
Chapter 1 Introduction and Literature review

1.1 Diabetic wound: Silent Killer?

Diabetes is often linked to various long-term complications, including chronic wounds, which pose significant challenges for healthcare systems globally. In the United States alone, an estimated 2.4 to 4.5 million individuals experience diabetic wounds[1]. India, often called the "diabetes capital of the world," faces a serious problem with diabetic foot ulcers[2]. With more than 80 million people living with diabetes as depicted in figure 1.1, many are at risk of developing these long-lasting wounds[3]. This can lead to amputations and high healthcare costs. These wounds disrupt the skin's natural healing processes, complicating recovery and often leading to infections, particularly in the limbs, such as the feet. The prevalence of infections in diabetic wounds is concerning, as it increases the risk of chronic conditions, amputations, and even mortality. Additionally, these wounds typically persist for an average of 12 to 13 months, with a recurrence rate of 60% to 70%, resulting in substantial healthcare costs[4]. Treating diabetic wounds alone constitutes about 12% to 15% of total diabetes-related expenses in the country[5].

The challenges in treating diabetic wounds stem from a combination of factors, including an imbalanced inflammatory response, oxidative stress, elevated blood glucose levels, inadequate angiogenesis, and a high susceptibility to bacterial infections[6]. Historically, wound care relied on dry dressings aimed at keeping the area dry to prevent infections[7]. While traditional dry dressings, such as gauze, can absorb exudate, they do not actively adapt to changes in the wound environment or provide ongoing treatment, leading to potential adhesion issues and limited efficacy[8].



Figure 1.1 Estimated Number of people with Diabetes worldwide and per region in 2015 and 2040 (20-79 years)[6]

1.2 Wound Healing Mechanism

Wound healing is the complex biological process through which the body repairs damaged tissue after an injury. This process is essential for restoring the integrity and function of the skin and underlying tissues. Wound healing can be categorized into four main phases[9]: hemostasis, inflammation, proliferation, and remodeling as shown in figure 1.2.

Hemostasis Phase

The hemostasis phase is the immediate response to injury, occurring within minutes of tissue damage. When a wound occurs, blood vessels constrict to reduce blood loss, and platelets aggregate at the site, forming a temporary plug. These platelets release signalling molecules

that attract other immune cells to the area, initiating the inflammatory response. The formation of a fibrin clot provides a scaffold for incoming cells, and this phase is critical for preventing excessive bleeding while setting the stage for the next phase of healing.

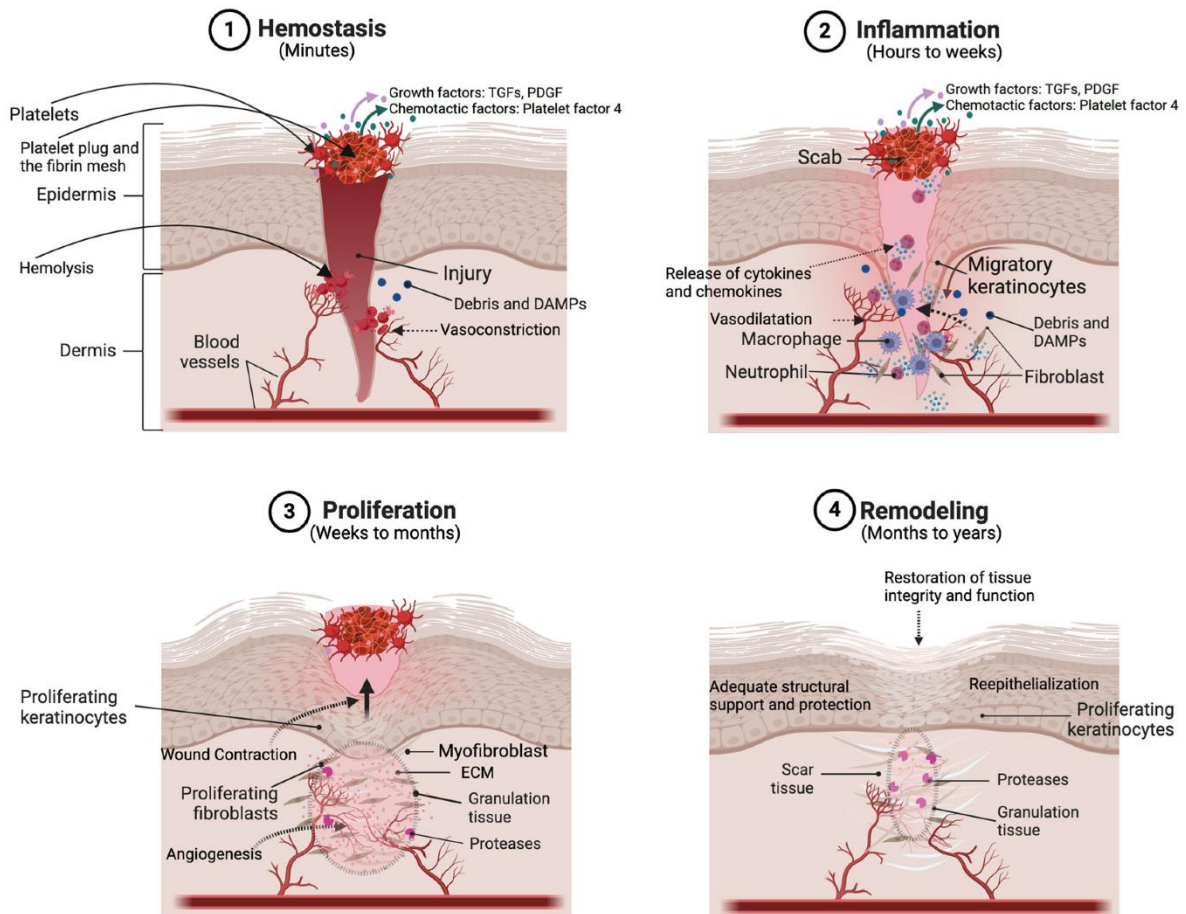


Figure 1.2 Schematic illustrating the various phases of the wound healing process [10]

Inflammatory Phase

The inflammatory phase follows hemostasis and can last for several days. During this time, the wound area becomes infiltrated with immune cells, particularly neutrophils and macrophages. Neutrophils are among the first responders, combating pathogens and clearing debris. As they die off, macrophages take over, playing a pivotal role in orchestrating the

healing process. They not only help in clearing dead tissue but also release growth factors and cytokines that stimulate fibroblast activity and angiogenesis (the formation of new blood vessels). While inflammation is a necessary part of healing, chronic inflammation can delay recovery and contribute to complications, especially in diabetic wounds.

Proliferative Phase

The proliferative phase begins a few days after the injury and can last from several days to weeks, depending on the wound severity. This phase is characterized by tissue formation and the rebuilding of the extracellular matrix. Fibroblasts are activated and migrate to the wound site, where they produce collagen and other components essential for structural integrity. Angiogenesis occurs to restore blood supply, and new epithelial cells migrate across the wound bed to cover the area. Granulation tissue, a soft, pink tissue rich in collagen and blood vessels, forms and helps protect the wound. This phase is critical for closing the wound and restoring skin integrity.

Remodelling Phase

The remodeling phase, also known as the maturation phase, can last from weeks to months or even years after the initial injury. During this phase, the collagen matrix laid down during the proliferative phase is remodeled and strengthened. The collagen fibers reorganize, cross-link, and become more structured, increasing the tensile strength of the wound. Although the wound appears healed on the surface, it may still be weaker than the surrounding tissue. This phase can result in scar formation, which may change in appearance and texture over time as the remodeling continues.

In summary, normal wound healing progresses through these four distinct phases—hemostasis, inflammation, proliferation, and remodeling—each critical for effective tissue repair and restoration of function. In diabetic patients, disruptions in these phases can significantly impair healing, leading to chronic wounds that are difficult to treat.

1.3 Diabetic Wound healing

In contrast diabetic wound healing is frequently disrupted due to several interrelated factors, which hinder the normal progression through the healing phases. One of the primary issues is hyperglycemia, or elevated blood sugar levels, which leads to increased oxidative stress and the production of advanced glycation end products (AGEs)[10]. These compounds can damage tissues and impair cellular function, particularly affecting fibroblasts and endothelial cells essential for tissue repair and angiogenesis. Additionally, diabetes often results in poor circulation due to vascular damage, which reduces the delivery of oxygen and nutrients to the wound site.

The inflammatory phase, which is typically a short and efficient response in healthy individuals, becomes prolonged in diabetic patients[11]. This chronic inflammation can result in an excessive presence of immune cells, such as macrophages, which instead of promoting healing may contribute to further tissue damage and inhibit the transition to the proliferative phase. During this phase, the production of collagen and new blood vessels is impaired, leading to inadequate tissue formation.

Moreover, the remodeling phase is also affected, as the collagen that is produced may be disorganized and less effective due to impaired cellular signaling and growth factor availability. These disruptions culminate in chronic wounds that are resistant to healing,

increasing the risk of complications such as infections, and potentially leading to more severe outcomes, including amputations. Effective management of diabetic wounds requires addressing these underlying metabolic issues, optimizing blood sugar levels, and employing targeted wound care strategies to facilitate healing.

Furthermore, the high blood glucose levels typical of diabetic patients can severely impact the formation of new blood vessels and the overall healing process. The lack of sufficient blood flow creates a hypoxic environment that further complicates recovery. Understanding these challenges is essential for developing effective treatment strategies to enhance healing in diabetic wounds.

1.4 Existing Solutions for Diabetic Wound Healing

Diabetic wounds present unique challenges in healing due to factors such as impaired blood circulation, neuropathy, and increased susceptibility to infections. As a result, several treatment modalities have been developed to address these specific needs.

One of the primary solutions for managing diabetic wounds is the use of advanced dressings. These dressings, including hydrocolloids, alginates, and foam dressings, are designed to maintain a moist wound environment, which is essential for optimal healing[12]. Hydrocolloid dressings, for example, can absorb excess exudate while providing a protective barrier against bacteria. Their ability to create a moist environment promotes autolytic debridement, where the body's own enzymes help break down necrotic tissue. However, while these dressings are beneficial for moderate exudating wounds, they may not be suitable for wounds with heavy drainage, as they can become saturated and lose their effectiveness.

Table 1.1 shows the existing treatment methods for curing diabetic wound along with their drawbacks.

Table 1.1 Existing solutions for treatment of diabetic wound and their drawbacks [15]

S. No.	Solutions/Products	Drawbacks
1	Antibiotic ointments (Bacitracin, Hydrocortisone cream, Urea cream)	Allergic reactions, Resistance, Interactions with other medications, Potential toxicity
2	Growth Factor therapy	Expensive, require specialized training to apply and remove, Uncomfortable for sensitive skin
3	Offloading devices	Costly, require regular maintenance, difficult to apply, uncomfortable to wear,
4	Negative pressure wound therapy (NPWT)	Poor bioavailability, Inefficient,

Another critical intervention is the use of topical antiseptics and antibiotics. These agents help reduce the bacterial load in wounds, a crucial step for preventing infections, especially in diabetic patients who are at higher risk. Topical antibiotics like silver sulfadiazine or honey-based treatments have shown promise in managing infections and promoting healing.

However, the overuse of antibiotics can lead to resistance, and certain antiseptics may be cytotoxic to healthy tissue, potentially hindering the healing process.

Negative pressure wound therapy (NPWT) is another advanced treatment used for diabetic wounds[13]. This technique involves applying suction to the wound through a sealed dressing, which helps remove excess fluid and promotes blood flow to the area. NPWT has been shown to accelerate healing by enhancing granulation tissue formation and reducing bacterial colonization. Nevertheless, the cost and complexity of NPWT systems may limit their availability in some clinical settings, and proper training is required to avoid complications.

Additionally, growth factor therapies have gained attention in the management of diabetic wounds. Products that contain recombinant growth factors aim to enhance cellular proliferation and migration, facilitating tissue repair. While these therapies can be effective, their high cost and variable patient responses can be significant barriers to widespread use.

Despite the availability of these various treatments, challenges remain in the comprehensive management of diabetic wounds. Factors such as patient compliance, the presence of comorbidities, and the specific characteristics of each wound can influence healing outcomes. As a result, a multidisciplinary approach that combines various treatment modalities is often necessary to achieve optimal healing in diabetic patients.

1.5 Hydrogel as an Emerging Solution

Hydrogels have emerged as a novel and promising material for diabetic wound management. These hydrophilic polymers can absorb and retain moisture, creating a moist environment that is conducive to healing. This property is particularly beneficial for diabetic wounds, as it helps

facilitate autolytic debridement and reduce pain during dressing changes. In addition to moisture retention, hydrogels can be formulated to incorporate therapeutic agents, such as antibiotics, anti-inflammatory drugs, or growth factors, allowing for localized treatment. This targeted approach can enhance the healing process by addressing specific challenges associated with diabetic wounds, such as infection and impaired tissue regeneration.

The biocompatibility of hydrogels minimizes the risk of irritation, making them suitable for prolonged use in sensitive wound areas. Furthermore, their soft and pliable nature conforms well to the wound surface, promoting comfort for the patient.

However, hydrogels also face some limitations, including their mechanical strength and handling difficulties compared to traditional dressings. Despite these challenges, the unique properties of hydrogels position them as a valuable tool in the evolving landscape of diabetic wound care, offering new hope for improved healing outcomes in this patient population.

1.6 Hydrogel: Basic Concept and Working

Hydrogels are three-dimensional, hydrophilic polymeric networks that can retain a significant amount of water within their structure without dissolving as shown in figure 1.3. This unique property stems from their chemical composition, which typically includes natural or synthetic polymers with cross-linking points. The high-water content allows hydrogels to mimic biological tissues, making them particularly useful in medical applications, including wound healing.

The fundamental working mechanism of hydrogels revolves around their ability to absorb moisture and provide a suitable environment for wound healing. When applied to a wound,

hydrogels maintain a moist environment that facilitates autolytic debridement, a process in which the body's own enzymes and moisture break down necrotic tissue. This moist environment not only promotes healing but also reduces pain and discomfort associated with dry, crusted wounds.

Hydrogels can also be engineered to include bioactive components, such as growth factors, antimicrobial agents, or anti-inflammatory drugs. This allows for localized delivery of therapeutic agents directly to the wound site, enhancing the healing process. The release of these agents can be controlled by the hydrogel's structure and composition, allowing for sustained therapeutic effects over time.

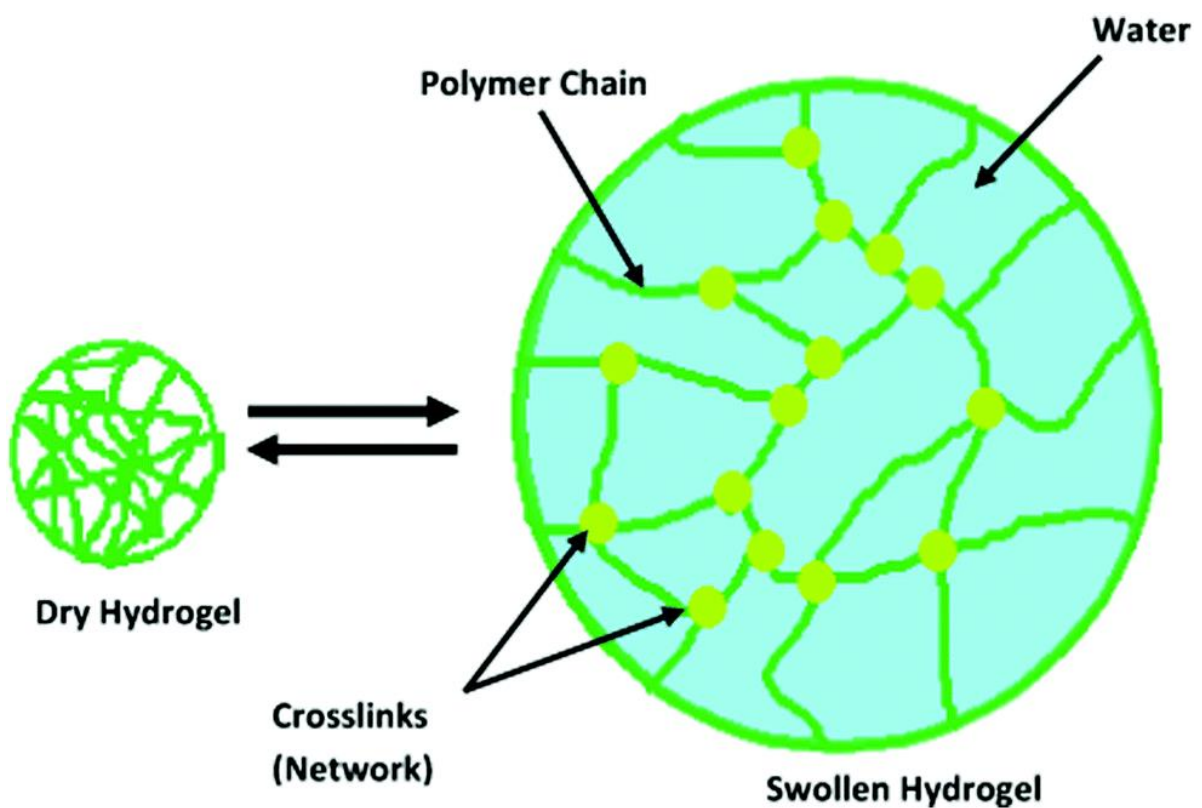


Figure 1.3 Transition (reversible) of a hydrogel from the dry to the swollen state in an aqueous medium and the crosslinked (network) structure of a hydrogel.

1.6.1 Features of Hydrogels

Hydrogels possess several key features that make them suitable for wound healing applications:

1. **High Water Content:** Hydrogels typically contain 90% or more water, which is essential for maintaining a moist wound environment. This high-water content supports cellular activities crucial for healing.
2. **Biocompatibility:** Many hydrogels are made from natural polymers, such as gelatin, alginate, or hyaluronic acid, which are biocompatible and non-toxic. This minimizes the risk of irritation or adverse reactions when applied to wounds.
3. **Soft and Flexible Texture:** The pliable nature of hydrogels allows them to conform to the irregular shapes of wounds, providing a comfortable fit that promotes patient compliance.
4. **Porous Structure:** Hydrogels often have a porous network that facilitates the exchange of gases and nutrients. This permeability is vital for cellular respiration and metabolic processes in the healing tissue.
5. **Customizable Properties:** Hydrogels can be tailored in terms of their mechanical strength, swelling capacity, and drug release profiles. This customization allows for the creation of specific formulations optimized for different wound types and stages of healing.
6. **Controlled Drug Delivery:** The incorporation of therapeutic agents into hydrogels allows for the sustained release of medications, enhancing their effectiveness while

minimizing systemic side effects. This feature is particularly beneficial for chronic wounds that require ongoing treatment.

7. **Antimicrobial Properties:** Some hydrogels can be designed to have intrinsic antimicrobial properties or be loaded with antimicrobial agents, providing protection against infections that are common in diabetic and chronic wounds.

1.6.2 Applications of Hydrogels

Hydrogels are polymeric networks that can retain significant amounts of water, making them highly valuable for a range of biomedical applications. Their unique characteristics—biocompatibility, structural similarity to human tissue, and the ability to be engineered for various functional roles—have led to their widespread adoption in wound healing, drug delivery, tissue engineering, diagnostics, and implantable devices depicted in figure 1.4.. Here is an in-depth look at these applications:

1. Wound Healing

One of the most impactful applications of hydrogels is in wound care, particularly for chronic wounds, burns, and surgical incisions. Hydrogels maintain a moist wound environment, which is critical for optimal healing. This moisture retention reduces dehydration, which can lead to cell death, and minimizes pain by soothing the wound site. Importantly, a moist environment also accelerates tissue regeneration, reducing the risk of scarring and supporting the migration and proliferation of skin cells.

For chronic wounds like diabetic ulcers, hydrogels offer additional benefits. Chronic wounds are typically characterized by inflammation and a tendency to become infected. Hydrogel

dressings can be designed to include bioactive molecules, such as growth factors, which can encourage cell growth and repair. Furthermore, antimicrobial agents can be incorporated to combat infection, which is particularly crucial in diabetic wounds where poor circulation hinders natural healing processes. Hydrogels are also highly absorbent, capable of managing wound exudate and reducing the need for frequent dressing changes, making them practical and effective for long-term wound care.

2. Drug Delivery Systems

Hydrogels are exceptional carriers for drug delivery due to their high porosity and ability to encapsulate therapeutic molecules. Their network structure allows for the controlled, sustained release of drugs, which is ideal for chronic diseases requiring consistent dosing over time. By tailoring the cross-linking density and polymer composition, scientists can precisely control the rate at which drugs are released, improving therapeutic efficiency and reducing the risk of side effects.

In the case of targeted delivery, hydrogels can be engineered to respond to specific stimuli, such as pH, temperature, or enzymatic activity. For example, some tumors create an acidic microenvironment; pH-sensitive hydrogels can be engineered to release anticancer drugs specifically in these areas, sparing healthy tissue from the toxic effects of chemotherapy. Similarly, thermosensitive hydrogels can respond to body temperature, allowing them to deliver drugs at a controlled rate once injected. In implantable drug delivery systems, hydrogels can be placed at specific sites to provide localized and sustained drug release, improving patient compliance by reducing the need for repeated doses or invasive procedures.

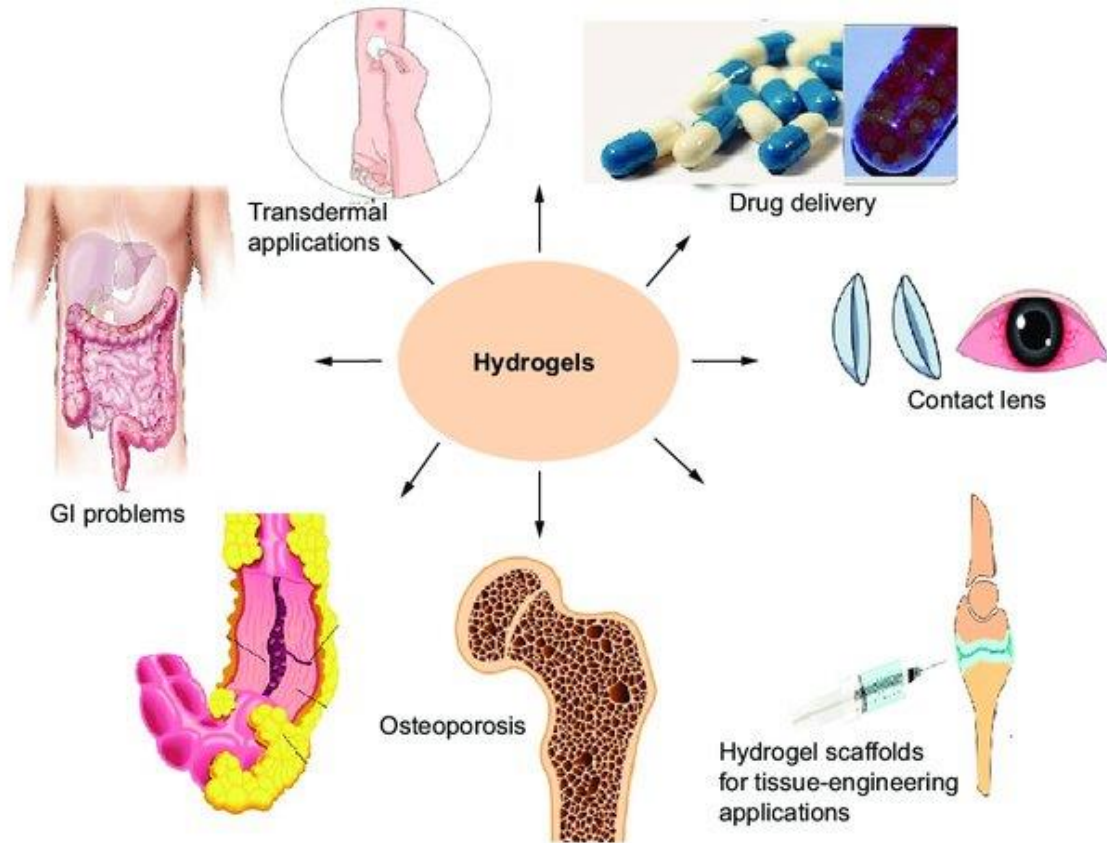


Figure 1.4 Biomedical applications of Hydrogels [19]

3. Tissue Engineering and Regenerative Medicine

Hydrogels are widely used as scaffolds in tissue engineering and regenerative medicine because they closely mimic the extracellular matrix (ECM), providing a supportive environment for cells to grow and form tissue. Hydrogels' high-water content and permeability to oxygen and nutrients make them ideal for cultivating cells and enabling tissue repair. The flexibility in modifying the structural properties of hydrogels—such as porosity, stiffness, and biodegradability—allows researchers to design specific environments for different types of tissue regeneration.

For bone and cartilage regeneration, hydrogels can be embedded with growth factors or even stem cells to support the formation of dense, load-bearing tissues. In these applications, the hydrogel scaffold provides a framework that cells can use to build up the new tissue. Similarly, hydrogels are used in cardiac and neural tissue engineering, where they act as a substrate for cells to attach and proliferate. The ability to culture cells within a hydrogel matrix provides a way to repair damaged heart muscle after a heart attack or to regenerate neurons in cases of brain or spinal cord injury.

Moreover, 3D bioprinting—a technique that uses hydrogels as “bioinks”—has revolutionized tissue engineering by enabling the precise layering of cells and biomaterials to construct complex tissue structures. These bioinks can be customized to create tissues with specific architectures, which is essential for building organs or functional tissue units. This approach holds promise for developing transplantable tissues and organs, potentially addressing shortages in donor organs for transplants.

4. Contact Lenses and Ophthalmic Applications

Hydrogels are a standard material for making soft contact lenses due to their high water content, which provides comfort, and their oxygen permeability, which is essential for corneal health. The moisture-retaining properties of hydrogels help prevent dry eyes, making them suitable for extended wear. This makes them ideal for contact lenses that can be worn for long periods without causing irritation or damage to the eye.

Advances in hydrogel technology have also enabled the development of drug-releasing contact lenses, which represent a novel method for treating eye conditions like glaucoma, infections, and allergies. In traditional eye drop treatment, most of the medication is washed

away by tears, resulting in limited drug absorption and potential side effects. In contrast, hydrogel-based contact lenses can release drugs slowly and steadily, enhancing drug bioavailability to the eye while minimizing systemic exposure. This approach offers a more efficient, patient-friendly alternative for delivering eye medication, particularly for chronic conditions requiring regular treatment.

5. Biosensing and Diagnostic Devices

Hydrogels are increasingly used in biosensing devices because of their sensitivity to changes in the environment. In glucose monitoring for diabetes, hydrogels sensitive to glucose concentrations can detect fluctuations in glucose levels and provide feedback, enabling real-time, continuous monitoring. These glucose-sensitive hydrogels can swell or change optical properties in response to glucose, making it possible to track levels noninvasively.

In point-of-care diagnostic tools, hydrogels can encapsulate reagents that detect specific biomarkers, making them suitable for quick, accurate disease detection. For instance, hydrogel-based diagnostic devices can detect biomarkers for infections or metabolic diseases by producing a visible color change or other detectable signal. This has proven especially useful for at-home or remote diagnostics, where stability and ease of use are paramount. Hydrogels provide a stable environment for sensitive reagents, improving the reliability and accuracy of diagnostic tests, which is essential for timely treatment decisions.

6. Injectable Hydrogels for Minimally Invasive Therapies

Injectable hydrogels have garnered interest in minimally invasive therapeutic approaches, as they can be administered in liquid form and then solidify within the body. This feature allows hydrogels to be used as fillers in irregular defects or cavities, making them valuable for

reconstructive surgeries or trauma repair. Injectable hydrogels are particularly useful in cell therapy, where they protect cells during injection and provide a nurturing matrix that supports cell survival and integration.

In cancer treatment, injectable hydrogels offer a way to deliver drugs directly to tumors while minimizing exposure to healthy tissue. Some hydrogels are designed to release anticancer drugs only in response to the tumour's specific microenvironment, such as an acidic pH or the presence of tumour-specific enzymes. This targeted release reduces side effects and increases treatment efficacy, providing a localized approach to chemotherapy.

7. Medical Implants and Prosthetics

Hydrogels also play a role in enhancing the performance and biocompatibility of medical implants and prosthetics. Their cushioning properties make them suitable as soft tissue fillers in prosthetics, providing comfort and reducing friction, which is crucial for long-term use. Hydrogels can also be coated on implants, such as pacemakers or orthopedic devices, to create a biocompatible interface between the implant and surrounding tissues. This coating reduces immune reactions and prevents biofouling, which can lead to infection and device failure. Some hydrogel-coated implants are designed to release therapeutic agents over time, aiding in infection prevention and enhancing integration with surrounding tissues. This drug-releasing feature is especially valuable for implants that need to remain in the body for extended periods, as it minimizes the need for additional procedures or systemic treatments, thereby improving patient outcomes and quality of life.

1.6.3 Protein-based hydrogels

Protein-based hydrogels are an exciting development in the field of biomaterials, particularly for their applications in wound healing, tissue engineering, and drug delivery as shown in table 1.2. Made from natural proteins such as collagen, gelatin, silk fibroin, elastin, keratin, and fibrin, these hydrogels form a three-dimensional network capable of absorbing large amounts of water. Their similarity to the body's extracellular matrix (ECM) supports cell adhesion, proliferation, and migration, which are essential processes in tissue repair and regeneration. Their biocompatibility and biodegradability make them highly suitable for medical applications, as they break down naturally without causing toxicity or adverse immune reactions, unlike many synthetic materials.

In the context of wound healing, protein-based hydrogels offer a range of benefits that make them ideal for treating complex, chronic wounds. They create a moist wound environment, which promotes cell migration and prevents the formation of scabs, leading to faster healing. Furthermore, these hydrogels can be loaded with bioactive agents, including antibiotics, growth factors, and anti-inflammatory compounds, allowing for a controlled and sustained release directly at the wound site. This characteristic is especially valuable in managing infections and stimulating tissue repair in chronic wounds. Some proteins, such as silk fibroin and keratin, even have mild inherent antibacterial properties, which further reduces the risk of infection.

Table 1.2 Materials, synthesis methods, and properties of protein hydrogels [19]

Protein	Additional materials	Synthesis method	property	Potential applications
BSA	Phosphate/Glutathione	Chemical cross-linking	Self-healing	Biomedical, Tissue Engineering
BSA	Ca ²⁺	Physical	Self-healing	Biomedical
GE and GK	—	Chemical	Self-healing, injectable	Tissue engineering, drug delivery
Human serum albumin	DNA building blocks	Chemical	Self-healing	Tissue engineering
LOV2, zdk1	—	Chemical	Light-responsive	Cell proliferation, drug/cell delivery, and tissue engineering
Silk-Elastin-Like	AdoB ₁₂	Chemical	Light-responsive	Shape-change materials, drug and cell delivery and tissue regeneration
SPI	Corn fiber gel, GDL	Chemical, enzymic	pH-responsive	Drug delivery
casein	Transglutaminase	Chemical	Swelling	Food industry
whey and egg white	—	Chemical	Swelling	Food industry
gelatin	Biotin,avidin	Chemical	Swelling	Biomaterial functionality and protein release
SPI	GA	Chemical	Swelling	Food industry
Silk fibroin, collagen	—	Chemical	Mechanical	cell delivery and tissue engineering
SF-gelatin	Kosmotropic	Chemical	Mechanical	tissue engineering
Prolamin	Acetic acid, AG	Chemical, Physical	Mechanical	Biopolymer, biocompatible materials
BSA	—	Physical	Biological	Drug delivery
SF	PLA			

The ECM-mimicking structure of these hydrogels supports cellular processes crucial for wound healing by providing scaffolding that encourages cells to attach, migrate, and proliferate. For instance, collagen and gelatin-based hydrogels are well-regarded for supporting the attachment and growth of cells necessary for tissue regeneration. Silk fibroin hydrogels, on the other hand, offer the added benefit of mechanical strength, making them suitable for applications where longer-lasting support is required. However, protein-based hydrogels are not without limitations. Their mechanical strength is often lower than that of synthetic hydrogels, limiting their use in load-bearing applications. Additionally, their rapid biodegradability in some cases may require structural modifications to ensure sustained support for chronic wounds.

1.6.4 Amyloid Fibrils: Therapeutic Opportunity?

While protein-based hydrogels offer a promising approach in biomedical applications due to their biocompatibility, tunable properties, and potential to mimic natural extracellular matrices, they face certain limitations. Proteins like collagen, gelatin, and silk can be sensitive to environmental changes, such as pH and temperature, which may compromise the hydrogel's stability and mechanical strength. Additionally, some protein hydrogels lack the robustness needed for long-term applications, especially under physiological stress, and may degrade too quickly or fail to maintain their structural integrity, limiting their use in sustained or load-bearing applications.

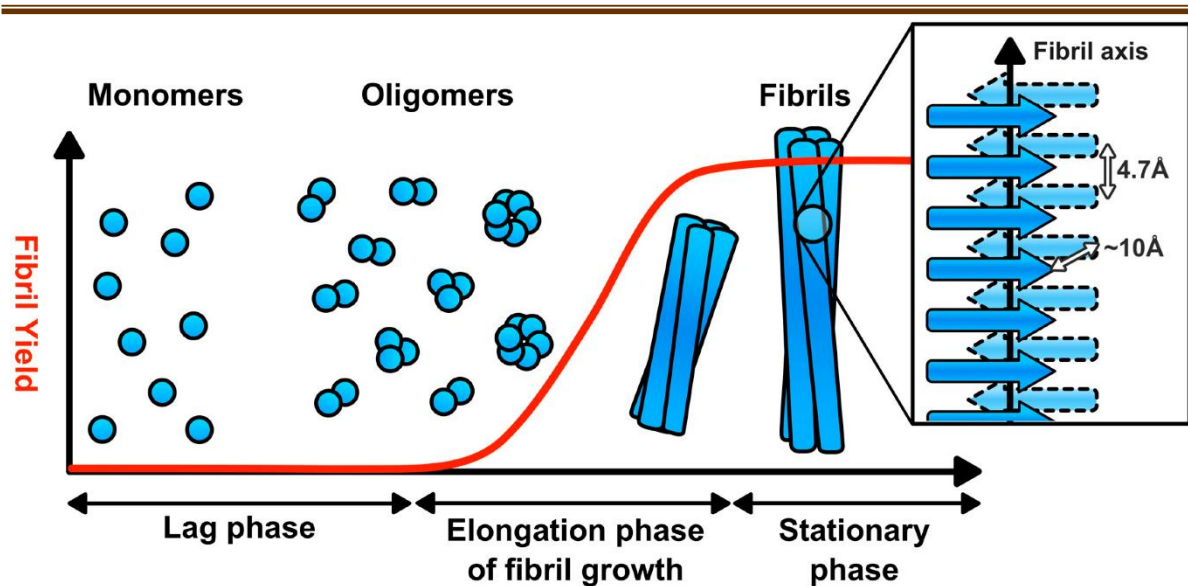


Figure 1.5 Formation of Amyloid fibrils over the time [20]

To address these challenges, researchers are turning to amyloid fibrils as a compelling alternative for enhancing protein hydrogel properties. Amyloid fibril formation begins with the conversion of soluble proteins into misfolded monomers, which expose hydrophobic regions normally buried in the native structure as shown in figure 1.5. These misfolded monomers aggregate to form small, unstable oligomers, which act as seeds for fibril formation. The process typically proceeds through a lag phase, where no visible fibril formation occurs as the misfolded proteins undergo structural rearrangements. Once the oligomers reach a critical concentration, they nucleate and promote the elongation phase, where additional monomers align into β -sheet structures, forming protofibrils. As more monomers are added, these protofibrils grow into mature, highly ordered amyloid fibrils with a characteristic cross- β structure. Amyloid fibril-based hydrogels leverage the robustness and rigidity of the fibrils to form networks that are stable under physiological conditions. These hydrogels can self-assemble through non-covalent interactions, offering control over

mechanical properties and the ability to tune them for specific applications. The nano-architecture of amyloid fibrils provides a scaffold conducive to cell adhesion and proliferation, making them suitable for biomedical applications, particularly in wound healing and tissue engineering. Their biocompatibility, combined with the ease of incorporating bioactive molecules, positions amyloid fibril-based hydrogels as promising candidates for creating advanced, multifunctional wound dressings and other regenerative materials. Additionally, their ability to form stable, responsive hydrogels makes amyloid fibril-based hydrogels highly suitable for controlled drug release and targeted therapeutic applications.

1.7 Scope of the thesis

The scope of this thesis centers on developing innovative BSA (bovine serum albumin)-based hydrogels tailored specifically for diabetic wound healing applications. Addressing the limitations of conventional wound care methods, this work focuses on enhancing healing efficiency through a series of progressive, novel designs. Each study within this thesis builds upon prior research, with a primary aim to create hydrogels that not only expedite wound repair but also reveal the underlying mechanisms through pathway studies.

In the first phase, a 3D-printable BSA-Aloe vera hydrogel was formulated, providing a foundational bioactive dressing with promising healing properties. Recognizing the need for even more effective healing agents, the second phase refined the hydrogel by isolating wound-healing constituents of Aloe vera, particularly hyaluronic acid. This refined 3D-printed hydrogel enabled customized dressing designs, demonstrating a significant enhancement in diabetic wound healing compared to the first hydrogel. Additionally, molecular pathway

analysis was conducted to better understand the biological mechanisms involved in this hydrogel's therapeutic action.

The third and final phase introduced conductive and photothermal capabilities to the BSA-based hydrogel, integrating real-time monitoring through advanced imaging techniques. This multifunctional hydrogel not only offers enhanced diabetic wound healing through conductivity and controlled heat application but also facilitates a comprehensive molecular biology study of the healing process. By incorporating such dynamic elements, this thesis establishes a foundational understanding of hydrogel interactions with diabetic wound environments, paving the way for future advancements in wound care.

In summary, this thesis presents a progressive development of BSA-based hydrogels specifically designed for diabetic wound healing, each phase addressing and overcoming challenges identified in previous studies. From a 3D-printed hydrogel base to an advanced conductive, photothermal variant with real-time monitoring, these hydrogels exhibit enhanced healing properties and reveal critical insights into their underlying mechanisms, establishing a pathway for future therapeutic applications.