PEPTIDE-BASED HYDROGELS: BUILDING BLOCKS FOR ADVANCED BIOMEDICAL TECHNOLOGIES

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INTRODUCTION

Peptides, which are naturally occurring short chains of amino acids, possess highly ordered assembly structures that enable them to perform specific biological functions. Through self-assembly processes, peptides can form various nanoarchitectures, including nanotubes, nanoparticles, nanofibers, and nanobelts, by noncovalent interactions such as hydrogen bonding, ionization, hydrophobicity, and π-π stacking. Peptides possess distinctive qualities, including their biological origins, biocompatibility, bioactivity and biodegradability, which render them highly promising in various applications. These peptide-based nanoarchitectures show potential in fields such as drug delivery, photothermal/photoelectric conversion, photocatalysis, and optical waveguides..

Recently, there has been a substantial rise in the interest surrounding peptide-based hydrogels, which are made up of short chains of amino acids, or peptides, that can self-assemble into a three-dimensional (3D) fibrous network structure, crosslinked through chemical or physical bonding. These hydrogels are characterized by their ability to form a 3D fibrous network, which

imparts them with unique mechanical properties, such as viscoelasticity and self-healing behavior. The fibrils are associated with various morphologies such as α-helices, β-sheets, micelles, vesicles, tapes, tubes, ribbons, fibers,, and coiled structures and these network structure allows for the incorporation of various molecules, such as drugs or growth factors, which can be released over time as the hydrogel degrades. These hydrogels are known for their distinct characteristics, which include a microporous structure, high-water content,adjustable mechanical stability,tissue-like elasticity , injectability, biocompatible, meaning they do not elicit harmful responses from living tissues, and are biodegradable, which allows for safe degradation and elimination from the body over time. The most important property is their low toxicity, which makes them suitable for biomedical applications. Furthermore, these hydrogels can be conveniently decorated or functionalized by modifying the side chains and incorporating them into the backbone structure and the tunability of peptide-based hydrogels, through modifications in peptide sequences or crosslinking strategies, allows for fine-tuning of their properties to suit specific applications. The combination of these properties makes peptide-based hydrogels highly suitable for a wide range of biomedical applications, including drug delivery, biosensors, protein separation, wound healing and tissue engineering.

Additionally, peptide hydrogels can be easily tuned to exhibit multi-stimuli responsive behavior, responding to external cues such as changes in temperature, pH, or light, which is one of the main reasons for the growing attention of researchers towards peptide-based hydrogels making them versatile for a diverse array of biomedical applications.

PEPTIDE BASED HYDROGEL: PROPERTIES

Peptide-based hydrogels have emerged as a promising class of materials with diverse applications in various fields, such as biomedicine, tissue engineering, drug delivery, and soft

robotics. These hydrogels are composed of nanofibrous networks interconnected by chemical or physical bonds, and exhibit unique characteristics such as microporous structure, mechanical stability, biocompatibility, tissue-like elasticity ,high water content, and injectability.

There are different types of peptide-based hydrogels that are classified based on their secondary structures, including β-sheet-rich hydrogels and α-helix-rich hydrogels. β-sheet-rich hydrogels are composed of antiparallel β-sheets, where nonpolar and polar amino acids alternate in the peptide sequence. These hydrogels form a 3D network of fibers that entangle and result in a self-supporting hydrogel. However, α-helix-rich hydrogels are stabilized by intermolecular interactions mediated by hydrophobic and electrostatic interactions, as well as hydrogen bonding. These hydrogels are assembled from longer α-helix sequences and offer opportunities for precise control over binding strength, assembly order, stimulus-response and stoichiometry.
One of the major advantages of peptide-based hydrogels is their biocompatibility, as they are composed of amino acids that are natural building blocks of proteins in living organisms. This renders them well-suited for various biomedical applications, including but not limited to drug delivery, tissue engineering, and regenerative medicine.

Peptide-based hydrogels can mimic the extracellular matrix (ECM), creating a favorable environment for proliferation,cell adhesion and differentiation.. These hydrogels can also be tailored by modifying the peptide sequence, allowing for customization to meet specific requirements, such as mechanical properties, degradation rate, and bioactivity.
Peptide-based hydrogels also offer high water content, which can facilitate nutrient and oxygen transport to encapsulated cells or tissues. They exhibit tissue-like elasticity, which can provide mechanical support to the surrounding tissues and organs. Additionally, peptide-based hydrogels can be easily injected through a needle due to their injectability, Enabling their suitability for minimally invasive procedures and tissue engineering using bioprinting techniques..

They are primarily composed of antiparallel β-sheets that have a specific architecture where nonpolar and polar amino acids alternate. The non-polar amino acids contribute to the strength and stability of the β-sheet-rich fibers, whereas the polar amino acids play a role in the interactions between fibers and the process of aggregation. As the concentration of fibers reaches a critical level, they entangle and form a 3D network, ultimately resulting in a self-supporting hydrogel.However, helix bundles stabilized by hydrophobic and electrostatic interactions, as well as hydrogen bonding, are responsible for the assembly of helix-rich fibers and hydrogels. Charged α-helix peptides have the ability to assemble into diverse micro- and nanoscale structures, which are dependent on factors such as pH and salt concentrations. Assembly of helix bundles is more complex compared to β-sheet assembly, as it requires longer α-helix sequences. However, these longer α-helix sequences offer opportunities for attaching fluorescent labels, reactive groups, and precise control over binding strength, stoichiometry, assembly order and stimulus-response.

Despite their many advantages, peptide-based hydrogels also have some limitations. One limitation is their potential for enzymatic degradation, as peptide-based hydrogels can be susceptible to protease cleavage in vivo, which may affect their stability and longevity. Moreover, the mechanical properties of peptide-based hydrogels may not always be sufficient for certain applications, and may require further modifications or reinforcements to achieve the desired mechanical strength. Peptide-based hydrogels can also have limited shelf-life and stability, as they may undergo structural changes or degradation over time, which could affect their performance and functionality

BIOMEDICAL APPLICATIONS OF PEPTIDE-BASED HYDROGELS

BIOSENSING

Peptide hydrogel biosensors have become a topic of interest because of their ability to quickly react to external stimuli, like changes in temperature and pH, and their strong adherence to cells, stability, and ability to self-repair. There are two primary categories of peptide hydrogels: (1) those that can sense variations in their surroundings and are employed independently of bioreceptors, and (2) those that have porous structures and are combined with sensing biomolecules. The latter category is particularly noteworthy because it prevents non-specific adsorption and enhances their suitability for ultra-sensitive biosensors.Peptide hydrogel biosensors have demonstrated enormous potential in a range of applications, viz. environmental monitoring, food safety and medical diagnosis. These biosensors provide several benefits compared to conventional sensing technologies, such as superior sensitivity, specificity, selectivity, biocompatibility, low cost, and simple fabrication. Additionally, they can be readily customized with a variety of sensing biomolecules, making them highly flexible and adaptable to various target analytes. In light of this, the advancement of novel peptide hydrogel biosensors with improved performance and selectivity is a promising area of research, particularly for applications such as cancer diagnosis, detection of infectious diseases, and monitoring environmental pollutants. Additionally, peptide hydrogels can be customized with a range of biomolecules, such as enzymes, antibodies, and aptamers, to enable precise detection of specific analytes. As a result, peptide hydrogel biosensors are highly flexible and can be tailored to suit a variety of sensing requirementsThe incorporation of aptamers in peptide hydrogel biosensors has also opened up new avenues for the development of point-of-care diagnostic tools that can rapidly and accurately generate results with minimal sample preparation. For instance, a peptide hydrogel biosensor equipped with aptamers has been created by scientists for the detection of thrombin, a protein that aids in blood clotting. The biosensor exhibited high sensitivity and selectivity for thrombin and could detect concentrations of the protein as low as 1 nM in blood

samples. This technology could have implications in the diagnosis and tracking of thrombotic conditions like deep vein thrombosis and pulmonary embolism.

Researchers have utilized self-assembled hydrogels made of Fmoc-Phe-Phe dipeptide for various biosensing and disease detection applications. Yang and colleagues used the hydrogel to detect H2O2 levels released from HeLa cells, while Park and colleagues demonstrated the detection of varying levels of phenolic and glucose compounds using enzyme-based optical biosensors. Alves and colleagues used the hydrogel for the detection of Leishmaniasis disease in patient samples, while Miller and colleagues developed a biosensor for identifying susceptibility to specific diseases. Peptide-based hydrogels such as Puramatrix and Matrigel are already commercialized and are in use, while others are undergoing clinical trials. These suggests that peptide-based hydrogels, particularly short and ultrashort peptides, exhibit superior chemical, biophysical, and biocompatible properties compared to synthetic organic and inorganic polymers, making them highly suitable for various biomedical applications such as drug delivery, tissue engineering, bioprinting, and bioimaging. Given the significant interest and ongoing research in this area, it is expected that many more novel peptide-based hydrogels will be developed for diverse biomedical purposes in the future.

DRUG DELIVERY AND ANTITUMOR THERAPY

Peptide hydrogels have water-filled micropore structures, work under mild conditions, respond to external stimuli, and are biocompatible. These properties make them suitable as drug carriers with high capacity. Achieving controlled and prolonged drug release at specific sites is crucial for enhancing anticancer efficiency. Traditional drug administration methods have drawbacks such as solubility problems, burst release, low bioavailability, degradation, and nonspecific distribution. To address these challenges, researchers have explored various drug delivery techniques utilizing polymer-based nanostructures. These nanostructures allow for the encapsulation of both hydrophilic and hydrophobic drugs, providing protection from the external environment and

controlled delivery. Peptide-based nanostructures, particularly injectable hydrogels, have gained significant attention in drug delivery research. Researchers have utilized these nanostructures for various applications, including anticancer drug delivery. Traditional cancer chemotherapy often leads to adverse side effects because drugs are delivered indiscriminately to healthy cells, causing damage.

For instance, the self-assembly of modified dipeptides can be observed through tests like the tube inversion test. These peptide-based nanostructures offer the potential for controlled drug release, improving drug delivery to cancerous cells while minimizing harm to healthy tissue
To minimize harmful side effects and improve the effectiveness of therapies, researchers have utilized peptide-based hydrogels as drug delivery systems. These hydrogels possess advantageous properties such as biocompatibility, the ability to accommodate various drugs through tunable structures, water-filled mesoporous structures, and responsiveness to external stimuli for controlled drug release.

Peptide-based hydrogels can enhance the biochemical characteristics of anticancer drugs, including their chemical stability, solubility, and bioavailability. Drugs are typically incorporated into hydrogels using two main approaches: chemical interactions or physical interactions.
Chen et al. conducted a study where they developed a short peptide-based hydrogel that responds to MMP-2 (a protein overexpressed by HeLa cells) for targeted delivery of anticancer peptides [87]. They designed and synthesized self-assembled fibrillar Ac-I3SLGK-NH2 hydrogels that could be degraded in the presence of HeLa cells. This property was utilized for controlled release of an anticancer peptide, G(IIKK)3I-NH2, specifically in cancer cells.

In the context of topical drug delivery through the skin, there are significant limitations imposed by physiological barriers between the skin and tumors, which can greatly reduce the therapeutic effectiveness.

To improve the efficiency of transdermal drug delivery, hydrogels can be employed to enhance the penetration of anticancer drugs through the skin. The biocompatibility and adjustable structure of hydrogels allow for the incorporation of various modifiers and drugs, thereby enhancing their skin penetration capabilities.

In vitro studies on peptide-based hydrogels have demonstrated their ability to enhance the pharmacokinetic properties of both hydrophilic and hydrophobic drugs. Additionally, these hydrogels have shown promise in reducing the effective dosage required for achieving anticancer effects.

However, further research is necessary to strengthen and advance this emerging field of smart drug carriers before they can be clinically approved for use.

TISSUE ENGINEERING

Tissue engineering aims to create functional tissue substitutes for the repair or replacement of damaged or diseased tissues. Central to tissue engineering is the development of suitable scaffolds that can mimic the native extracellular matrix (ECM) and provide a supportive environment for cell growth, proliferation, and differentiation. Peptide-based hydrogels have emerged as promising materials in tissue engineering due to their unique properties and versatility. One of the key advantages of peptide-based hydrogels is their ability to mimic the ECM, which plays a crucial role in tissue development and regeneration. The ECM provides mechanical support, signaling cues, and a reservoir for growth factors, creating a microenvironment that guides cellular behavior. Peptide-based hydrogels can replicate the

structural and functional properties of the ECM, allowing them to interact with cells and provide a suitable niche for tissue regeneration. In tissue engineering applications, peptide-based hydrogels are commonly used as scaffolds to support cell adhesion, proliferation, and differentiation. The hydrogel architecture can be engineered to provide a porous structure that facilitates nutrient and oxygen diffusion, as well as the exchange of waste products. The tunable mechanical properties of peptide-based hydrogels allow for the customization of scaffold stiffness to match the target tissue, providing mechanical cues that influence cell behavior and tissue development.

Furthermore, peptide-based hydrogels can be functionalized with bioactive motifs, such as cell adhesion peptides or growth factors, to enhance cellular interactions and tissue regeneration. These bioactive peptides can be incorporated into the hydrogel matrix or presented on the hydrogel surface, promoting specific cell adhesion, migration, and differentiation. The controlled release of bioactive molecules from peptide-based hydrogels can also be achieved, providing sustained delivery of growth factors or other therapeutic agents to support tissue regeneration.

Peptide-based hydrogels have been successfully applied in various tissue engineering applications, including bone regeneration, cartilage repair, nerve regeneration, and vascular tissue engineering. For example, in bone regeneration, peptide-based hydrogels can provide a three-dimensional environment that supports osteoblastic cell differentiation and mineralization. The incorporation of bioactive peptides, such as those derived from bone morphogenetic proteins (BMPs), can further enhance bone formation.

In cartilage tissue engineering, peptide-based hydrogels offer a biocompatible and biodegradable platform for chondrogenic cell encapsulation and cartilage matrix synthesis. The hydrogel properties can be optimized to mimic the mechanical properties of native cartilage,

while the inclusion of cartilage-specific bioactive peptides can stimulate chondrogenesis and matrix deposition.

In nerve regeneration, peptide-based hydrogels have shown promise as scaffolds to guide axonal growth and support neuronal cell survival. The hydrogel architecture can be tailored to provide topographical cues and release neurotrophic factors, facilitating nerve regeneration and functional recovery.

In vascular tissue engineering, peptide-based hydrogels have been utilized to promote neovascularization and enhance tissue perfusion. The incorporation of angiogenic factors, such as vascular endothelial growth factor (VEGF), into the hydrogel can stimulate the formation of new blood vessels, while the scaffold provides mechanical support and a matrix for endothelial cell attachment and organization.

ADVANTAGES AND DISADVANTAGES

Peptide hydrogels offer several advantages in biomedical applications. Firstly, they provide a protective environment for cells and drugs, shielding them from degradation and maintaining their bioactivity. Additionally, peptides can be easily modified to incorporate cell adhesion ligands, enabling targeted interactions with specific cell types. Moreover, peptide hydrogels can be administered as liquid formulations due to their thixotropic properties, which undergo gelation at body temperature or pH. Furthermore, peptide molecules are typically biocompatible and exhibit low toxicity. However, a limitation of peptide hydrogels is their mechanical weakness, which can pose challenges in their handling and structural integrity. Loading drugs and cells into peptide hydrogels and subsequently crosslinking them in vitro can be challenging. To overcome this, it is beneficial to develop mechanically robust peptide hydrogels with thixotropic properties.

This can be achieved through various approaches such as incorporating nanofillers or using long chain peptides that promote numerous hydrogen bonding interactions with the -CONH- groups. Furthermore, introducing aromatic moieties into the peptide chain can enhance mechanical strength through π-stacking interactions. However, it is crucial to ensure that the modified peptides remain biocompatible and exhibit low toxicity. Synthetic end-capping groups, such as naphthalene, fluorenylmethyloxycarbonyl, or anthracene, can also be employed to improve both mechanical properties and gelation capabilities of small peptides.The aforementioned issues are also resolved by peptide-based hybrid, co-assembled, metal coordinated hydrogels. Peptide hydrogel biosensors have gained significant attention in recent years due to their remarkable properties. These biosensors exhibit high responsiveness to external stimuli such as pH and temperature. Additionally, they demonstrate good cell adhesion, offer a well-established chemistry for structural modification, possess long-term chemical and mechanical stability, exhibit antifouling properties, and have tunable viscoelastic characteristics. Moreover, the self-healing ability of peptide hydrogel biosensors adds to their appeal and versatility in various applications. Hydrogels can be classified into two main groups based on their chemical makeup: (1) peptide hydrogels with high environmental sensitivity, such as to pH, temperature, or electrical fields, which are used on their own without the aid of bioreceptors or other auxiliary sensing components, and (2) peptide hydrogels with high porosity and large internal surfaces, which are used in conjunction with sensing biomolecules like enzymes, DNA strands, and other molecules.

CONCLUSION

In general, peptide-based hydrogels are composed of nanofibrous networks that are linked together by chemical or physical bonds. These hydrogels exhibit several important characteristics, such as microporous structure, mechanical stability, biocompatibility, tissue-like elasticity, high water content and injectability. Peptide-based hydrogels can be readily

customized by modifying the side chains of amino acids and incorporating specific amino acids into the backbone structure. This flexibility allows for tailoring the hydrogels to meet specific requirements and desired functionalities. Hydrogelation can be achieved through non-covalent self-assembly, resulting in the formation of nanofibers that elongate and ultimately form fibrillar networks in 3D. These networks can trap water, resulting in a self-supporting hydrogel. An example of a non-covalent interaction utilized to create peptide-based hydrogels is the synthesis of a biomimetic physical hydrogel with shear-thinning and self-healing properties. This characteristic is advantageous for minimally invasive procedures and tissue engineering applications involving bioprinting, as the hydrogel can flow and recover its structure after shearing forces are applied. These hydrogels can be injected directly from a needle due to their properties,this can enhance cell viability by minimizing mechanical forces that may otherwise harm cell membranes., making them suitable for stem cell transplantation in tissue engineering. Overall, peptide-based hydrogels have unique properties that make them ideal for a variety of applications in drug delivery, regenerative medicine and tissue engineering. Their ability to be easily customized and their biocompatibility make them a promising material for future research and development in the field.