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Review paper

Surface Property Modification of Hyaluronic Acid Scafold and Loading of Mesenchymal Stem Cells on Hyaluronic Acid Scafold: A Mini Review

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Abstract

Background and aim: Hyaluronic acid (HA) scaffolds have emerged as versatile biomaterials for tissue engineering and regenerative medicine due to their biocompatibility and tunable properties. However, optimizing their surface properties is essential to enhance cell adhesion, proliferation, and differentiation, which are crucial for successful tissue regeneration. This review aims to provide an overview of the methods and strategies employed to modify the surface properties of HA scaffolds.

Methods: A comprehensive literature search was conducted to gather relevant studies on surface property modification of HA scaffolds. Various techniques, including chemical modifications, cross-linking, nanoparticle integration, and bioactive coatings, were analyzed for their effectiveness in altering the surface characteristics of HA scaffolds. Additionally, we explored the impact of these modifications on mesenchymal stem cells loading and proliferation.

Results: The review highlights a range of methods used to modify the surface properties of HA scaffolds, each offering unique advantages and limitations. Chemical modifications enable precise control over surface chemistry, while cross-linking enhances mechanical stability. Integration of nanoparticles imparts additional functionalities, and bioactive coatings promote specific cellular responses. These modifications have demonstrated improved stem cells adhesion, proliferation, and differentiation, ultimately enhancing tissue regeneration potential.

Conclusion: Surface property modification of hyaluronic acid scaffolds is a key strategy in tissue engineering. Various methods offer precise control over surface properties, enhancing cell interactions and tissue regeneration potential. Future research should focus on multifunctional HA scaffolds, advancing tissue engineering possibilities.

Keywords: *Hyaluronic acid, Scaffold, Surface modification, Mesenchymal stem cell*

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Introduction

Hyaluronic acid (HA) is a linear polysaccharide comprised of repeating disaccharide units composed of glucuronic acid and N-acetylglucosamine. These units are connected through beta (1,4) and beta(1,3) glycosidic bonds [1]. Under physiological conditions, HA exists as a sodium salt, giving it a negative charge, and is referred to as sodium hyaluronate. Its molecular weight ranges from 106 to 108 Daltons, depending on the specific enzyme involved in its synthesis [1], [2], [3]. Due to its substantial molecular weight and strong intermolecular interactions, aqueous HA solutions exhibit high viscosity and shear-thinning behavior, rendering them suitable for biomedical applications. HA is naturally present in various organisms, including animals and bacteria. It is most concentrated in the extracellular matrix of connective tissues. Notably, HA is abundant in synovial fluid, skin dermis, and the vitreous humor of the eye, where it serves as a lubricant and shock absorber [1]. The unique properties of HA, such as its biocompatibility and its role in tissue repair, make it a versatile biomaterial with applications spanning from skin augmentation to viscosupplementation for damaged joints and wound healing [4], [5]. Importantly, HA possesses an appropriate half-life in organisms as it undergoes natural enzymatic degradation [1]. Recent research underscores the potential of HA for developing novel biomaterials through various chemical and physical modifications while preserving biocompatibility and biodegradability [6]. One study focused on preparing HA-based hydrogel scaffolds for drug delivery applications [7]. This biocompatible material rapidly forms a gel within minutes, allowing for dry storage and quick transformation into a flexible hydrogel within seconds. Characterization of these hydrogel scaffolds involved scanning electron microscopy (SEM) and spectrophotometric techniques, revealing their favorable porous properties [7].

In tissue engineering, the quest for innovative materials is guided by the need to physically support tissue growth and trigger specific cellular functions [8]. Therefore, the synthesis of biocompatible materials capable of enhancing desired cellular responses and participating in tissue regeneration is of paramount importance [8]. In this context, one study developed biologically active polysaccharide-based composites containing HA [8]. The study employed techniques such as electron microscopy for surface topography, X-ray photoelectron spectroscopy for surface chemistry analysis, and reflectance infrared spectroscopy to assess chemical composition. The results indicated that these composites hold promise for tissue engineering and wound healing applications [8]. Surface properties of biomaterials have gained significant recognition due to their multifaceted roles [9]. The surface plays a pivotal role in biomedical applications of biomaterials, necessitating various approaches for surface modification, including physical, chemical, mechanical, and laser treatments [9], [10], [11], [12].

Methods of Hyaluronic Acid Scaffold Modification

Modifying HA scaffolds is a common and essential practice in the field of tissue engineering and regenerative medicine. These modifications aim to fine-tune the properties of HA scaffolds to suit specific applications and optimize their performance. Here, we delve into various methods of HA scaffold modification, each offering distinct advantages:

- *Chemical Modification*:

Chemical modification involves altering the chemical structure of HA to introduce specific properties. Common chemical modifications include esterification, cross-linking, and conjugation with molecules like peptides or proteins. These modifications can enhance the mechanical strength, stability, and biological activity of HA scaffolds [13].

- *Cross-Linking*:
Cross-linking is a technique that strengthens HA scaffolds' mechanical properties and resistance to degradation. This can be achieved through different approaches, including chemical cross-linking with agents such as glutaraldehyde or physical cross-linking via temperature or pH variations. Cross-linking allows for precise control over scaffold degradation rates and stiffness, which is crucial for tissue engineering applications [14].
- *Hydrogel Formation*:
HA can be modified to form hydrogels, which are three-dimensional networks of polymer chains capable of retaining a substantial amount of water. This modification creates an environment that mimics the natural extracellular matrix, providing an ideal milieu for cellular growth and tissue regeneration [15].
- *Incorporation of Biological Molecules*:
Biological molecules like growth factors, cytokines, or drugs can be incorporated into HA scaffolds to promote specific cellular responses, facilitate tissue regeneration, or enable controlled drug delivery. These molecules can either be physically entrapped within the scaffold or chemically linked to HA, depending on the desired application [16].
- *Nanoparticle Integration*:
Integration of nanoparticles, such as metal, ceramic, or polymer nanoparticles, into HA scaffolds can impart unique properties. This includes enhanced electrical conductivity, improved mechanical strength, or superior drug delivery capabilities. These modified HA scaffolds find applications in various fields, including neural tissue engineering and drug delivery systems [17].
- *Microfabrication and 3D Printing:*
HA can be employed in microfabrication and 3D printing techniques to create scaffolds with precise structures and controlled dimensions. These advanced manufacturing methods offer unparalleled control over the scaffold's architecture, enabling tailored solutions for specific tissue regeneration challenges [18].
- *Surface Modification*:
Surface modification of HA scaffolds is a strategic approach to enhance cell-scaffold interactions. Techniques such as plasma treatment or the application of bioactive coatings can be employed to modify the scaffold's surface properties. These modifications encourage cell adhesion, proliferation, and differentiation, ultimately facilitating tissue regeneration [19].
- *Degradation Control*:
Controlling the degradation of HA scaffolds is vital to align their lifespan with tissue regeneration rates. This control can be achieved by adjusting the scaffold's molecular weight or cross-linking density. Fine-tuning degradation rates ensures that the scaffold provides structural support for tissue growth without degrading too quickly or too slowly [20].
In summary, HA scaffold modification is a multifaceted field offering a diverse range of techniques to optimize these biomaterials for specific applications. Researchers and engineers continue to explore and innovate in this area to develop HA scaffolds that meet the ever-evolving demands of tissue engineering and regenerative medicine.
The membrane of mesenchymal stem cells (MSCs) is comprised of a lipid bilayer embedded with various types of proteins. This lipid bilayer consists of two layers of phospholipid molecules, with hydrophobic tails facing each other and hydrophilic heads facing outward. The membrane-bound proteins serve a multitude of functions, including cellular signaling, transport, and structural support. In a specific case study, mesenchymal stem cells derived from bone marrow were cultured on scaffolds made of graphene foam and transplanted into surgically created wounds in rats. The

outcomes of these studies demonstrated that the transplanted mesenchymal stem cells exhibited proper engraftment and proliferation on the scaffold, leading to reduced scar formation and enhanced skin quality during wound healing. Additional experiments have showcased that mesenchymal stem cells have been successfully cultured on various scaffolds for skin regeneration in cases of burns or trauma. These scaffolds, serving as temporary carriers for cells, offer numerous advantages that enhance regenerative potential when compared to direct cell injection. Among the most significant advantages is providing a substrate for cell adhesion, a crucial factor in cell viability that reduces cell death. Another advantage lies in the promotion of angiogenesis. Porous scaffolds facilitate the transport of nutrients and growth factors, thereby expediting angiogenesis. In contrast, situations where a substrate for cell adhesion is absent and hindered transport of nutrients and growth factors results in slow angiogenesis, leading to minimal treatment efficacy [21]. In another study, a three-dimensional porous hyaluronic acid scaffold was synthesized and evaluated for its ability to induce the proliferation and chondrogenic differentiation of adipose-derived mesenchymal stem cells. Research findings highlighted the potential of hyaluronic acid scaffolds as suitable platforms for expanding adipose-derived mesenchymal stem cells [22]. Furthermore, research outcomes exploring the chondrogenic differentiation potential of mesenchymal stem cells within hyaluronic acid hydrogels suggested that mesenchymal stem cells may interact with hyaluronic acid through cell surface receptors, and these hydrogels could impact mesenchymal stem cell proliferation and differentiation [23]. This implies that using hyaluronic acid as a stem cell substrate can be complex due to the biochemical effects of the scaffold on cellular properties [24].

In summary, tissue engineering leverages the principles of natural sciences and engineering techniques to create substitutes that, when placed in the body, effectively contribute to the reconstruction and repair of damaged tissues in a more natural manner. In this context, the use of modified scaffolds that can accommodate stem cells effectively and provide a conducive environment for their proliferation is of paramount importance. This approach offers significant advantages, such as eliminating the need for graft donors, thus avoiding complications related to graft donor sites, and producing tissue with the desired clinical dimensions under laboratory conditions, starting from a small-sized tissue sample. Therefore, despite the high costs associated with the equipment and materials used in this technique, the increasing adoption and expansion of tissue engineering technology can be attributed to its substantial benefits. Materials employed in tissue engineering, which act as scaffolds, must meet several criteria, including non-toxicity, tissue compatibility, non-carcinogenicity, sterilizability, and suitability for clinical applications. Additionally, they should possess essential physical properties, such as permeability, stability, elasticity, and flexibility, along with the ability to absorb and retain growth factors [25].

Advantages and Challenges of Previous Research

The use of hyaluronic acid offers multiple advantages in cell therapy, particularly in the proliferation and differentiation of stem cells and their transplantation to areas of wounds and injuries, especially for wound and burn treatment. However, the application of this polymer as a suitable scaffold for the transfer of stem cells to damaged tissue areas has faced significant challenges primarily stemming from the topographical issues of the polymer [26], [27], [28], [29]. On the other hand, studies indicate that surface topography affects cell adhesion. Biological tissues in the body possess various surface morphological characteristics, and various micro-morphological features have specific effects on cell properties, such as proliferation and viability [30], [31]. Surface roughness is an important factor influencing cell behavior. For example, in

human venous endothelial cells, increasing surface roughness at the nanometer scale can enhance cell adhesion and growth on rough surfaces [32], [33]. Additionally, the mechanical properties of the substrate's surface have significant effects on cellular structure and protein expression in cells [34], [35]. Surface wettability (hydrophobicity and hydrophilicity) can also impact cell adhesion and, consequently, cell proliferation [36], [37]. In this regard, previous studies on the surface modification of hyaluronic acid scaffolds, which alter the polymer's topography, and subsequent studies on cell therapy are very limited. Today, on one hand, mesenchymal stem cells derived from human adipose tissue are significantly relevant in biomedical engineering, especially for skin wound healing. On the other hand, hyaluronic acid polymer, due to its unique properties, has found widespread use in cell therapy to improve skin elasticity, moisture, and wrinkle reduction. However, due to the inadequate surface properties of hyaluronic acid-based scaffolds, the proliferation and viability of stem cells have been problematic in many cases. This may impede the success of cell therapy [38], [39]. Previous studies on hyaluronic acid have mainly focused on investigating its physicochemical properties, emphasizing chemical and physical characteristics of the polymer. Fortunately, surface modification techniques have made it possible to modify the topographical, mechanical, morphological, roughness, and wettability properties of polymers and scaffolds. This allows for the enhancement of cell adhesion and proliferation on modified surfaces. In fact, one of the most significant challenges facing tissue engineering researchers in cell therapy is ensuring the stability and viability of cells on scaffolds, preserving their natural morphological and physiological properties to an acceptable extent. Unfortunately, this has proven to be a considerable challenge in experimental cases.

Conclusion

In this context, studies regarding the viability of mesenchymal stem cells, derived from adipose tissue, when cultured on hyaluronic acid scaffolds, especially modified hyaluronic acid scaffolds, are exceedingly limited. Given the previous research background on this topic, to the best of our knowledge, no research has been conducted on the viability of mesenchymal stem cells derived from adipose tissue cultured on modified hyaluronic acid scaffolds. Based on this, as the use of hyaluronic acid for transferring stem cells to damaged areas, especially skin wounds, is of special importance, this research aims to first prepare hyaluronic acid as a polymeric scaffold. Subsequently, the surface of the polymeric scaffold is modified, and ultimately, mesenchymal stem cells derived from human adipose tissue are cultured on this modified polymer. The viability of these cultured cells on the modified hyaluronic acid polymer is compared with the viability of adipose-derived mesenchymal stem cells cultured on unmodified hyaluronic acid polymer. It is expected that surface modification will increase the viability of mesenchymal stem cells. This can justify the use of modified hyaluronic acid polymer for transferring mesenchymal stem cells to damaged areas, especially skin wounds, in the form of wound dressings.

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Conflict of interests

The author declares that there are no competing interests.

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