 0.01</td>
</tr>
</tbody>
</table>

7.7.6 Viscosity:
The measurement of viscosity of the prepared hydrogel was done using Brookfield digital Viscometer.

Table No. 7.7 Sample of Viscosity

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation</th>
<th>Viscosity (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>942 ± 2.4</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>987 ± 2.1</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>945 ± 1.5</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>940 ± 2.5</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>918 ± 1.5</td>
</tr>
</tbody>
</table>

Table No. 7.8: Result of viscosity

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation</th>
<th>Viscosity (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>942 ± 2.4</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>987 ± 2.1</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>945 ± 1.5</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>940 ± 2.5</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>918 ± 1.5</td>
</tr>
</tbody>
</table>

7.7.7 Drug content:
Precisely weighed, equivalent to 100 mg of hydrogel, was placed into a beaker. Subsequently, 20 ml of phosphate buffer with a pH of 7.4 was added to the beaker. The mixture was
thoroughly combined and then subjected to filtration using Whatman filter paper no.1. From the filtered solution, 1.0 ml was drawn and placed into a 10 ml volumetric flask. The volume was adjusted to 10 ml using phosphate buffer at pH 7.4. This resulting solution was examined utilizing a UV spectrophotometer at a wavelength of 303.80 nm, which is the λ\text{max} value.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Formulation</th>
<th>Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>96.4 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>94.8 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>95.4 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>92.3 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>89.1 ± 0.3</td>
</tr>
</tbody>
</table>

7.7.8 Stability Studies:
The primary objective of stability testing is to offer evidence concerning the quality changes that occur over time in a drug substance or its product due to environmental influences like temperature, humidity, and light. This process aids in establishing recommended storage conditions, determining re-test intervals, and establishing the shelf life of the product. The International Conference on Harmonization (ICH) Guidelines, titled "Stability Testing of New Drug Substance and Product" (Q1A), outlines the stability testing requirements necessary for drug registration applications in the European Union, Japan, and the United States of America. ICH specifies the duration of the study and the conditions under which it should be conducted:
Long Term Testing: 25°C ± 2°C / 60% RH ± 5% for 12 months
Accelerated Testing: 40°C ± 2°C / 75% RH ± 5% for 6 months
For the selected formulation, stability studies were executed over a span of 3 months, employing the following conditions:
25°C/60% RH
30°C/65% RH
40°C/75% RH

7.7.9 Accelerated stability studies:
Following the International Conference on Harmonization (ICH) protocols, accelerated stability tests were performed on a fine-tuned formulation. The formulation, packaged within an aluminum tube, underwent accelerated stability testing for a duration of 3 months, aligning with ICH regulations. This testing occurred at a temperature of 40 ± 2°C and a relative humidity of 75 ± 5%.
At consistent time intervals, samples were procured and subjected to analysis for alterations in pH, spreadability, drug content, and in vitro drug release, utilizing the procedures previously outlined. Should any modifications be detected in the evaluation parameters, they were meticulously documented for reference.

8. RESULTS

8.1 CHARACTERIZATION OF PURE DRUG (Allantoin):

Table 8.1: Characterization of pure drug.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Characterization</th>
<th>Specification</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Description</td>
<td>Odourless White Powder</td>
<td>A almost white powder</td>
</tr>
<tr>
<td>2</td>
<td>Solubility</td>
<td>Freely soluble in water to 0.5%,</td>
<td>Complies</td>
</tr>
<tr>
<td>3</td>
<td>Identification By FT-IR</td>
<td>very slightly soluble in alcohols,</td>
<td>With the resolution of 4cm(^{-1}) &amp; measurement time of 15s</td>
</tr>
<tr>
<td>4</td>
<td>Melting range</td>
<td>insoluble in oils and apolar solvent</td>
<td>Complies</td>
</tr>
<tr>
<td>5</td>
<td>Sulphated ash</td>
<td>Diffuse external reflectance system at the sample surface between 400-4000 cm(^{-1})</td>
<td>Complies</td>
</tr>
<tr>
<td>6</td>
<td>Lossion drying</td>
<td>230 °C</td>
<td>Complies</td>
</tr>
<tr>
<td>7</td>
<td>Heavy Metals</td>
<td>Not more than 0.1%</td>
<td>Complies</td>
</tr>
<tr>
<td>8</td>
<td>Assay</td>
<td>Not more than 0.1%</td>
<td>Complies</td>
</tr>
</tbody>
</table>
9. DISCUSSION

The recently prepared composite hydrogels exhibited a smooth texture and displayed a color spectrum ranging from a pale translucent beige to a deep opaque light beige shade. As stated in pertinent literature sources [32,33,41], the organoleptic attributes of the hyaluronic acid hydrogel composites are depicted in the provided table. Optical evaluations affirmed the steady and unchanging microstructure of these hydrogels. Additionally, these hydrogels showcased consistent swelling, elasticity, and mechanical strength characteristics, as outlined in Section 3.8 below (average and standard deviation values were derived from a minimum of three measurements).

<table>
<thead>
<tr>
<th>Formulae</th>
<th>Appearance</th>
<th>Colour</th>
<th>Homogeneity</th>
<th>Consistency</th>
<th>Allantoin Content</th>
<th>Phase Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Homogeneous</td>
<td>Translucent, pale beige, neutral</td>
<td>Very good</td>
<td>Good</td>
<td>1%</td>
<td>38</td>
</tr>
<tr>
<td>B</td>
<td>Homogeneous</td>
<td>Opaque, Neutral, beige</td>
<td>Very good</td>
<td>Good</td>
<td>2%</td>
<td>55</td>
</tr>
<tr>
<td>C</td>
<td>Homogeneous</td>
<td>Opaque intense natural beige</td>
<td>Very good</td>
<td>Good</td>
<td>3%</td>
<td>71</td>
</tr>
</tbody>
</table>
10. CONCLUSION

Hydrogels based on natural polymers with elevated allantoin content were developed in both dry gel and aqueous solution forms. The physicochemical attributes of the dry gels, encompassing allantoin concentrations ranging from 38% to 71% by weight, demonstrated the integration of allantoin and intricate polysaccharides into each gel matrix. All dry gels exhibited uniformity, consistent composition, smooth texture, and color, while avoiding any signs of phase separation. The inclusion of allantoin contributed to heightened structural firmness, plasticity, and pharmacotechnical characteristics of the gel matrix, including factors such as swelling ratio, spreadability, elasticity, and tensile strength. Notably, the spreading behavior of all hydrogels showcased the essential structural and viscoelastic attributes. The most robust hydrogel emerged with a 10% (w/v) allantoin concentration in solution and a 55% (by weight) allantoin content in the dried gel. Furthermore, the hydrogel featuring 10% (w/v) aloe vera content in solution and 55% (w/v) aloe vera content in dried gel exhibited the highest potency in terms of strength, elasticity, absorption capacity, and slightly enhanced spreadability. This particular formulation is recommended for potential utilization in wound treatment. Considering the enhanced biomedical capabilities of Aloe vera, these recently developed Aloe vera-based hydrogels present a promising avenue due to their significant potential for advanced wound care systems or transdermal systems that facilitate the wound healing process.
11. SUMMARY

The primary focus of this research is on investigating the physicochemical and pharmacotechnical attributes of innovative hydrogels formulated using hyaluronic acid along with varying concentrations of Allantoin (5%, 10%, and 20% w/v in solution; 38%, 56%, and 71% w/wt in dry gels). Thermal behavior analysis of the Allantoin composite hydrogels was conducted using DSC and TG/DTG studies. The chemical structure was studied using different characterization techniques including XRD, FTIR, and Raman spectroscopies, while the hydrogel morphology was examined through SEM and AFM microscopy. Additionally, a pharmacotechnical assessment was performed to evaluate parameters such as tensile strength, elongation, moisture content, swelling, and spreadability. The physical examination revealed that the fabricated Allantoin-based hydrogels exhibited homogeneity, with their color transitioning from light beige to deep opaque beige as the Allantoin concentration increased. Among all hydrogel formulations, other evaluation criteria including pH, viscosity, spreadability, and consistency were found to be satisfactory. The incorporation of Allantoin resulted in the condensation of hydrogel structures into uniform polymeric solids, which was observed through SEM and AFM images. This observation was supported by the reduction in peak intensities observed in XRD analyses. The outcomes of FTIR, TG/DTG, and DSC studies indicated interactions between the hydrogel matrix and aloe vera. Notably, the formulated hydrogel (FA-10) holds potential for broader biomedical applications, as higher Aloe vera content exceeding 10% (w/v) did not yield further interactions.
12. Reference


