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In-situ forming nanogels based on chitosan for drug delivery and wound healing

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Abstract:

In-situ forming nanogels based on chitosan have emerged as a promising platform for drug delivery and wound healing applications due to their biocompatibility, biodegradability, and versatility. This study explores the design and development of chitosan-based nanogels that form in response to physiological conditions, offering controlled release of therapeutic agents. Chitosan, a natural polymer derived from chitin, is ideal for creating nanogels due to its positive charge, which enhances its interaction with negatively charged cell membranes, and its ability to gel in aqueous environments. The nanogels are capable of encapsulating a variety of drugs, including antibiotics, anti-inflammatory agents, and growth factors, thereby promoting localized and sustained therapeutic effects. The ability to form in-situ allows for easier application in wound healing, where they conform to irregular wound surfaces and provide a protective barrier, preventing infection and promoting tissue regeneration. In addition, the nanogels facilitate the controlled release of bioactive molecules, enhancing the healing process and reducing the need for frequent dressing changes. The physical properties, drug release kinetics, and biological performance of chitosan-based nanogels were thoroughly evaluated to ensure their potential as a robust drug delivery system and an effective wound healing agent. The results indicate that these nanogels offer an innovative, non-invasive approach to treating chronic wounds, improving therapeutic efficacy while minimizing side effects. In-situ forming chitosan-based nanogels have garnered significant attention as innovative drug delivery systems for enhancing wound healing, owing to their unique properties such as biocompatibility, biodegradability, and the ability to respond to physiological stimuli. Chitosan, a natural polysaccharide derived from chitin, exhibits excellent mucoadhesive properties, making it an ideal candidate for the development of nanogels that can be applied directly to wound sites. These nanogels undergo self-assembly and gelation in response to environmental factors like pH, temperature, or ionic strength, which enables them to conform to the wound surface upon application, ensuring intimate contact with damaged tissues. This in-situ gelation mechanism not only ensures ease of application but also provides localized drug release over an extended period.

Keywords: Chitosan, In-situ forming nanogels, Drug delivery, Wound healing, Biocompatibility, Biodegradability, Controlled release, Drug encapsulation, etc.

Introduction:

Wound healing remains a significant challenge in modern medicine, particularly for chronic wounds, such as diabetic ulcers, burns, and pressure sores, which require efficient therapeutic strategies to accelerate the healing process and reduce complications. Conventional wound care methods often fail to provide optimal healing outcomes, highlighting the need for advanced drug delivery systems that can target the wound site more effectively. Nanotechnology, particularly the development of nanogels, has emerged as a promising approach to address these limitations. Nanogels are crosslinked polymer networks that possess the ability to encapsulate therapeutic agents and release them in a controlled manner, offering advantages such as high drug loading capacity, enhanced stability, and localized delivery.[12] Among various materials used for nanogel fabrication, chitosan, a natural biopolymer derived from chitin, stands out due to its unique properties, including biocompatibility, biodegradability, non-toxicity, and the ability to form gels under physiological conditions. Chitosan's inherent mucoadhesive properties also facilitate prolonged retention at the application site, making it an ideal

candidate for wound healing applications. The use of chitosan-based nanogels offers numerous advantages, such as promoting controlled release of bioactive molecules, reducing the risk of infection, minimizing inflammation, and accelerating tissue regeneration. In-situ forming nanogels, which undergo gelation in response to environmental stimuli such as pH, temperature, or ionic strength, have further enhanced the versatility of chitosan-based systems. This ability to form a gel directly at the wound site ensures better conformity to irregular wound surfaces, improving the therapeutic outcomes while reducing the frequency of dressing changes. Additionally, these nanogels can be engineered to release a range of therapeutic agents, including antibiotics, anti-inflammatory drugs, growth factors, and antioxidants, in a sustained and localized manner, thus enhancing the efficacy of wound care and promoting faster healing. This introduction aims to highlight the potential of chitosan-based in-situ forming nanogels as a cutting-edge solution for drug delivery and wound healing, exploring their unique properties, formulation strategies, and therapeutic applications. By combining the advantages of nanotechnology and natural biopolymers, these nanogels hold promise for revolutionizing wound care and offering effective solutions for chronic wound management.[23]

In-situ:

"In-situ" is a Latin term that means "in its original place" or "in position." In scientific and medical contexts, it refers to processes, reactions, or phenomena occurring in the natural or original location without the need for relocation or external interventions. In the context of in-situ forming nanogels, the term refers to the ability of the nanogel to form or gel directly at the site of application—such as a wound—without needing pre-formation or mixing before application. These nanogels typically undergo a transformation (e.g., gelling) in response to specific environmental conditions, such as changes in temperature, pH, or ionic strength, allowing them to conform to the surface of the wound and provide controlled, localized drug delivery.

For example, in wound healing, in-situ forming nanogels are advantageous because they can be applied as a liquid or semi-liquid and then gel once in contact with the wound surface, allowing the nanogel to fit the wound's irregular shape and provide a sustained release of therapeutic agents. This on-site formation offers ease of application, better adaptability to the wound site, and a more controlled release of the drug, enhancing therapeutic efficacy.[34]

Application:

1. Wound Healing: In-situ forming chitosan-based nanogels are particularly effective for promoting wound healing, especially for chronic wounds such as diabetic ulcers, burns, and pressure sores. Upon application, these nanogels gel at the wound site, conforming to its irregular surface and forming a protective barrier. They can encapsulate therapeutic agents such as antibiotics, anti-inflammatory drugs, growth factors, and antioxidants, releasing them in a controlled manner to accelerate tissue regeneration, prevent infection, and reduce inflammation. The nanogels also maintain a moist environment, which is critical for optimal wound healing.[45]

2. Drug Delivery Systems: Chitosan-based nanogels provide a versatile platform for drug delivery across various therapeutic fields. Their ability to encapsulate a wide range of hydrophobic and hydrophilic drugs, coupled with their in-situ gelation properties, enables sustained and localized release of drugs at the target site. This feature is particularly beneficial for the treatment of diseases that require long-term or controlled release of therapeutic agents, such as cancer, cardiovascular diseases, and neurological disorders.[56]

3. Cancer Treatment: In cancer therapy, chitosan-based nanogels can be used to deliver chemotherapy agents directly to the tumor site, improving drug bioavailability while minimizing systemic toxicity. The in-situ gelation ability helps the nanogels to maintain prolonged residence time at the tumor site, ensuring continuous release of the therapeutic agent. This targeted drug delivery also enhances the therapeutic efficacy of the treatment, allowing for higher drug concentrations at the tumor while reducing side effects to healthy tissues.[11

4. Ocular Drug Delivery: In-situ forming nanogels are increasingly explored for ophthalmic drug delivery, where they can be applied to the eye as a liquid solution and form a gel once in contact with the ocular surface. This gelation improves the residence time of drugs on the eye, allowing for sustained release and better therapeutic outcomes in the treatment of ocular diseases such as glaucoma, dry eye syndrome, and retinal disorders.[24]

5. Gene Delivery: Chitosan-based nanogels also show promise in gene therapy by encapsulating genetic material, such as DNA or RNA, and facilitating its delivery to target cells. Their mucoadhesive properties and the ability to form gels in-situ enhance the stability and bioavailability of genetic agents, making them ideal candidates for gene delivery in tissues that require localized treatment, such as the skin, lungs, or mucosal membranes.[43]

6. Tissue Engineering: The use of chitosan-based nanogels in tissue engineering allows for the localized release of growth factors, cytokines, or other bioactive molecules to promote tissue regeneration. These nanogels can support the growth of new tissue by providing a scaffold that encourages cellular attachment, migration, and proliferation, making them useful in the regeneration of skin, cartilage, bone, and other tissues.

7. Bone Regeneration: In bone tissue engineering, in-situ forming chitosan-based nanogels can deliver growth factors or bioactive molecules that stimulate osteoblast differentiation and bone formation. Their ability to form a gel at the injury site ensures that the active agents are precisely delivered to the bone regeneration area, aiding in the repair of bone fractures or defects.[10]

8. Oral Drug Delivery: Chitosan-based nanogels have potential for use in oral drug delivery systems, where they can protect drugs from the acidic environment of the stomach, allowing for controlled release in the intestines. This system can be applied to enhance the bioavailability of poorly soluble drugs and provide sustained therapeutic effects, especially in the treatment of chronic conditions like diabetes, arthritis, and gastrointestinal diseases.

9. Skin Delivery: The skin is another target for chitosan-based nanogel applications, where the nanogels can be used to deliver both hydrophilic and lipophilic drugs to the epidermis and dermis. This is particularly valuable in treating skin infections, inflammation, or for cosmetic purposes like anti-aging formulations, as the gelation process enhances the adhesion and release of active ingredients on the skin surface.[11]

Approaches for Developing In-Situ Forming Chitosan-Based Nanogels (Short Summary):

1. pH-Responsive Nanogels: Chitosan forms gels in response to pH changes, useful for localized drug delivery in acidic or basic environments, such as wounds or the gastrointestinal tract.

2. Temperature-Responsive Nanogels: Chitosan derivatives gel at specific temperatures, like body temperature, making them ideal for injectable formulations.

3. Ionic Strength-Dependent Nanogels: Gelation occurs due to ionic changes, enabling controlled release in environments like mucosal surfaces.[34]

4. Crosslinking: Crosslinking chitosan with other polymers or agents enhances stability, drug loading, and controlled release, useful in chronic wound healing and tissue repair.

5. Encapsulation of Bioactive Compounds: In-situ nanogels can encapsulate drugs, growth factors, or genes for sustained release at the target site.

6. Surface Modification for Targeting: Nanogels can be surface-modified with targeting ligands to improve drug localization at specific tissues or cells.

7. Multifunctional Nanogels: Combining multiple therapeutic agents (e.g., antibiotics and growth factors) in one nanogel for enhanced treatment effects.[20]

8. Hybrid Nanogels: Combining chitosan with other materials (e.g., lipids or PLGA) for better stability, drug encapsulation, and release profiles.

9. Dual-Responsive Nanogels: Responsive to both pH and ionic strength, offering precise control over drug release in complex environments, like tumors or inflamed tissues.

These approaches enable chitosan-based nanogels to be tailored for efficient, site-specific drug delivery and therapeutic outcomes.[54]

Nano-gels:

Nano-gel in situ refers to a type of nano-gel material that is created or applied directly at the site of interest (in situ) rather than being prepared and then transported or used elsewhere. In-situ nano-gel systems are used in various scientific and industrial fields, including biomedical applications, material science, and environmental engineering.[13]

FIG.Nanogel

Key Aspects of Nano-gels:

1. Nano-gels: These are nanoscale, cross-linked polymeric materials that can absorb large amounts of water or other solvents. They have a gel-like structure but are small enough (typically in the range of 1-1000 nanometers) to interact with cells, tissues, or molecules at a microscopic scale. Their properties, such as swelling behavior, drug release capacity, and biocompatibility, make them versatile for many applications.

2. In situ formation or application: In this context, "in situ" refers to the preparation or application of nano-gels at the specific site or location where they are needed. This could involve:[22]

o In situ synthesis: The nano-gel is formed directly within a system or environment, often via chemical reactions or physical changes, at the site of interest. This method eliminates the need for the nano-gel to be synthesized and stored beforehand.

o In situ delivery: The nano-gel is applied to the site of action, for example, for drug delivery in the human body or for environmental remediation. In this case, the nano-gel may form or swell in response to environmental triggers (like pH or temperature) at the site of delivery.[56]

Applications of In-situ Nano-gels:

1. Biomedical Applications: In drug delivery, in situ nano-gels are highly beneficial because they can be injected or applied as a liquid solution and then form a gel at the desired site (for example, inside the body). This can improve localized drug release, increase bioavailability, and reduce side effects.

2. Tissue Engineering: Nano-gels can be used for in situ scaffolding to support cell growth or repair tissues. The gel can provide a supportive matrix that promotes cell adhesion and growth while offering controlled release of growth factors.

3. Environmental Remediation: In situ nano-gels can be applied in contaminated environments (e.g., water or soil) where they can absorb pollutants or toxins, then degrade or release agents that neutralize contaminants.[7]

Approaches for in situ nano-gels:

1. Thermal-responsive: Nano-gels change from liquid to gel when exposed to a specific temperature (e.g., PNIPAM polymers). Used in drug delivery and tissue engineering.

2. pH-responsive: Nano-gels alter their structure in response to pH changes. Applied in cancer therapy and wound healing.

3. Ionic-strength-responsive: Changes in salt concentration trigger the nano-gel's behavior. Used in drug release and environmental applications like water purification.

4. Light-responsive: Nano-gels change structure when exposed to light. Utilized in controlled drug release and phototherapy.[8]

Types:

1. Polymeric Nano-gels: Made from synthetic or natural polymers (e.g., PEG, chitosan) for drug delivery and tissue engineering.

2. Thermo-responsive: Change structure with temperature (e.g., PNIPAM). Used in drug delivery and tissue scaffolds.

3. pH-responsive: Alter structure with pH changes. Used in cancer therapy and targeted drug delivery.

4. Ionic-strength-responsive: React to changes in salt concentration. Used in drug release and water purification.

5. Light-responsive: Change properties under light exposure. Applied in controlled drug release and phototherapy.[1]

6. Enzyme-responsive: Respond to specific enzymes. Used for targeted drug delivery.

7. Magnetic-responsive: React to magnetic fields (e.g., with iron oxide). Used in targeted drug delivery and imaging.

Chitosan:

Chitosan is a natural biopolymer derived from chitin, which is found in the shells of crustaceans like shrimp and crabs. It is widely used in various fields, especially in biomedicine, pharmaceuticals, food industry, and environmental applications due to its biodegradable, non-toxic, and biocompatible properties.[19]

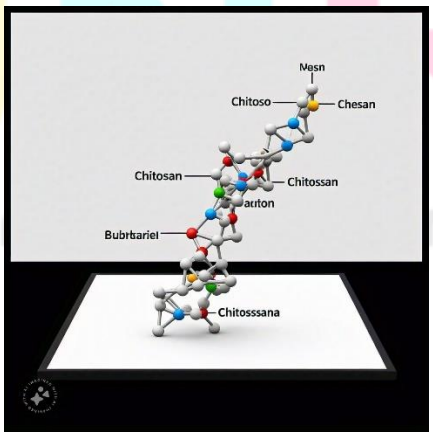

FIG.Structure of Chitosan

Applications of Chitosan:

1. Drug Delivery: Used in nano-gels and microparticles for controlled drug release, particularly in the oral delivery of drugs and vaccines.

2. Wound Healing: Chitosan is used in dressings due to its ability to promote cell growth and tissue regeneration.

3. Food Industry: Acts as a preservative, food packaging material, and fat replacer.

4. Water Treatment: Used for adsorption of heavy metals and other pollutants due to its ability to bind with toxins.

5. Cosmetics: Included in products for its moisturizing and anti-aging properties.[21]

Origin:

Chitosan originates from chitin, a natural biopolymer found in the exoskeletons of arthropods (e.g., shrimp, crabs, and lobsters) and the cell walls of certain fungi. Chitin is the second most abundant natural polymer on Earth, after cellulose.

Process of Obtaining Chitosan:

1. Extraction of Chitin: Chitin is extracted from the shells of crustaceans (shrimp, crabs) or fungi by removing proteins, minerals, and pigments.

2. Deacetylation to Chitosan: Chitosan is derived from chitin through a chemical process called deacetylation. This involves the removal of acetyl groups (-COCH3) from the chitin molecules, typically by treating it with an alkaline solution (like sodium hydroxide).[32]

The resulting chitosan has amino groups (-NH2) that make it more reactive and functional compared to chitin, allowing it to be modified and used in various applications.

Wound:

A **wound** is damage to the skin or underlying tissues. The main types include:

1. **Acute Wounds**: Heal quickly, e.g., cuts, abrasions, and surgical incisions.

2. **Chronic Wounds**: Do not heal as expected, often due to conditions like diabetes or poor circulation, e.g., pressure ulcers and diabetic ulcers.

3. **Closed Wounds**: No break in the skin, but underlying tissue is damaged, e.g., bruises or hematomas.

4. **Open Wounds**: Skin is broken, exposing underlying tissues, e.g., abrasions, lacerations, punctures, and avulsions.[43]

5. **Burns**: Caused by heat, chemicals, or electricity, classified by severity (1st, 2nd, or 3rd degree).

Each type requires different care and treatment based on its nature and severity.

Mechanism to generate in-situ nano-gels:

In situ nano-gels are generated using external stimuli to trigger their formation at the desired site. Key mechanisms include:

1. **Temperature-triggered**: Gelation occurs at specific temperatures (e.g., body temperature).

2. **pH-triggered**: Gelation happens in response to changes in pH, useful for targeting acidic areas like tumors.

3. **Ionic strength-triggered**: Changes in salt concentration lead to gel formation.

4. **Light-triggered**: Light exposure causes gelation via photo-sensitive polymers.

5. **Enzyme-triggered**: Enzymes cleave specific linkages, inducing gel formation.

6. **Self-assembly**: Molecules spontaneously form a gel structure under certain conditions.

These mechanisms enable precise, site-specific gel formation for drug delivery, tissue engineering, and other applications.[27]

In-situ gelling systems based on chitosan include:

1. **pH-responsive gels**: Gel formation occurs in acidic environments, used for drug delivery in mucosal or ocular applications.

2. **Thermosensitive gels**: Form gels at body temperature for injectable drug delivery systems.

3. **Ionic gelation**: Chitosan forms gels in the presence of ions like tripolyphosphate, used in wound healing and drug delivery.

4. **Polysaccharide-based gels**: Chitosan combined with other polysaccharides (e.g., alginate) for controlled drug release and tissue regeneration.

5. **Enzyme-responsive gels**: Chitosan gels degrade in response to specific enzymes, useful for targeted drug delivery.

6. **Chitosan nanoparticle-based gels**: Nanoparticles aggregate to form gels, applied in controlled release and vaccination.[37]

These systems offer versatile, localized gelation for applications in **drug delivery**, **wound care**, and **tissue engineering**.

In-situ covalent crosslinking systems

In-situ covalent crosslinking systems are gel systems that form by **covalently bonding polymer chains** at the site of interest, usually triggered by external factors (e.g., light, temperature, pH, or chemical agents). This type of gelation involves **permanent** crosslinks, which stabilize the gel structure.

Key Features:

1. **Covalent Bond Formation**: Crosslinking occurs through the formation of covalent bonds between polymer chains, making the gel structure permanent.
2. **External Stimuli**: Crosslinking can be triggered by external stimuli like UV light, heat, or specific chemical agents.[64]
3. **Applications**:
   - Drug delivery (controlled release)
   - Tissue engineering (scaffolds)
   - Wound healing (creating stable gel barriers)

In-situ phase separation systems

In-situ phase separation systems are gelation systems that form through the **separation of phases** (e.g., liquid-liquid or solid-liquid) when an external stimulus (like temperature, pH, or solvent composition) is applied, leading to the formation of a gel or a gel-like structure.

Key Features:

1. **Phase Separation**: The system involves the **separation of components** (e.g., solvent and polymer) to create two distinct phases—one being a gel phase and the other a liquid phase.
2. **External Stimuli**: The phase separation can be triggered by factors such as:
   - **Temperature** (thermoresponsive systems)
   - **pH** (pH-responsive systems)
   - **Ionic strength** (electrolyte concentration)
3. **Gel Formation**: Once phase separation occurs, one phase forms a gel, while the other remains in liquid form. The gel typically forms in situ when the stimulus is applied to the system, such as in vivo.[47]

Thermal-sensitive gelation system

A **thermal-sensitive gelation system** forms a gel in response to temperature changes. The polymer is in a liquid form at lower temperatures and transitions to a gel when exposed to higher temperatures (like body temperature, 37°C).

Key Features:

- **Thermo-responsive polymers** (e.g., PNIPAM) change from sol to gel as temperature increases.
- **Injectable** in liquid form and **gelates in situ** at the target site.

Applications:

- **Injectable drug delivery** for controlled release.
- **Wound healing** and **tissue engineering**.

These systems allow for non-invasive, controlled, and localized gel formation.

pH-sensitive gelation system

A **pH-sensitive gelation system** forms a gel in response to changes in the pH of the surrounding environment. These systems typically use **polymers** that undergo a conformational change (e.g., ionization or protonation) at specific pH levels, leading to gelation.[23]

Key Features:

1. **Polymers**: pH-sensitive polymers (e.g., **polyacrylic acid**, **chitosan**, **alginate**) contain functional groups (like carboxyl or amino groups) that ionize at specific pH values.
2. **Gelation Mechanism**: At certain pH values, these polymers either become insoluble or undergo crosslinking, forming a gel.

3. **Reversible Gelation**: The gel can dissolve or revert to a sol state when the pH changes.

Applications:

- **Targeted drug delivery**: Used to release drugs at specific sites (e.g., acidic tumors or the stomach).
- **Wound healing**: Form gels at injury sites where pH may be altered.
- **Tissue engineering**: Provide scaffolds that respond to pH changes in biological environments.

Example:

- **Chitosan-based systems**: Gelate in acidic environments (like the stomach) for controlled drug release.

In short, pH-sensitive gelation systems offer **site-specific drug delivery**, **wound healing**, and **biomedical applications** by forming gels in response to pH changes.[25]

In-situ gelation system through electrostatic interactions

An **in-situ gelation system through electrostatic interactions** forms a gel when **electrostatic forces** (attraction between oppositely charged molecules or ions) cause polymer chains to crosslink or self-assemble. This type of gelation does not require external triggers like temperature or pH but relies on the natural electrostatic properties of the materials involved.

Key Features:

1. **Electrostatic Interactions**: Oppositely charged groups on polymers or molecules (e.g., **cationic** and **anionic** polymers) attract each other, leading to the formation of a gel structure.

2. **Ion-Mediated Crosslinking**: Electrostatic forces can lead to physical gelation or reversible crosslinking between polymer chains in the system.[15]

3. **Reversible**: Electrostatic gelation can be reversible if the ionic strength or pH of the environment changes.

Applications:

- **Drug delivery**: Used for controlled release, where the gelation occurs at the site of administration (e.g., mucosal tissues).
- **Wound healing**: Forms gels at the wound site to protect and promote healing.
- **Tissue engineering**: Provides scaffolds for cell growth that respond to electrostatic interactions.

Example:

- **Chitosan-alginate systems**: Chitosan (cationic) and alginate (anionic) form gels when they come into contact, useful in drug delivery and wound care.

In-situ gelation through electrostatic interactions involves the formation of a gel at the target site due to the electrostatic attraction between oppositely charged polymer chains or molecules. This type of gelation relies on ionic interactions rather than external stimuli like temperature or pH, making it a more straightforward and controlled method for localized gel formation.[17]

Mechanisms of Electrostatic In-situ Gelation:

1. **Polyelectrolyte Complexation**:

   o The electrostatic interaction between **positively charged (cationic)** and **negatively charged (anionic)** polymers leads to gel formation. These interactions result in **polymer crosslinking**, creating a gel structure.

   o For example, **chitosan (cationic)** and **alginate (anionic)** can form a gel due to their opposite charges, leading to the formation of a stable network.

2. **Ion-Mediated Crosslinking**:

   o **Multivalent ions** (like **calcium** or **magnesium**) can act as bridges between oppositely charged polymer chains, inducing gelation.

   o This is common in systems like **alginate-based gels**, where calcium ions crosslink the polymer chains to form a stable gel structure.

3. **Charge-Driven Self-Assembly**:

   o In some cases, the electrostatic interactions lead to **self-assembly** of molecules or nanoparticles into a gel-like structure, often without the need for additional crosslinking agents.[19]

Advantages:

- **Non-invasive and simple**: No external triggers like heat or pH changes are needed, making it an easy-to-administer system.

- **Biocompatible and biodegradable**: Polymers like **chitosan**, **alginate**, and **dextran** are commonly used and are biocompatible, reducing the risk of adverse effects.

- **Reversible**: Gelation can be reversed or modified based on changes in the ionic environment, providing flexibility in drug delivery and other applications.

- **Controlled release**: Electrostatic gelation allows for **sustained release** of drugs at the site of application, making it ideal for localized treatment.

Applications:

1. **Drug Delivery**:

   - **Local drug delivery**: Electrostatic gelation systems can be used for the controlled release of drugs at a specific site (e.g., mucosal, ocular, or subcutaneous delivery).

   - **Targeted drug release**: They can be designed to release drugs in response to the **ionic environment** of the target area, enhancing the specificity and efficacy of the drug.

2. **Wound Healing**:

   - Electrostatic gels can form directly at the wound site, providing a protective barrier and enabling sustained release of antimicrobial agents or growth factors that promote healing.[25]

3. **Tissue Engineering**:

   - Electrostatic gels provide **biodegradable scaffolds** for cell growth and tissue regeneration. The ionic interactions can be fine-tuned to control scaffold formation and degradation.[13]

4. **Ocular Drug Delivery**:

   - In ocular drug delivery, gels that form in the eye at the site of application can enhance the residence time of the drug and ensure its sustained release to treat eye diseases.

Example Systems:

- **Chitosan-Alginate System**: Chitosan (positively charged) and alginate (negatively charged) are widely used to form **polyelectrolyte complexes** for drug delivery, especially in gastrointestinal and mucosal delivery.

- **Calcium-Alginate Gel**: **Calcium ions** crosslink **alginate** polymers to form gels, which are commonly used in controlled drug release applications and tissue scaffolding.

Limitations:

- **Stability**: The ionic strength of the environment can influence the stability of the gel, so systems may be sensitive to changes in the surrounding environment.

- **Ionic interference**: High concentrations of competing ions could disrupt electrostatic interactions and affect gel formation.[24]

In summary, **electrostatic in-situ gelation systems** offer **biocompatible, flexible**, and **controlled drug release** solutions with broad applications in **drug delivery**, **wound healing**, and **tissue engineering**, making them highly useful for targeted, localized treatments.

In-situ forming chitosan hydrogels

In-situ forming chitosan hydrogels are hydrophilic materials that can transition from a liquid to a gel state upon exposure to certain environmental stimuli (like temperature, pH, or ionic strength). These hydrogels are commonly used in a variety of biomedical and pharmaceutical applications due to chitosan's biocompatibility, biodegradability, and ability to form stable gels.

Key Applications of In-Situ Forming Chitosan Hydrogels:

1. **Drug Delivery Systems**:[29]

   - **Localized drug delivery**: Chitosan hydrogels can be injected in a liquid form and gel at the target site (e.g., in the gastrointestinal tract, eyes, or wounds), allowing for controlled and sustained release of drugs.

   - **Targeted delivery**: The pH or temperature sensitivity of the hydrogel allows drugs to be released at specific locations (e.g., acidic tumors, gastrointestinal sites).

   - **Pain relief**: Chitosan-based hydrogels can be used to deliver analgesic drugs directly to the site of pain, providing targeted relief.

2. **Wound Healing**:

o **Wound dressings**: Chitosan hydrogels can be applied to wounds where they form a gel upon contact with body fluids. These hydrogels promote healing by creating a moist environment and can also release antimicrobial agents to prevent infection.

o **Burn treatment**: Chitosan-based hydrogels can form a protective layer over burns, promoting skin regeneration and reducing pain and infection.

3. **Tissue Engineering**:

o **Scaffolds for cell growth**: Chitosan hydrogels can be used as scaffolds for tissue regeneration, providing a structure for cell attachment and growth. Their biodegradability ensures they degrade as the tissue regenerates, reducing the need for surgical removal.[41]

o **Bone and cartilage regeneration**: Modified chitosan hydrogels can be used as scaffolds for bone and cartilage tissue engineering, allowing for sustained release of growth factors to stimulate cell proliferation and differentiation.

4. **Ocular Drug Delivery**:

o **Eye drops and gels**: Chitosan hydrogels are used for ocular drug delivery, where they form a gel in the eye and extend the residence time of drugs, increasing their therapeutic efficacy. This is particularly useful for sustained release of drugs in the treatment of ocular diseases.[51]

5. **Gene Delivery**:

o **Gene therapy**: Chitosan hydrogels can be used as carriers for the delivery of genes or plasmids. The hydrogel can encapsulate the genetic material, protecting it from degradation and facilitating controlled release at the site of action.

6. **Vaccination**:

o **Vaccine delivery**: Chitosan hydrogels are explored for the delivery of vaccines, especially for mucosal or intradermal administration. They help improve the stability and release of antigens, stimulating a strong immune response.

7. **Dental Applications**:

o **Periodontal drug delivery**: Chitosan-based hydrogels can be used for the controlled release of drugs for periodontal therapy, improving the treatment of gum diseases.

8. **Cosmetic and Dermatological Applications**:

o **Skin moisturizers**: Chitosan hydrogels can be used in cosmetics for moisturizing and enhancing the skin's hydration, forming a gel-like layer that retains moisture.

o **Anti-aging products**: Chitosan hydrogels are used in facial masks and anti-aging creams, promoting skin regeneration and hydration.

Advantages of Chitosan Hydrogels:

- **Biocompatibility and biodegradability**: Chitosan is naturally derived, safe for use in the body, and degrades over time.
- **Moisture retention**: These hydrogels retain moisture, making them ideal for wound healing and skin applications.
- **Ease of administration**: Can be administered through **injection**, **topical application**, or **implantation**, providing flexibility in treatment methods.
- **Controlled release**: Provides sustained and controlled release of active agents, enhancing therapeutic efficacy.[53]

Tissue generation

Tissue generation (or **tissue engineering**) refers to the process of developing **biological tissues** in the laboratory to replace or repair damaged or diseased tissues in the body. This field combines principles of **biology**, **engineering**, and **material science** to create functional tissues that can be used for therapeutic purposes. One of the key strategies in tissue generation involves using **scaffolds** and **biomaterials** to support the growth and differentiation of cells into functional tissue structures.[26]

Key Components of Tissue Generation:

1. **Scaffolds**:

o **Biodegradable materials** like **chitosan**, **collagen**, and **alginate** are commonly used to create scaffolds. These scaffolds mimic the extracellular matrix (ECM), providing support for cells to grow and form tissue.

o Scaffolds should be **biocompatible**, meaning they don't cause an immune response, and **biodegradable**, so they break down as the tissue regenerates, leaving behind newly formed tissue.

2. **Stem Cells**:

o **Stem cells** (e.g., **mesenchymal stem cells** or **induced pluripotent stem cells**) are used to seed the scaffolds because they have the ability to differentiate into various cell types.

o These cells can be cultured in a laboratory environment and then induced to form specific tissues such as bone, cartilage, or skin.[9]

3. **Growth Factors**:

o **Growth factors** (e.g., **bone morphogenetic proteins**, **vascular endothelial growth factor**) are proteins that stimulate cell growth, differentiation, and tissue formation. They can be incorporated into scaffolds or directly applied to support tissue generation.

4. **Bioreactors**:

o A **bioreactor** is a controlled environment where **cells** and **scaffolds** are cultured together. These systems can provide the necessary mechanical, biochemical, and environmental conditions (e.g., oxygen, nutrients, and temperature) to help tissues grow and mature.

o Mechanical stimulation in bioreactors can help **mimic the natural environment** (e.g., bone or cartilage tissues exposed to mechanical stress) to encourage proper tissue development.

Types of Tissue Generation:

1. **Bone Tissue Engineering**:

o Involves the creation of **bone-like tissues** using scaffolds and stem cells to treat bone defects, fractures, or diseases like osteoporosis.

o **Hydroxyapatite**, **collagen**, and **chitosan** are commonly used materials to support bone regeneration.

2. **Cartilage Tissue Engineering**:

o Used to repair or regenerate damaged cartilage, which is common in conditions like **osteoarthritis**.

o **Chitosan**, **alginate**, and **collagen-based** scaffolds are often used to support cartilage regeneration.[6]

3. **Skin Tissue Engineering**:

o The development of artificial skin for burn victims or patients with chronic wounds.

o Scaffolds made from **collagen**, **fibronectin**, or **chitosan** can support skin cell growth and differentiation.

4. **Cardiac Tissue Engineering**:

o Focuses on regenerating **heart tissue** to repair damage caused by **heart attacks**.

o Scaffold materials may include **gelatin**, **collagen**, and **chitosan**, and stem cells (like **cardiomyocytes**) may be used to generate functional heart tissue.[16]

5. **Vascular Tissue Engineering**:

o Aims to regenerate blood vessels, which can be used for bypass surgery or to repair vascular damage.

o **Polylactic acid (PLA)**, **poly(lactic-co-glycolic acid) (PLGA)**, and **collagen** are common materials for creating blood vessel scaffolds.

6. **Nerve Tissue Engineering**:

o Focuses on regenerating nerve cells for treating conditions like **spinal cord injury** or **peripheral nerve damage**.

o **Chitosan** has been used for nerve regeneration due to its ability to support neural cell growth.

Role of Chitosan in Tissue Generation:

Chitosan, a natural polymer derived from chitin, plays an important role in tissue engineering because of its **biocompatibility**, **biodegradability**, and **non-toxicity**. It can be used in various forms such as **hydrogels**, **nanoparticles**, and **scaffolds**. Chitosan-based materials are especially useful for:

- **Wound healing**: By forming hydrogels at the site of injury, chitosan can promote tissue regeneration.
- **Bone and cartilage regeneration**: Chitosan's osteoconductive properties help in the formation of bone-like tissue.
- **Drug delivery**: It can carry growth factors or other agents to the site of tissue injury, promoting regeneration.

Applications:

- **Clinical use in regenerative medicine**: Generating tissues for transplant or repair of damaged tissues (e.g., skin grafts, cartilage repair).
- **Cosmetic surgery**: Skin regeneration and anti-aging treatments using tissue-engineered skin.
- **Organ repair**: Attempts to create lab-grown organs (e.g., liver, kidney) are ongoing with tissue engineering techniques.[19]

Challenges in Tissue Generation:

- **Vascularization**: Developing large, complex tissues that require blood vessels for nutrient and oxygen supply remains a major challenge.
- **Integration with host tissue**: Ensuring that engineered tissues properly integrate with surrounding tissues after transplantation.
- **Scaffold degradation**: Balancing scaffold degradation with tissue formation so that scaffolds degrade at the right rate without affecting tissue regeneration.

Wound dressing material for wound healing:

Wound dressing materials play a crucial role in the wound healing process by protecting the wound from infection, maintaining a moist environment, and promoting tissue regeneration. These materials vary widely, depending on the wound type, severity, and the healing environment needed. Here are some common wound dressing materials used for wound healing:

1. Hydrocolloids:

- Composition: Hydrocolloids are made from a combination of polymers like carboxymethyl cellulose and gelatin, with a backing material.
- Properties: They form a gel-like substance when they come into contact with wound exudate, maintaining a moist environment. They provide moderate absorption and are semi-occlusive, which helps prevent infection.
- Uses: Ideal for light to moderately exuding wounds, like venous ulcers, pressure sores, and superficial burns.[10]

2. Hydrogels:

- Composition: Hydrogels are composed of water-based polymers like polyvinyl alcohol, carbomer, or chitosan.
- Properties: They provide moisture to dry or necrotic wounds and help in debridement. Hydrogels are non-adhesive, making them gentle on fragile skin.
- Uses: Suitable for dry wounds, burns, and abrasions. They also help in rehydrating necrotic tissue.

3. Foam Dressings:

- Composition: These dressings are made of polyurethane or silicone foam.
- Properties: Highly absorbent, they help manage moderate to heavy exudating wounds while maintaining a moist wound environment. Foam dressings are soft and comfortable and provide a cushioning effect.
- Uses: Ideal for diabetic ulcers, pressure ulcers, and surgical wounds with high exudate.

4. Alginate Dressings:

- Composition: Derived from brown seaweed, alginate dressings are made of calcium or sodium salts of alginic acid.[20]
- Properties: These dressings are highly absorbent and promote hemostasis (helping stop bleeding). They form a gel when they come into contact with wound exudate.
- Uses: Best for moderate to heavily exuding wounds, such as venous ulcers, donor sites, and pressure sores.

5. Chitosan-Based Dressings:

- Composition: Chitosan, a natural polysaccharide derived from chitin (found in shellfish), is used to make wound dressings.
- Properties: Antibacterial, biodegradable, and biocompatible, chitosan promotes healing by providing a moist environment, preventing infection, and stimulating tissue regeneration. It also accelerates wound closure.
- Uses: Suitable for a variety of wounds, including chronic ulcers, surgical wounds, and burns.

6. Silver-Impregnated Dressings:

- Composition: These dressings are impregnated with silver salts, typically silver sulfadiazine, or silver nanoparticles.[31]

- Properties: Silver has antibacterial properties and helps to prevent infection in the wound. These dressings provide antimicrobial protection while promoting healing.
- Uses: Ideal for infected wounds, burns, chronic ulcers, and wounds at risk for infection.

7. Collagen-Based Dressings:

- Composition: Collagen dressings are made from bovine, porcine, or human collagen.
- Properties: Collagen stimulates the body's natural wound-healing processes by promoting cell growth, angiogenesis (formation of new blood vessels), and tissue regeneration.
- Uses: Effective for chronic wounds (e.g., diabetic ulcers, pressure ulcers) and surgical wounds.

8. Nanofiber and Nanogel Dressings:

- Composition: These dressings use nanomaterials (e.g., nanocellulose, nanoparticles, or nanofibers) to create highly porous and lightweight dressings.
- Properties: High surface area, moisture retention, and controlled release of growth factors or drugs (e.g., antimicrobial agents). Nanofibers promote tissue regeneration and can help control inflammation.
- Uses: Suitable for chronic wounds, burns, and infections. They can also be used for drug delivery to accelerate healing.[42]

9. Petrolatum Gauze:

- Composition: A gauze dressing coated with a layer of petrolatum (petroleum jelly).
- Properties: Keeps the wound moist and prevents the dressing from sticking to the wound, minimizing pain upon removal.
- Uses: Effective for minor burns, abrasions, and post-surgical wounds.

10. Transparent Film Dressings:

- Composition: Made of polyurethane or polyethylene films with adhesive layers.
- Properties: These are waterproof, moisture-retentive, and provide breathability. They allow for easy monitoring of the wound without removal of the dressing.
- Uses: Suitable for superficial wounds, abrasions, skin tears, and post-surgical sites.

11. Hydrogel-Silver Dressings:

- Composition: A combination of hydrogel and silver particles.
- Properties: They provide the moisture-retentive properties of hydrogels along with the antibacterial benefits of silver, making them effective for wounds prone to infection.
- Uses: Ideal for burns, infected chronic ulcers, and post-surgical wounds.[51]

12. Xeroform Dressings:

- Composition: Xeroform is made of gauze impregnated with a petrolatum-based compound and bismuth tribromophenate.
- Properties: Provides moisture to the wound, protects the wound from infection, and promotes healing. Bismuth has mild antimicrobial properties.
- Uses: Often used for surgical wounds, burns, and abrasions.

Key Considerations for Selecting Wound Dressings:

- Wound Exudate: The amount of fluid coming from the wound (light, moderate, or heavy exudate) determines the dressing type. Absorbent dressings are needed for highly exuding wounds, while less absorbent dressings are used for low-exudate wounds.
- Infection Risk: If infection is a concern, antimicrobial dressings (e.g., silver-impregnated or iodine dressings) should be used.
- Wound Type: Dressings should be chosen based on the type of wound (e.g., chronic, acute, surgical, or burn wounds).
- Moisture Balance: Maintaining a moist wound environment accelerates healing, prevents scab formation, and reduces pain.
- Ease of Removal: Non-adherent or pain-free dressings are ideal for wounds with sensitive or fragile skin.[57]

Cell scaffold in tissue engineering:

A **cell scaffold** is a crucial component in **tissue engineering**, serving as a three-dimensional structure that supports the growth, organization, and differentiation of cells into functional tissues. These scaffolds mimic the **extracellular matrix (ECM)**, which is the natural network of proteins and other molecules surrounding cells in tissues. The primary purpose of a cell scaffold is to provide mechanical support for tissue formation and guide cells to form complex tissue structures.

Key Functions of Cell Scaffolds in Tissue Engineering:

1. **Cell Support and Anchorage**:

o Scaffolds provide a **surface for cells** to adhere to, multiply, and organize into tissues. Cells rely on the scaffold for structural support during growth.

o The scaffold material should have suitable surface properties, such as the ability to promote cell attachment and prevent cell detachment.

2. **Guidance for Tissue Formation**:

o Scaffolds guide cells to form tissues by offering **3D architecture** that mimics the natural tissue's structure. This helps to ensure that the cells grow in the correct shape and function as they would in vivo.[23]

3. **Nutrient and Oxygen Transport**:

o Scaffolds create pores or channels that allow for the diffusion of nutrients, oxygen, and waste products to and from the cells. This is especially important for larger tissue constructs, where diffusion alone might not be sufficient to sustain cell viability.

4. **Controlled Release of Bioactive Factors**:

o Scaffolds can be engineered to release **growth factors**, **cytokines**, or **drugs** in a controlled manner. These bioactive molecules can promote cell proliferation, differentiation, and the formation of specific tissue types (e.g., cartilage, bone, skin).

5. **Mechanical Support**:

o Scaffolds provide mechanical support to newly forming tissues. This is particularly important in **load-bearing tissues** like bone or cartilage, where the scaffold needs to maintain structural integrity until the tissue matures.[39]

6. **Biodegradability**:

o A good scaffold is **biodegradable**, meaning it breaks down over time as the tissue regenerates. This eliminates the need for surgical removal and allows the newly formed tissue to take over the function previously provided by the scaffold.

Types of Cell Scaffolds in Tissue Engineering:

1. **Natural Biomaterial Scaffolds**:

o **Collagen**: The primary structural protein in connective tissues. Collagen scaffolds are biocompatible, biodegradable, and promote cell adhesion and tissue formation. They are commonly used for **skin**, **bone**, and **cartilage** regeneration.

o **Chitosan**: Derived from chitin, chitosan is a biopolymer with antimicrobial properties and is used in scaffolds for **wound healing** and **cartilage regeneration**.

o **Alginate**: A polysaccharide derived from seaweed, often used to form hydrogels. **Alginate scaffolds** are biocompatible and can encapsulate cells, making them useful for applications in **liver**, **pancreatic**, and **cartilage** engineering.

o **Gelatin**: A hydrolyzed form of collagen, gelatin scaffolds are commonly used for **vascular** and **skin tissue** engineering because they are easy to process and promote cell growth.

2. **Synthetic Biomaterial Scaffolds**:

o **Poly(lactic acid) (PLA)**: A biodegradable polymer commonly used for bone, cartilage, and nerve tissue engineering. PLA scaffolds can be tailored for controlled degradation and mechanical properties.

o **Poly(lactic-co-glycolic acid) (PLGA)**: A copolymer of lactic acid and glycolic acid used in tissue engineering due to its **biodegradability** and **mechanical strength**. PLGA scaffolds are often used for **bone** and **cartilage** regeneration.

o **Polycaprolactone (PCL)**: A biodegradable synthetic polymer that is flexible and has excellent mechanical properties, often used in **bone** and **nerve tissue engineering**.

o **Polyethylene glycol (PEG)**: A synthetic polymer used to create hydrogels. PEG scaffolds can be functionalized with bioactive molecules to enhance cell adhesion and tissue formation.[17]

3. **Composite Scaffolds**:

o **Combination of natural and synthetic materials**: Composite scaffolds are designed by combining natural polymers (e.g., collagen or chitosan) with synthetic polymers (e.g., PLGA or PCL). These scaffolds aim to combine the **biocompatibility** of natural materials with the **tailorability** and **mechanical strength** of synthetic materials.

- **Ceramic composites**: For **bone regeneration**, ceramics like **hydroxyapatite** or **tricalcium phosphate** are used to create scaffolds with a composition similar to bone mineral. These composites can also be combined with polymers to enhance mechanical properties and promote cell growth.

4. **Hydrogels**:

- **Water-based scaffolds**: Hydrogels are soft, water-absorbent materials that can mimic the hydrated nature of tissues. Hydrogels can be made from natural or synthetic materials and provide an environment conducive to cell growth and tissue development.

- **Use in soft tissues**: They are particularly useful for **soft tissue engineering**, including **cartilage** and **skin** regeneration, and can be used for **drug delivery** applications.

5. **Decellularized Extracellular Matrix (dECM)**:

- **Decellularization** is the process of removing all cellular components from a tissue, leaving behind the **extracellular matrix (ECM)**. The ECM provides the natural microenvironment for cells to grow and organize.

- **dECM scaffolds** maintain the original tissue's architecture, biochemical composition, and mechanical properties, making them ideal for **tissue-specific engineering**, such as **heart**, **liver**, and **kidney** regeneration.[49]

Scaffold Fabrication Techniques:

1. **Electrospinning**: A technique used to produce nanofiber scaffolds that mimic the **fibrous** nature of the ECM. These scaffolds are highly porous, allowing for cell infiltration and nutrient exchange.

2. **3D Bioprinting**: Involves **layer-by-layer deposition** of bioinks (containing cells, growth factors, and biomaterials) to build highly intricate scaffolds with precise control over geometry and cellular distribution.

3. **Solvent Casting and Particulate Leaching**: A process where a polymer solution is cast into a mold, and then a particulate material (e.g., salt) is leached out to create a porous structure for cell growth.

4. **Freeze-Drying (Lyophilization)**: This method involves freezing a polymer solution and then sublimating the frozen solvent, leaving behind a porous scaffold suitable for cell growth.

5. **Self-Assembly**: In some cases, **cells themselves** can organize and assemble into scaffold-like structures by providing the right biochemical and mechanical cues.[52]

Applications of Cell Scaffolds in Tissue Engineering:

1. **Bone Regeneration**: Scaffolds made from **hydroxyapatite** and **collagen** or synthetic polymers like **PLGA** are commonly used for regenerating bone tissue, including in cases of **fractures**, **bone defects**, and **osteoporosis**.

2. **Cartilage Repair**: Scaffolds that mimic the **extracellular matrix (ECM)** of cartilage, such as **chitosan** and **collagen-based scaffolds**, are used to repair **joint cartilage** damaged by **osteoarthritis** or trauma.

3. **Skin Regeneration**: **Collagen**, **chitosan**, and **silk-based scaffolds** are used to engineer **skin grafts** for burn victims or patients with **chronic wounds** like **diabetic ulcers**.

4. **Cardiac Repair**: Scaffolds made from **gelatin** or **collagen** are used in the regeneration of heart tissue after **myocardial infarction** (heart attack) to improve tissue healing and prevent further damage.

5. **Vascular Engineering**: **Polymeric scaffolds** combined with **growth factors** can help generate **blood vessels** for use in **bypass surgery**, **organ transplantation**, and **vascular disease treatments**.

6. **Nerve Regeneration**: **Chitosan** and **polymer-based scaffolds** are used to regenerate **nerve tissue** for conditions like **spinal cord injury** or **peripheral nerve damage**.[56]

Challenges and Future Directions:

- **Vascularization**: One of the biggest challenges is creating larger tissues that can be sustained over time. Developing scaffolds that promote the formation of blood vessels (vascularization) within engineered tissues is crucial for their success.

- **Immune Response**: Even though scaffolds are often designed to be biocompatible, **immune rejection** can still occur, especially in the case of **xenogeneic materials** (derived from other species).

- **Mechanical Properties**: The scaffold must have mechanical properties that match the target tissue (e.g., **bone scaffolds** need to be strong and stiff, while **skin scaffolds** need to be flexible).

- **Long-Term Integration**: Ensuring that the engineered tissue fully integrates with the host tissue without causing complications remains a significant challenge.[36]

Conclusion:

Cell scaffolds are fundamental components in tissue engineering, playing a crucial role in supporting cell growth, guiding tissue formation, and ensuring the regeneration of functional tissues. They mimic the extracellular matrix (ECM) and provide structural, mechanical, and biochemical cues that promote cell adhesion, proliferation, and differentiation. Scaffolds are created from a variety of natural and synthetic materials, such as collagen, chitosan, alginate, and synthetic polymers like PLGA and PCL, each offering unique benefits suited for different tissue types. In tissue engineering, the scaffold's design is essential to ensure proper cellular interaction, nutrient diffusion, and biodegradability. Advanced fabrication techniques like electrospinning, 3D bioprinting, and freeze-drying have improved the precision and effectiveness of scaffold creation, making them capable of supporting more complex tissue structures.

References:

1.Buwalda SJ, et al. Hydrogels in a historical perspective: From simple networks to smart materials. J Control Release. 2014;190:254–273. : 10.1016/j.jconrel.2014.03.052.

2.Zhu JM, Marchant RE. Design properties of hydrogel tissue-engineering scaffolds. Expert Rev Med Devic. 2011;8:607–626. : 10.1586/Erd.11.27.

3.Yang JY, Xu CY, Kopeckova P, Kopecek J. Hybrid hydrogels self-assembled from HPMA copolymers containing peptide grafts. Macromol Biosci. 2006;6:201–209. : 10.1002/mabi.200500208.

4.Li JY, Illeperuma WBK, Suo ZG, Vlassak JJ. Hybrid Hydrogels with Extremely High Stiffness and Toughness. Acs Macro Lett. 2014;3:520–523. : 10.1021/mz5002355.

5.Elisseeff J, et al. Transdermal photopolymerization for minimally invasive implantation. Proc Natl Acad Sci U S A. 1999;96:3104–3107. : 10.1073/pnas.96.6.3104.

6.Cai L, Dewi RE, Heilshorn SC. Injectable Hydrogels with In Situ Double Network Formation Enhance Retention of Transplanted Stem Cells. Adv Funct Mater. 2015;25:1344–1351. : 10.1002/adfm.201403631.

7.Griffin DR, Kasko AM. Photoselective Delivery of Model Therapeutics from Hydrogels. Acs Macro Lett. 2012;1:1330–1334. : 10.1021/mz300366s.

8.Hou S, Wang XF, Park S, Jin XB, Ma PX. Rapid Self-Integrating, Injectable Hydrogel for Tissue Complex Regeneration. Adv Healthc Mater. 2015;4:1491–1495. : 10.1002/adhm.201500093.

9.Liow SS, et al. Thermogels: In Situ Gelling Biomaterial. ACS Biomaterials Science & Engineering. 2016;2:295–316. : 10.1021/acsbiomaterials.5b00515.

10.Hoare TR, Kohane DS. Hydrogels in drug delivery: Progress and challenges. Polymer. 2008;49:1993–2007. : http://dx..org/10.1016/j.polymer.2008.01.027.

11.Ifkovits JL, Burdick JA. Review: photopolymerizable and degradable biomaterials for tissue engineering applications. Tissue Eng. 2007;13:2369–2385. : 10.1089/ten.2007.0093.

12.Koutsopoulos S. Self-assembling peptide nanofiber hydrogels in tissue engineering and regenerative medicine: Progress, design guidelines, and applications. J Biomed Mater Res A. 2016;104:1002–1016. : 10.1002/jbm.a.35638.

13.Zhang SG. Fabrication of novel biomaterials through molecular self-assembly. Nat Biotechnol. 2003;21:1171–1178. : 10.1038/nbt874.

14.Webber MJ, Appel EA, Meijer EW, Langer R. Supramolecular biomaterials. Nat Mater. 2016;15:13–26. : 10.1038/nmat4474.

15.Hirobe T. Keratinocytes regulate the function of melanocytes. Dermatol Sin. 2014;32:200–204. : 10.1016/j.dsi.2014.05.002.

16.McGrath JA, Eady RAJ, Pope FM. Rook's Textbook of Dermatology. Blackwell Publishing, Inc.; 2008. pp. 45–128.

17.Archer CB. Rook's Textbook of Dermatology. Blackwell Publishing, Inc.; 2008. pp. 129–140.

18.Sinno H, Prakash S. Complements and the wound healing cascade: an updated review. Plast Surg Int. 2013;2013:146764. : 10.1155/2013/146764.

19.Xue ML, Jackson CJ. Extracellular Matrix Reorganization During Wound Healing and Its Impact on Abnormal Scarring. Adv Wound Care. 2015;4:119–136. : 10.1089/wound.2013.0485.

20.Grinnell F, Zhu MF. Fibronectin degradation in chronic wounds depends on the relative levels of elastase, alpha 1-proteinase inhibitor, and alpha 2-macroglobulin. J Invest Dermatol. 1996;106:335–341. : 10.1111/1523-1747.ep12342990. : 10.1111/1523-1747.ep12342990.

21.Lauer G, et al. Expression and proteolysis of vascular endothelial growth factor is increased in chronic wounds. J Invest Dermatol. 2000;115:12–18. : 10.1046/j.1523-1747.2000.00036.x. : 10.1046/j.1523-1747.2000.00036.x.

22.Falanga V. Wound healing and its impairment in the diabetic foot. Lancet. 2005;366:1736–1743. : 10.1016/S0140-6736(05)67700-8.

23.Metcalfe AD, Ferguson MWJ. Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration. J R Soc Interface. 2007;4:413–437. : 10.1098/rsif.2006.0179.

24.Turturro MV, et al. MMP-Sensitive PEG Diacrylate Hydrogels with Spatial Variations in Matrix Properties Stimulate Directional Vascular Sprout Formation. Plos One. 2013;8 : 10.1371/journal.pone.0058897. :ARTNe5889710.1371/journal.pone.0058897.

25.Madden LR, et al. Proangiogenic scaffolds as functional templates for cardiac tissue engineering. Proceedings of the National Academy of Sciences. 2010;107:15211–15216. : 10.1073/pnas.1006442107.

26.Matsuno T, et al. Preparation of injectable 3D-formed beta-tricalcium phosphate bead/alginate composite for bone tissue engineering. Dent Mater J. 2008;27:827–834. : 10.4012/dmj.27.827.

27.Bidarra SJ, Barrias CC, Granja PL. Injectable alginate hydrogels for cell delivery in tissue engineering. Acta Biomater. 2014;10:1646–1662. : 10.1016/j.actbio.2013.12.006.

28.Friedl P, Gilmour D. Collective cell migration in morphogenesis, regeneration and cancer. Nat Rev Mol Cell Biol. 2009;10:445–457. : 10.1038/nrm2720.

29.Friedl P, Mayor R. Tuning Collective Cell Migration by Cell-Cell Junction Regulation. Cold Spring Harb Perspect Biol. 2017;9 : 10.1101/cshperspect.a029199.

30.Pierschbacher MD, Ruoslahti E. Cell Attachment Activity of Fibronectin Can Be Duplicated by Small Synthetic Fragments of the Molecule. Nature. 1984;309:30–33. : 10.1038/309030a0. : 10.1038/309030a0.

31.Pierschbacher MD, Ruoslahti E. Variants of the Cell Recognition Site of Fibronectin That Retain Attachment-Promoting Activity. P Natl Acad Sci-Biol. 1984;81:5985–5988. : 10.1073/pnas.81.19.5985. : 10.1073/pnas.81.19.5985.

32.Ruoslahti E. RGD and other recognition sequences for integrins. Annu Rev Cell Dev Bi. 1996;12:697–715. : 10.1146/annurev.cellbio.12.1.697. : 10.1146/annurev.cellbio.12.1.697.

33.Maheshwari G, Brown G, Lauffenburger DA, Wells A, Griffith LG. Cell adhesion and motility depend on nanoscale RGD clustering. J Cell Sci. 2000;113:1677–1686. : 10.1242/jcs.113.10.1677.

34.Lee TT, et al. Light-triggered in vivo activation of adhesive peptides regulates cell adhesion, inflammation and vascularization of biomaterials. Nature Materials. 2015;14:352–360. : 10.1038/Nmat4157.

35.Li S, et al. Hydrogels with precisely controlled integrin activation dictate vascular patterning and permeability. Nat Mater. 2017 : 10.1038/nmat4954. advance online publication. http://www.nature.com/nmat/journal/vaop/ncurrent/abs/nmat4954.html#supplementary-information.

36.Alijotas-Reig J, Fernandez-Figueras MT, Puig L. Late-Onset Inflammatory Adverse Reactions Related to Soft Tissue Filler Injections. Clin Rev Allerg Immu. 2013;45:97–108. : 10.1007/s12016-012-8348-5.

37.Griffin DR, Weaver WM, Scumpia PO, Di Carlo D, Segura T. wound healing by injectable microporous gel scaffolds assembled from annealed building blocks. Nature Materials. 2015;14:737-+. : 10.1038/nmat4294.

38.Bencherif SA, et al. Injectable preformed scaffolds with shape-memory properties. P Natl Acad Sci USA. 2012;109:19590–19595. : 10.1073/pnas.1211516109.

39.Harris JM, Chess RB. Effect of pegylation on pharmaceuticals. Nat Rev Drug Discov. 2003;2:214–221. : 10.1038/nrd1033.

40.MacDonald ML, et al. Identifying off-target effects and hidden phenotypes of drugs in human cells. Nat Chem Biol. 2006;2:329–337. : 10.1038/nchembio790.

41.Brudno Y, Mooney DJ. On-demand drug delivery from local depots. J Control Release. 2015;219:8–17. : 10.1016/j.jconrel.2015.09.011.

42.Huebsch N, et al. Ultrasound-triggered disruption and self-healing of reversibly cross-linked hydrogels for drug delivery and enhanced chemotherapy. P Natl Acad Sci USA. 2014;111:9762–9767. : 10.1073/pnas.1405469111.

43.Buwalda SJ, Vermonden T, Hennink WE. Hydrogels for Therapeutic Delivery: Current Developments and Future Directions. Biomacromolecules. 2017;18:316–330. : 10.1021/acs.blomac.6b01604.

44.Heskins M, Guillet JE. Solution Properties of Poly(N-isopropylacrylamide) Journal of Macromolecular Science: Part A - Chemistry. 1968;2:1441–1455. : 10.1080/10601326808051910.

45.Gharakhanian EG, Deming TJ. Role of Side-Chain Molecular Features in Tuning Lower Critical Solution Temperatures (LCSTs) of Oligoethylene Glycol Modified Polypeptides. J Phys Chem B. 2016;120:6096–6101. : 10.1021/acs.jpcb.6b02065.

46.Loh XJ, Cheng LWI, Li J. Micellization and Thermogelation of Poly(ether urethane)s Comprising Poly(ethylene glycol) and Poly(propylene glycol) Macromol Sy. 2010;296:161–169. : 10.1002/masy.201051024.

47.Shinde UP, Moon HJ, Ko du Y, Jung BK, Jeong B. Control of rhGH Release Profile from PEG-PAF Thermogel. Biomacromolecules. 2015;16:1461–1469. : 10.1021/acs.biomac.5b00325.

48.Ci TY, et al. Effects of amphiphilic block copolymers on the equilibrium lactone fractions of camptothecin analogues at different pHs. Biomater Sci-Uk. 2013;1:1235–1243. : 10.1039/c3bm60152h.

49.Gao Y, et al. A thermo-sensitive PLGA-PEG-PLGA hydrogel for sustained release of docetaxel. J Drug Target. 2011;19:516–527. : 10.3109/1061186x.2010.519031.

50.Gupta MK, et al. Cell Protective, ABC Triblock Polymer-Based Thermoresponsive Hydrogels with ROS-Triggered Degradation and Drug Release. J Am Chem Soc. 2014;136:14896–14902. : 10.1021/ja507626y.

51.Ci T, Chen L, Yu L, Ding J. Tumor regression achieved by encapsulating a moderately soluble drug into a polymeric thermogel. Sci Rep. 2014;4:5473. : 10.1038/srep05473.

52.Qiu ML, et al. Platelet-Rich Plasma-Loaded Poly(d, l-lactide)-Poly(ethylene glycol)-Poly(d, l-lactide) Hydrogel Dressing Promotes Full-Thickness Skin Wound Healing in a Rodent Model. Int J Mol Sci. 2016;17 : 10.3390/ijms17071001. :UNSP100110.3390/ijms17071001.

53.Jeong B, Bae YH, Kim SW. Drug release from biodegradable injectable thermosensitive hydrogel of PEG-PLGA-PEG triblock copolymers. J Control Release. 2000;63:155–163. : 10.1016/s0168-3659(99)00194-7. : 10.1016/S0168-3659(99)00194-7.

54.Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins: basic science and product development. J Pharm Pharmacol. 2010;62:1607–1621. : 10.1111/j.2042-7158.2010.01030.x.

55.Gan TT, Zhang YJ, Guan Y. In Situ Gelation of P(NIPAM-HEMA) Microgel Dispersion and Its Applications as Injectable 3D Cell Scaffold. Biomacromolecules. 2009;10:1410–1415. : 10.1021/bm900022m.

56.Yun EJ, Yon B, Joo MK, Jeong B. Cell Therapy for Skin Wound Using Fibroblast Encapsulated Poly(ethylene glycol)-poly(L-alanine) Thermogel. Biomacromolecules. 2012;13:1106–1111. : 10.1021/bm2018596.

57.Hayakawa T, Hashimoto Y, Myokei Y, Aoyama H, Izawa Y. Changes in Type of Collagen during the Development of Human Post-Burn Hypertrophic Scars. Clin Chim Acta. 1979;93(79):119–125. 90252–3. : 10.1016/0009-8981(79)90252-3. : 10.1016/0009-8981.

58.Tan HP, et al. Thermosensitive injectable hyaluronic acid hydrogel for adipose tissue engineering. Biomaterials. 2009;30:6844–6853. : 10.1016/j.biomaterials.2009.08.058.

59.Kim EJ, Choi JS, Kim JS, Choi YC, Cho YW. Injectable and Thermosensitive Soluble Extracellular Matrix and Methylcellulose Hydrogels for Stem Cell Delivery in Skin Wounds Biomacromolecules. 2016;17:4–11. : 10.1021/acs.biomac.5b01566.

60.Li JJ, et al. A chitosan-glutathione based injectable hydrogel for suppression of oxidative stress damage in cardiomyocytes. Biomaterials. 2013;34:9071–9081. : 10.1016/j.biomaterials.2013.08.031.

61.Garripelli VK, Namgung R, Kim WJ, Jo S. Drug release from a pH-sensitive multiblock co-polymer thermogel. J Biomater Sci Polym Ed. 2012;23:1505–1519. : 10.1163/092050611X584414.

62.Ferreira L, Vidal MM, Gil MH. Evaluation of poly(2-hydroxyethyl methacrylate) gels as drug delivery systems at different pH values. Int J Pharm. 2000;194:169–180. : 10.1016/s0378-5173(99)00375-0. : 10.1016/S0378-5173(99)00375-0.

63.Jones EM, Cochrane CA, Percival SL. The Effect of pH on the Extracellular Matrix and Biofilms. Adv Wound Care (New Rochelle) 2015;4:431–439. : 10.1089/wound.2014.0538.

64.Gethin G. The significance of surface pH in chronic wounds. Wounds uk. 2007;3:52.