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The Application and Research Progress of Hydrogel Loading Stem Cells in Bone Regeneration

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Abstract:

Nowadays, osteogenesis technology is one of the hot topics in the current orthopedic field. Globally, improving the stability and efficiency of bone regeneration has become a problem that society has been studying. Due to its good tunability, compatibility, and combination ability, hydrogel is gradually applied to bone regeneration technology. Stem cells can differentiate and copy, and have certain clinical potential in the regeneration and treatment of bone tissue. In recent years, many new types of hydrogel materials have been developed, and some are expected to be applied to clinical trials. Likewise, stem cell therapy is also at the forefront of medical research today. Many types and good characteristics make part of them smoothly applied to bone regeneration. In this review, the experimental data and research results of cutting-edge and developmental hydrogel-loading stem cell technology in the field of bone regeneration are collected, and the development and application of this technology will be summarized. The article contains the nature, preparation, and development of hydrogel; discusses the effective role of hydrogel and stem cells in the field of bone regeneration; combined with biological factor therapy and the future development of new technologies. The article also focuses on the development and application of different types of new composite hydrogel. At the same time, these hydrogels make bone regeneration technology more efficient and stable through stem cells with different characteristics. Therefore, this review tells the far-reaching impact of this technology on the future of bone regeneration technology, skeletal development, and even organizational repair.

Keywords: Bone regeneration; hydrogel; stem cells; mesenchymal stem cells.

1. Introduction

In recent years, skeletal disease has seriously impacted human health due to its complex diversity and specific instability. Nowadays, the frequency of fractures caused by osteoporosis among people over 50 years of age due to osteoporosis is as high as every 20 seconds [1]. In addition, osteoarthritis leads to a wide range of degenerative diseases, affecting more than 303 million people around the world [2], and it is huge in terms of the number of diseases and frequency of disease. Many skeletal diseases are not fatal, but they have tortured the body for a long time, causing serious threats to global social health. More importantly, the repair and treatment of skeletal disease have not yet achieved the ideal effect. The efficiency of biological factors applied in the process of bone regeneration the stability of the required materials, and even the time cycle of patient recovery and the problem of economic costs did not meet the needs of society. Fortunately, the development of this field has been relatively perfect for the new field that society thinks. This field contains advanced and efficient material science, stem cell science, biology, clinical transformation, etc., to achieve regeneration of complex bone tissue and organ systems [3]. Thus, bone regeneration is bracketed through stable and appropriate biomolecular materials, combined with high-efficiency cytokines, and uses its characteristics to repair. This method has become a solution to bone regeneration problems in recent years.

The bone repair process is a complex dynamic process. It is not just a simple bone formation and bone absorption process but also involves the interaction between many molecular signal pathways, combining biological technology and engineering methods to regenerate bone tissue. In terms of brackets, water gel has become a good bone regeneration material due to its excellent structure, biocompatibility, and active factors. The combination of hydrogels through different preparation methods and a variety of materials produces emerging composite hydrogels and new biopiplazing hydrogels [4]. According to studies, hydrogels can be processed into microdomains with cells or active factors. In terms of the cell types involved, stem cells can regenerate human tissues due to their differentiation and replication potential and have clinical potential in fracture healing. Therefore, hydrogel-loaded stem cells can work together on bone regeneration, and by enhancing cell-cell interactions and promoting the exchange of substances, bone tissue can be repaired in a more targeted manner.

With the feasibility of hydrogel-loaded stem cell technology proven by experiments, it is gradually being applied in clinical treatment for bone regeneration and has become a social trend in bone repair treatment. The research is complex and lacks a systematic review to guide development. Hence, this review summarizes the new stable hydrogel types and the highly efficient stem cell types they carry, and organizes the optimization effects of the combination of the two technologies on bone regeneration, hoping to provide a reference guide for the response and treatment in the field of bone regeneration and bone repair. All in all, this article will introduce and summarize the current status of bone repair from the stent hydrogel composition, the type of stem cell type, the hydrogels of the load stem cells, the development of bone regeneration research technology, and challenge thinking.

2. Scaffold Hydrogel Composition

2.1 Development and Preparation of Bone Regeneration-related Hydrogels

2.1.1 Properties of hydrogels and the need for them in bone regeneration

Hydrogel is a polymer material with a three-dimensional network structure using water as the dispersion medium. Hydrogels contain up to 90% water, are very soft in texture, and have excellent diffusion and biocompatibility. Hydrogels can therefore be loaded with different materials and are ideally suited for cell inoculation and tissue implantation techniques. Structurally [5], hydrogels can mimic the basic three-dimensional biological, chemical, and mechanical properties of natural tissues due to their physical properties similar to biological tissues. These abilities are ideal for medicine to do basic research. In addition, the numerous side groups of the polymer chain can alter the way the hydrogel interacts with the cells. Therefore, hydrogels are an excellent class of biomaterials because of their adjustable biological and chemical properties as well as their mechanical properties, which allow them to change their shape according to different needs.

The specificity of bone repair makes it necessary for the repair process to be carried out more safely and efficiently and for the materials required to be more stable and targeted. Thus, for bone regeneration to be successfully achieved, hydrogels need to fulfill their specific requirements [4]. Firstly, the hydrogel needs to be able to be loaded with growth-related factors such as nanoparticles, characterizing drugs, and active cytokines and the quantitative release of these factors can be controlled to meet the needs of bone healing. Secondly, hydrogels can be compounded to be moderately stiff and conductive for targeted repair of different types of bone defect sites. Thirdly, the base material has a strong in vivo degradation ability, so as not to hinder the formation of new bone, and to prevent inflammatory reactions caused by residual. Fourthly, to avoid mechanical defects during regeneration, the hydrogel scaffold must have a good load-bearing capacity to avoid damaging the bone tissue. Fifthly, the porosity should be of moderate size to help control the release rate of internal bone-promoting factors and enhance the stability of the novel structure. By meeting these criteria, hydrogels can be used as effective biomaterials for bone regeneration.

2.1.2 Classification of hydrogels and the evolution of their use in bone tissue therapy

In the last decade, hydrogels have gone from being developed to expanding in variety and technology, to gradually being used in the treatment of various diseases. Its development has been rapid and relatively complete. Hydrogels can be classified according to different classifications [6]. Firstly, according to the classification of material source, it is divided into natural hydrogel and synthetic hydrogel. Natural classes are mainly synthesized from collagen and fibrous proteins, such as collagen and hyaluronic acid. The synthetic category consists mainly of hydrophilic polymers cross-linked, such as polyethylene glycol and polyacrylic acid. Secondly, the polymers are classified according to their composition into homopolymers, copolymers, and polymers. Characterized by differences in the way the polymers are crosslinked and composed and a gradual increase in the number of monomers. Thirdly, the shapes are very different when categorized in terms of either physical structure or chemical composition. Fourthly, classified from the different ways of cross-linking, physical cross-linking is produced by hydrogen bonding, ionic interactions, etc.; chemical cross-linking builds up a three-dimensional network through chemical bonding to make its structure more stable. In addition to the above classifications, it is still possible to classify conventional hydrogels and smart hydrogels in terms of their response to the environment. Conventional hydrogels as the name suggests cannot sense external conditions and inevitably do not exhibit different solubility behaviors. On the contrary, emerging smart hydrogels can adapt to their environment by changing their morphology through external stimuli, such as temperature, PH, and magnetic field, and have great potential for application in the field of flexible sensing.

Meanwhile, composite hydrogels, which are emerging together with smart hydrogels, have a part of them that not only has the capability that smart hydrogels have but also has a more stable structure. With continuous research, it is gradually being applied to treatments in the field of bone regeneration. From the timeline [4], in 2018, the American Chemical Society reported Ag/Cu nanoparticle composite hydrogels. In 2019, polymer microsphere composite hydrogels and bioactive glass nanocomposite hydrogels emerged. In 2020, hucmsc-derived exosome composite hydrogels were introduced. In 2021, novel molecular technologies are emerging, namely reduced graphene oxide-reinforced hydrogels and bisphosphonate-modified supramolecular hydrogels. Then today, in 2023 and 2024, stromal cell-derived factor-1α composite hydrogels, piezoresistant MXene/silk fibronectin nanocomposite hydrogels, and other composite types of hydrogels are gradually being developed. The emergence of these new composite hydrogels has made an important contribution to the development of the field of bone regeneration.

2.1.3 Various preparation techniques for hydrogels

It is most commonly prepared by cross-linking, which can be divided into two categories: physical cross-linking and chemical cross-linking. Different preparation methods can produce hydrogels with different properties and structures. Thus, different preparation methods can yield hydrogels for different needs. The hydrogel, when crosslinked in both ways, enhances the scaffold to form a three-dimensional porous structure that can create a suitable environment for cell growth and multiplication. The physical and chemical crosslinking aspects are described below [4]. One way of physical cross-linking is through hydrogen bonding cross-linking by introducing polymeric hydrophobic monomers or hydrophobic groups to form cross-linked structural domains. This method is usually injectable and is better suited for biomedical applications. A more common method is ionic interactions cross-linking, a method where the reaction conditions are relatively mild and the strength of the hydrogel can be adjusted in response to changes in ionic concentration. For example, hominate salts can form strong interactions with Ca^{2+}, Fe^{3+}, and Al3+ so the addition of metal ions usually enhances the strength of the gel. Physical cross-linking has the advantages of good biocompatibility, better tunability, and degradability. On the contrary, the weak stability due to poor interaction forces between gel molecules has become one of the loopholes of this method. Chemical cross-linking usually occurs through the chemical reaction of polymer functional groups with molecular monomers or cross-linking agents to form a cross-linked network. The combination of free radicals between the double bonds can efficiently generate a stable hydrogel network. Especially in alkaline environments, the imine bond formed by the aldehyde group and the amino group will be reorganized and transformed under stimulation, which gives the hydrogel excellent injectability and self-healing properties. Chemical cross-linking provides better mechanical strength and structural stability, but the legacy of unreacted monomers can easily lead to toxic reactions and adverse effects in organisms.

In recent years, 3D-printed hydrogel has emerged as a novel material that can well regulate the distribution and mechanical structure of osteoblasts. Due to the special characteristics of this material, researchers have been continuously improving methods and mining inks. However, 3D-printed hydrogels are too demanding in terms of materials, and some of the natural or synthetic compound materials cannot be successfully applied. Therefore, most cases use composite hydrogels containing modifiers, which are commonly nanoparticles, inorganic ions, and related polymers. At the same time, the composite hydrogels can interpenetrate each other thus improving their mechanical properties. The influence of the environment is also important, and the hydrogel ink is usually homogenized by adding heat and stirring. To prevent the ink from becoming too viscous, rheology modifiers such as ceramics, metallic nanoparticles, and other materials are often added to the ink to regulate its printability. In addition, to enhance the cross-linking rate between each branched layer, the cross-linking rate can be increased by UV cross-linking. Nowadays, the preparation method of this material is relatively simple and can be combined with the traditional preparation of hydrogel technology to avoid the disadvantages of a single preparation method and lag behind the increasing demand [7].

With the surge of interest in more novel hydrogels, conductive agent preparation, radiation technology preparation, and injection preparation are gradually being explored, especially for injectable hydrogels. This innovative injection method [4] does not require surgery to prefabricate the scaffold of hydrogel but rather injects it into the site of the bone defect through a minimally invasive procedure. The hydrogel material is in close contact with the defect site to better perform the healing function of osteogenic factors. These emerging technologies are still under constant experimentation and advancement and will surely become the future development and trend.

2.2 Introduction to New and Superior Hydrogels

2.2.1 Hydrogel microspheres and nanogels

With the development of nanomedicine technology, drug delivery has changed, with nanocarriers enabling more targeted and efficient delivery. Hydrogel microspheres (microgels) and nanogels, as a class of particulate hydrogels, have an inner diameter smaller than that of a syringe needle, making them easier to inject. Apart from that, the large relative surface area of the two not only allows them to easily pass through all types of tissue barriers but also enhances their ability to act on areas in need of regeneration. Also, natural clearance after treatment becomes easier.

Hydrogel microspheres have been widely used in the biomedical field. Gan et al [8] encapsulated MCS-EV in alginate hydrogel microspheres and also added a gelatin barrier to the outer layer, which was thought to avoid the corrosion of microspheres by gastric acid. The use of this composite microsphere structure in a mouse model of inflammatory bowel disease showed favorable therapeutic effects. In addition to this, microsphere therapy is more effective in repairing post-infarct cardiac tissues and weaker gastrointestinal tissues, which have been experimentally proven.

Nanogels have the properties of both nanoparticles and hydrogels. There are many types of nanogels, such as stimuli-responsive nanogels, multifunctional nanogels, and so on. Light responsiveness in the former, for example, can slow the progression of cancer. Shin et al [9] prepared a nanogel (CFN-gel) with rockweed polysaccharides as the base substance and combined it with photodynamic therapy to monitor colorectal cancer. This gel irradiates accurately and not only reduces the risk of damage to non-cancerous tissues but also monitors the progression of cancer and inhibits the rate of tumor growth. In addition, the polymer-based nanogels in the latter can be sensitive to pH, temperature, and infrared-ultraviolet light. Sahu et al [9] created a chitosan nano gel containing bleomycin, which is PH-sensitive and a self-assembling and biodegradable polymer. Besides, chitosan is hydrophilic, which enhances the diffusion of the drug. This gel is often used to target the dermal layer of the skin, allowing for targeted treatment.

Nanogels and microgels can be synthesized in a variety of ways and both can be synthesized using different routes depending on the different properties required. Thus, such hydrogels offer promising outputs in the delivery of drugs and can also be important in the diagnosis and prediction of diseases. In any case, some gels have been detected to contain small amounts of toxicity during treatment, and the adverse effects of prolonged presence in the body are not yet known. It is undeniable that such hydrogels may become the next generation of drug delivery systems, revolutionizing the treatment of various diseases.

2.2.2 Composite hydrogels of natural polymers combined with synthetic ones

Natural polymers are artificially post-synthesized into composite hydrogels, which are composite substances formed when natural polymer monomers are artificially polymerized, thus improving cell activity and adhesion. This type of hydrogel combines the excellent biocompatibility of natural hydrogels with the good mechanical properties of synthetic hydrogels to obtain a more versatile composite hydrogel.

Polyglycolic acid composite hydrogels have good biocompatibility, degradability, and plasticity. Lohan et al [10] conducted ex vivo experiments using polyglycolic acid and found that this substance can be used to repair cartilage tissue. Meanwhile, some researchers have implanted polyglycolic acid-loaded bone marrow mesenchymal stromal cells into the area of cartilage defects in rabbits and also achieved the desired results.

Gelatinized methacrylate (GelMA), which consists of methacrylic anhydride and gelatin, is a popular photosensitive hydrogel in recent years. Chen et al [11] created a directed hydrogel microfibre scaffold using electrospinning combined with GelMA hydrogel. It directs cell movement and axonal extension for targeted guidance of bone marrow regeneration. In addition, this hydrogel is a suitable environment for neuronal cell growth and metabolism. Such hydrogels not only promote the migration and differentiation of neural stem cells but also the formation of peripheral blood vessels.

Bio-natural polymers under synthetic hydrogels can be loaded with cytokines and stem cells, etc., which work together to repair the site of bone damage. Although there are some limitations to the use of polymers, they are bound to become one of the popular choices for bone repair.

2.2.3 Injectable self-healing and responsive hydrogels

Self-healing hydrogels are prepared based on the principle of non-covalent interactions. The advantages of these hydrogels are a high degree of self-healing ability to adapt to sites of injury and protect them, as well as a strong physical stability to encapsulate carried drugs or biological factors, which can be released at a controlled rate [8]. Stimulus-responsive hydrogels are smart hydrogels developed by researchers and scholars by selecting different types of materials combined with various physiological and biochemical stimuli. The advantage of this type of hydrogel is that it can release a large number of active substances used to aid in the repair of the specific body part that issued the response [8]. In addition, the method of injection allows for a more rapid and stable action on the regenerated area.

To obtain hydrogels that are both responsive and self-healing, researchers have developed PH-responsive injectable hydrogels, which are novel supramolecular hydrogels crosslinked with UPy-modified PEG [12]. This hydrogel can change its shape depending on the environmental pH and is in liquid form at a pH higher than 8.5 and in gel form at a neutral pH. This results in rapid hydrogel formation upon injection of the alkaline polymer precursor solution into a neutral solution. Simultaneously, the experimental results of the rheological cycling test proved the excellent healing effect of this hydrogel. Hence, these types of hydrogels can not only improve the mechanical resistance of hydrogels through self-repair but also deliver drugs to the repair site more accurately.

3. Loaded Stem Cell Types

3.1 Regenerative Stem Cell Types

A stem cell is a multipotential cell in the human body with the ability to differentiate and proliferate. According to the differentiation ability, stem cells can be classified into totipotent, pluripotent, and monopotent stem cells. Totipotent stem cells have the highest differentiation capacity and are capable of dividing and differentiating into whole organisms, and such cells have an important role to play in regenerative medicine. Pluripotent stem cells can differentiate into several different cell types, which narrows the range of differentiation but allows specialization in specific cell lineage types. Monopotent stem cells can only differentiate into a certain type of cell, but they are also one of the potential candidates for regenerative medicine due to their repetitive dividing properties [13].

Stem cells are widely used in tissue engineering and cell therapy due to their good regenerative properties, and are generally obtained from embryonic tissues or specific parts of adult organisms. Skin stem cells derived from the skin, which help regenerate the skin’s keratin protective layer; neural stem cells derived from neural tissues, which promote the regeneration of endogenous neurons; hematopoietic stem cells with their high degree of self-renewal and replication; and highly efficient regeneration in the restorative aspects of cosmetic and anti-aging as well as improvement of the cardiovascular system. All three of these stem cell types have contributed in no small way to the field of medical regeneration.

More importantly, most of the stem cells that play a role in the field of bone regeneration are derived from fat and bone marrow. Mesenchymal stem cells come from a variety of sources and can also differentiate into a variety of cell types, such as chondrocytes, osteoblasts, and so on. Mesenchymal stem cells can exist in all tissues and support the survival and growth of a wide range of cells. Due to its targeting ability and directional differentiation, as well as its ability to secrete a variety of cytokines and modulate the immune environment, it is now used to treat a variety of diseases, such as bone defects and cartilage injuries in skeletal disorders. In addition, adipose stem cells derived from adipose tissue can stimulate and repair tissue structure and function by secreting growth factors. It has been clinically demonstrated experimentally that adipose stem cells can promote self-repair and regeneration of the organism, and not only can they be obtained in significant quantities, but also in a relatively convenient manner, which is considered to be a very promising class of stem cells in the field of bone regeneration today [14].

3.2 Introduction to Loaded Highly Efficient Stem Cells

The majority of how stem cells are used in bone regeneration therapies is by direct application to damaged tissue. A better way is through loading materials or going in the form of extracellular vesicles for clinical application. The manner of loading will improve the degree of secretion and suitable release of growth factors and active molecules. Existing studies have identified two main stem cell types, mesenchymal stem cells (MSCs) and adipose-derived stem cells (ADSCs), that can be both loaded on top of hydrogels and well suited for bone regeneration techniques.

3.2.1 MSC-loaded hydrogels

MSCs possess good induction of differentiation and are usually implanted directly into the bone regeneration process in the clinic. However, conventional injection methods cause problems such as cell death, and a stable and suitable delivery system is needed today. Studies have shown that hydrogels with excellent stability and suitable mechanical strength are well-suited as scaffolds to protect cells. An example is the use of DNA supramolecular hydrogels, which can resist shear and friction forces from inside and outside the body and are one of the suitable choices for loading hydrogels [15]. At the same time, the selection of the type of MSCs is also very important. For example, bone marrow MSCs, dental pulp MSCs umbilical cord MSCs, etc., all promote cell proliferation and differentiation and can induce angiogenesis and cartilage repair. Therefore, different types of MSCs combined with excellent composite hydrogels are emerging technologies applied in the field of bone regeneration.

3.2.2 ASC-loaded hydrogels

ADSCs are commonly induced to differentiate towards osteoblasts in bone tissue engineering, thereby enhancing the rate of bone regeneration. To achieve this, hydrogels encapsulated with ADSCs can be injected into the regeneration area to enhance stem cell differentiation both in vivo and ex vivo. Similarly, hydrogels that are structurally stable and can promote osteogenic differentiation of ADSCs can be used as scaffolds. An example of this is cross-linked chitosan hydrogels, which make the structure more loose and porous, thus enhancing cell-cell interactions and allowing better access to oxygen and substances to the growing cells [16]. So, osteogenic differentiated adipose-like stem cells with strong osteoinductive properties can be firmly attached to suitable hydrogel scaffolds, which becomes one of the good application prospects to enhance the effect of bone regeneration.

4. Development of Hydrogels Loading Stem Cell for Bone Regeneration Applications

Hydrogel, with its development and research, possesses the excellent property of being able to act as a bone regeneration technique, but its lack of bioactivity has been an unavoidable disadvantage. In recent years, it has become a mainstream trend for researchers to further enhance the functionality of hydrogels by piggybacking them with drugs or active factors.

4.1 Important Types of Applications

4.1.1 Light-conditioned Gel-MA hydrogel loading ADSCs

The researchers and their team [17] experimented with the effect of loading adipose-derived stem cells (ADSCs) with gelatin methacrylate (Gel-MA) hydrogel under photobiological conditions on bone regeneration and osteogenic differentiation in organisms. The team chose the popular Gel-MA hydrogel. The advantage of this hydrogel is that it is a material for a new bioprinting system, and it can be added with photoinitiators for photocross-linking to promote regeneration. Furthermore, the full name of photomodulation is called photobiomodulation (PBM), which refers to the use of incoherent or co-phase light sources for regenerative purposes to externally stimulate the production of ATP in cells thereby enhancing self-healing at the tissue level. The experimenter divided 36 pre-treated (6 mm bone defects in the same location) rats into three experimental groups: blank control group, Gel-MA hydrogel group, and Gel-MA + ADSCs hydrogel group. Half of the mice in each group were randomly irradiated with polychromatic light from the near-infrared region, that is, subjected to PBM application. Tissue scans were evaluated macroscopically and microscopically after 20 weeks of ensuring the same conditions of administration. By observing the values of the levels of mineralization mechanism formation (MMF) (higher values represent better regeneration), it can be seen that the third group has a higher average, while the samples with non-PBM have a lower average than the samples with applied PBM. This experiment demonstrated that both ADSCs-loaded Gel-MA hydrogels and regular application of polychromatic light for PBM were beneficial for bone regeneration. On top of that, NIR light seems to have an osteoinductive effect on ADSCs, causing more growth factors to be released to act on the regeneration site after osteogenic differentiation. Moreover, the ADSCs + Gel-MA hydrogel system can be used to directly establish the location of this system in the intended clinical treatment using in-situ bioprinting methods to promote bone regeneration more precisely.

4.1.2 PCL/nHA + HPCH loading MSCs

The authors and their team [18] developed thermosensitive hydroxypropyl chitin hydrogel (HPCH)-loaded MSC scaffolds combining 3D printed poly(ε-caprolactone)/nanohydroxyapatite for bone regeneration as a new technique. The chitin derivative hydroxypropyl chitin is commonly used in bone regeneration techniques because of its excellent biocompatibility. However, due to their poor mechanical properties, they are usually injected into other substances to form hybrid scaffolds to enhance mechanical stability. PCL is a non-toxic and easily degradable polymer that can be printed at low temperatures. HA particles stimulate macrophages to release growth factors for blood vessels and bones. Consequently, it is feasible to cryoprint nHA combined with PCL into porous scaffolds to enhance force stability. Even more importantly, thermosensitive hydrogels can quickly migrate to the desired site at low temperatures, and then convert to gel form when the temperature rises back up. The article synthesizes a more stable and reproducible HPCH that binds sturdy scaffolds and carries MSCs thereby releasing growth factors. To verify feasibility, the degree of preservation of activity of MSCs and the mechanical properties of the scaffolds were tested. By this, the ability of nHA to stimulate the secretion of growth factors was also determined by matrix gel assay, and appropriate numbers of MSCs were cultured to enhance the immunomodulation of peripheral macrophages. After designing the complete scaffold, biological experiments, and various tests were performed on it. Looking at the post-experimental data and the imaging characterization of the scaffolds, the observers found that the hybrid scaffolds exhibited better mechanical properties and biofactor viability. MSCs successfully modulated the inflammatory response to HPCH and promoted bone differentiation. Thereby, the combination of PCL/nHA + HPCH-loaded MSCs achieves optimal bone regeneration. The excellent degree of compression resistance, the ability to induce growth factors to promote bone regeneration, and the activation of macrophage regeneration and secretion of this hybrid scaffold are outstanding, and it is a good demonstration of a novel bone regeneration scaffold.

4.1.3 3D printed composite scaffold hydrogel loading hDP-MSCs

This study [19] presented a 3D printed nanohydroxyapatite (nHA)/β-tricalcium phosphate (β-TCP)/collagen composite scaffold hydrogel. This hydrogel was combined with human dental pulp-derived mesenchymal stem cells (hDP-MSCs) to verify if there was an efficient positive effect on bone regeneration. It is well known that 3D scaffolds are one of the best technologies to achieve bone tissue reconstruction. HA/TCP with excellent osteoconductivity, suitable connectivity, and porosity combined with efficient 3D printing technology is beneficial to promote stable bone growth. The incorporation of HA promotes the stability of the scaffold and the involvement of TCP enhances the biodegradability. Collagen can provide a natural microenvironment for tissue cells and help bone regeneration to proceed smoothly. Parallel to this, combining ceramics and collagen not only results in a more composite and stable mechanical property, but also promotes cellular enhancement and differentiation, ultimately leading to bone regeneration. That is why the experimenter found the best ratio of HA/TCP to optimize the formulation of the printing ink and piggyback the hDP-MSCs. hDP-MSCs can differentiate into a wide range of cells by osteogenic differentiation and also modulate immunogenic effects. Ultimately, this scaffold was trialed in a rabbit model of mandibular defects with a critical size. After a suitable period, the investigators verified the angiogenesis, and the expression of induced osteogenic genes and analyzed the parameters of the scaffold to assess the performance. Paradoxically, all the criteria were met and the results were on target. It is concluded that this composite scaffold loaded with mesenchymal-derived stem cells has great potential as one of the future applications of bone regeneration. An important step in the future development of bone regeneration technology.

4.2 Future Trends, Challenges, and Reflections

Hydrogel-loaded stem cell technology has gradually entered the field of regenerative technology, and more and more ideas and experiments have been realized. In terms of future trends in the field of regeneration, the development of better composite hydrogel materials to carry suitable biofactors, stem cells, drugs, and other substances will be one of the most important trends. In addition, newer cell delivery systems will be developed, such as the existing exosomes and extracellular vesicles. Newer technologies such as nanoparticle carriers, optimized stem cells, viral carriers, etc. are expected to become new cellular therapies in the field of bone regeneration and even regeneration.

For the current scenario, the suitability of the selected materials becomes the most important challenge [2,20]. Firstly, some materials cause inflammatory reactions during regeneration, for example, PMA hydrogel has been banned by the State Drug Administration due to inflammation during breast augmentation. Secondly, hydrogels require increased bioadhesive properties; without good adhesion, even regenerated bone cannot be attached to the surrounding tissue. Thirdly, there is still a need for advances in the adaptability and personalization of materials, such as 3D printed scaffolds that are tough but require soft tissue for some defective areas, or example, difficulties such as cumbersome steps in the preparation of materials and cost control. Therefore, the authors have some thoughts and suggestions. First of all, in the future, materials, drugs, and protocols for treatment should be provided according to individual patient differences, and even personalized biomarkers should be developed for detecting or preventing diseases. Not only that, try to combine key factors in the human body and use environmental variability to combine therapies. For example, injecting hydrogel-loaded stem cell systems under hypoxic conditions may stimulate hypoxia-inducible factor (HIF-1α) to further target regenerated areas rapidly, and may even activate the surrounding immune system to accelerate the entire process of regeneration and the degree of defense of weaker areas. In addition, even better biomaterials may be developed and even contain targeting properties, thus combining more efficient substances for treatment. In the end, it is important to keep improving under the feedback data of real clinical applications, to try to meet the needs of the mass population while ensuring its safety, and to carry out the application of bone regeneration technology more stably and efficiently, and even more perfectly in other fields of medicine.

5. Conclusion

In this review, the article focuses on collating the existing developments and applications of different types of hydrogel-loaded stem cell technologies in the field of bone regeneration. In doing the work collation, ensure that the full text is closely related to bone regeneration techniques. First of all, the article is designed to ensure the selection of hydrogel scaffolds capable of meeting the needs of bone regeneration by familiarising themselves with the structure and properties of hydrogels. In fact, hydrogel materials are widely used in tissue engineering technologies due to their good biocompatibility, biodegradability, and bio tunability, among other properties, and can undoubtedly act as loading materials for new cell growth and release of active factors. Similarly, the article also highlights the different properties that different types of hydrogels have by describing the many ways in which they can be classified. According to recent information, it has been found that most of the hydrogels required in the field of bone regeneration are smart hydrogels, polymer hydrogels, and composite hydrogels. Therefore, the article describes the traditional cross-linking preparation to the 3D printing preparation with better application performance, and then to the injection preparation which changes the preparation environment and the mode of action, and other preparation methods, to better understand the principle properties of hydrogels and the reasons for their application in the field of bone regeneration. These hydrogels, which have both novel potential and applications in bone therapy, are exemplified and reported in detail in this review. Examples include nanogels that target and efficiently act on areas of bone regeneration, synthetic polymer composite hydrogels with more stable and advanced structures, and injectable self-repairing and stimulus-responsive hydrogels that protect the delivered drug and control the release rate. Thus, hydrogels are relatively well-developed and can be used as good scaffolding materials for bone regeneration.

The article first screens and introduces stem cells suitable for the field of bone regeneration by understanding the types of stem cells and their regenerative properties of different types of stem cells. For example, MSCs can secrete a variety of cytokines to promote osteogenesis, and the convenience and excellence of adipose stem cell acquisition are applied to bone regeneration experiments, both of which are excellent choices for bioactive factors for bone regeneration. As researchers discovered, most hydrogels have the disadvantage of lacking bioactivity. Therefore, the selection of stable and excellent hydrogels with bioactive factors that can promote bone regeneration has become one of the good solutions for bone regeneration techniques that are being developed and applied nowadays. The article gives examples of important types of applications of hydrogels that can be loaded with stem cells and are analyzed and considered. For instance, three examples are given in this article: photoconditioned GelMA hydrogels loaded with ADSCs, PCL/nHA + HPCH loaded with MSCs, and 3D-printed composite scaffold hydrogels loaded with hDP-MSCs. These technologies have been shown through various experiments to significantly improve the regenerative expression of bone repair, not only to promote the growth of bone and peripheral blood vessels but also to select different properties of stem cells with excellent hydrogel targeted to help promote bone growth. Hence, hydrogels can combine with stem cells to affect bone regeneration more stably and efficiently, contributing greatly to bone regeneration technology.

The authors provide a detailed compilation of information and a summary of knowledge on one of the emerging technologies that can optimize bone regeneration: hydrogel-loaded stem cells, to suggest references for research in the field of bone regeneration. What's more, society's attention to the significance of the important impact of this method will facilitate a more stable and efficient treatment of bone regeneration and even skeletal diseases. The promise of such materials and methods is accompanied by the emergence of difficulties and challenges. Issues such as poor bioadhesive properties, inflammatory reactions to some hydrogels, funding costs, and how to generalize to the public. For hydrogel-loaded stem cell technology to be used in a wider range of clinical applications, the performance characteristics of hydrogels need to be studied more closely, and the advantages of other materials adopted. Additionally, the combination of other bioactive factors, such as the activation of hypoxia-inducible factors by altering the oxygen content of the environment acts more efficiently at the site of bone regeneration. Apart from that, the release of the drug or active factor can be altered, e.g. utilizing extracellular vesicles for more efficient release of the substance. Eventually, it is hoped that advances in science and technology will lead to the development of even better bone regeneration technologies that will bring new directions and breakthroughs, prospects and results, and the future of several biomedical fields, including tissue engineering and regeneration fields in the future.

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