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Strategy of injectable hydrogel and its application in tissue engineering

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Tissue engineering (TE), which represents the technique of reconstituting or repairing organs and tissues by *in vitro* culture or construction using biologically active substances, has become one of the most commonly used approaches for tissue reconstruction and regeneration. It is an excellent, available biomaterial that is not only biocompatible and degradable, but can also efficiently transport cells and growth factors to the damaged tissue and provide support to the newly formed tissue until it matures.

Injectable hydrogels, as an appealing scaffold with structural similarity to the extracellular matrix of many tissues, can often be processed under mild conditions and delivered in a minimally invasive manner. Different from general hydrogels, injectable hydrogels are self-adaptive when used to repair complex shaped tissues and can be cured with body temperature. It is characterized by the possibility of implanting to the desired site by injection, forming a three-dimensional support *in situ* under certain conditions, and avoiding traumatic surgery, which is similar to the characteristics of the internal environment (allowing nutrient and waste to spread, providing cells with signal), and is beneficial to the differentiation and functional expression of cells and the regeneration of tissues.

Preparation, Classification, and Application of Injectable Hydrogels

Injectable hydrogels can be formed *in situ* by either chemical or physical crosslinking methods.

Chemical cross-linking injectable hydrogel

Crosslinking agent cross-linking

The crosslinking agents select a suitable monomer and are introduced into the body by injection, and then *in situ* crosslink at body temperature to form a hydrogel. The preparation of injectable hydrogels by crosslinking agents can be divided into two major categories: one is prepared with natural polymers or modified natural polymers; the other is prepared with synthetic polymers. The formation of chemically induced hydrogels occurs via covalent bonds between polymeric chains promoted by agents such as glutaraldehyde (GTA) or genipin and enzymes. GTA is predominantly used because it can react with functional groups in both proteins and carbohydrates and can provide materials with substantial improvement in tensile properties.

High-energy radiation crosslinking hydrogel

Gamma and electron beam polymerization involves high-energy electromagnetic irradiation as cross-linker. These high-energy radiations can cross-link water-soluble monomer or polymer chain ends without the addition of a cross-linker. During irradiation, using a gamma or electron beam, aqueous solutions of monomers are polymerized to form a hydrogel. Radiation cross-linking has an advantage over other crosslinking methods because it can be performed at room temperature and in physiological pH without using toxic and hard-to-remove crosslinking agents, such as potassium persulfate. However, the radiation used can cause damage to cells and tissues and is generally not used for *in vivo* TE.

Free radical crosslinking hydrogel

Chemically crosslinked hydrogels may be produced by free radical polymerization of polymerizable group from derivatized hydrophilic polymers, in addition to free radical polymerization of vinyl monomer mixtures. To synthesize gels via this route, natural, synthetic, and semisynthetic hydrophilic polymers were applied.

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Cross-linking using enzymes

Owning to their high specificity and mild reaction conditions, enzymatic crosslinking strategies have received considerable attention for the development of injectable hydrogels. Park *et al*[1] developed an *in situ* crosslinkable gelatin–poly(ethylene glycol)-tyramine (GPT) hydrogel. The results demonstrated that the GPT hydrogel, formed *in situ* via an enzyme-mediated reaction, was an excellent bioactive matrix for cellular behavior. Le Thi *et al*[2] used dual-enzymatic cross-linking of horseradish peroxidase (HRP) and tyrosinase for *in situ* formation of tissue-adhesive hydrogels. Singh *et al*[3] demonstrated that HRP can be used for the mild enzymatic cross-linking of thiofunctional polymers to produce hydrogels and nano-gels without the need for added hydrogen peroxide and the loading and functional release of the macroprotein β-galactosidase with nano-gels using HRP cross-linking.

Physical crosslinkable injectable hydrogel

The injectable hydrogel gel formation process does not involve chemical reactions, and the cross-linking between molecular chains is formed by intermolecular interaction forces, such as ionic interaction, crystallization, stereo-complex formation, hydrophobized polysaccharides, protein interaction, and hydrogen bond. In ionic interactions, hydrogels can be cross-linked under mild conditions, at room temperature, and in physiological pH.

Hydrophobic interactions cause the polymer to swell and uptake water that forms the hydrogel. Reports in the literature indicate that polysaccharides such as dextran, chitosan, carboxymethyl curdlan, and pullulan are used in the preparation of physically crosslinked hydrogels by hydrophobic modification. Protein interaction involves block copolymers that contain repetition of silk- and elastine-like blocks called Prolastin®. These biocompatible Prolastin® solutions may be mixed with drugs, and due to crystallization of the silk-like domains, may undergo an irreversible sol to gel transition (with time) in physiological conditions.

Tissue Regeneration Applications

Currently, the focus of most injectable hydrogels is on cartilage and bone, whereas other uses are intended for tissue repairs such as eye, liver, and heart, as well as drug release and delivery.

Angiogenesis

Angiogenesis, the formation of new blood vessels, is a critical process in tissue regeneration. However, inadequate vascularization of the injectable compound has long been a barrier, leading to necrosis or volume reduction after implantation. To resolve this problem, sustained release of certain growth factors such as vascular endothelial growth factor and basic fibroblast growth factor can be employed. An appropriate delivery system is needed to enhance the efficacy of growth factors for highly localized angiogenesis. Injectable scaffolds have been studied as an appropriate delivery vehicle due to their easy preparation and handling. For example, Ishihara *et al*[4] obtained a photo crosslinkable chitosan hydrogel containing azide groups and lactose moieties (Az-CH-LA) incorporating paclitaxel. As a local drug delivery carrier for agents (fibroblast growth factor 2 and paclitaxel), it can effectively inhibit tumor growth and angiogenesis in mice.

Bone repair

Injectable scaffolds have been extensively investigated for applications in bone tissue regeneration. Several factors, including macromonomer concentration, pre-treatment before injection, incorporation of cell adhesive peptide sequences, and controlled, localized release of growth factors within the injectable scaffolds, play an important role in bone formation. Vishnu Priya *et al*[5] developed an injectable hydrogel system consisting of chitin and poly (butylene succinate) loaded with fibrin nanoparticles and magnesium-doped bioglass. The results demonstrated early initiation of differentiation and higher expression of alkaline phosphatase and osteocalcin. They confirmed that this composite hydrogel can be used for regenerating irregular bone defects. Vo *et al*[6] designed an N-isopropylacrylamide/gelatin microparticle-composite hydrogel. The gelatin microparticles incorporated in the hydrogel enhance bony bridging and mineralization within bone implant and defect interface area. Huang *et al*[7] have fabricated an injectable nanohydroxyapatite/glycol chitosan/hyaluronic acid composite hydrogel. MC-3T3-E1 cells incorporated in the hydrogel attach and spread well after 7 days of co-incubation, thus suggesting hydrogel's potential application in bone TE. All of these outcomes suggest that hydrogel could be a potential candidate for irregular bone regeneration.

Cartilage regeneration

The limitation of cartilage tissue for self-repair and regeneration restricts the clinical application of TE cartilage. Kinard *et al*[8] used the oligomer oligo(poly(ethylene glycol) fumarate) as a backbone to produce 3D injectable hydrogel networks to deliver cells and growth factors for cartilage reconstruction. Glycosaminoglycan (GAG) content indicated that the hydrogel composite is a novel strategy for cartilage TE. In the study by Park *et al*,[9] a photo-initiating composite hydrogel, methacrylated glycol chitosan/hyaluronic acid, was shown to be cytocompatible with significantly increased cell proliferation and cartilaginous tissue. The biomaterial has the potential for use as a carrier of cells and bioactive molecules for treating cartilage damage. Therefore, careful control over the crosslinking density and structure of the macromonomers is necessary to achieve increased type II collagen synthesis and homogeneous distribution of GAG within the engineered cartilage.

Expectations of Injectable Materials for TE

Injectable hydrogels are promising scaffolds for TE. The bio-scaffolds play an important role as mediums for the dynamic extracellular signaling and impetus for cell aggregation, growth, and differentiation in tissue regeneration. In recent years, many studies have focused on synthesizing novel injectable hydrogels for TE.[10] To

acquire better injectable hydrogels, the strategies should be adopted.

First, these scaffolds need to provide a three-dimensional structure for cell attachment, growth, proliferation, differentiation, and migration of new cells, while allowing cells to maintain their normal physiological functions.[11] In addition, they act as filters to prevent harmful cells from invading specific tissue parts.

For certain tissues, like bone, a considerable amount of scaffold porosity is necessary to allow cell infiltration for extracellular matrix formation and secretion. Increased porosity and pore size can benefit the structure interconnectivity and the diffusion of nutrients and oxygen, especially in the absence of a pre-vascularized system.

The nature of the biological material itself is extremely important as it not only requires good mechanical strength and resistance to *in situ* forces, but it also controls whether the bioactive molecules (such as growth factors) can be well incorporated into the material (such as growth factors) to facilitate cell growth and differentiation in the tissue. Advanced fabrication methods require further development to improve the mechanical properties and physiological stability, and decrease cytotoxicity and adverse effects of the hydrogels *in vivo*.

The physiological degradability of cell scaffolds is an important index for TE applications. A slow degradation rate can inhibit new tissue function, as scaffold degradation can influence the deposition and the distribution of extracellular matrix molecules as well as cell migration.

In the future, in order to obtain better tissue regeneration materials, we need to conduct in-depth research on the optimization and control of gel dynamics parameter, material biocompatibility, biodegradability, growth factor release levels, and bioavailability.

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Conflicts of interest

None.

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