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Comparative Review on Crosslinking Mechanisms of Injectable Hydrogels and Their Research Progress in Minimally Invasive Tissue Repair

Ruifu Shi

Marine College, Shandong University (Weihai), Weihai, China
shiruifu3@gmail.com

Abstract. Injectable hydrogels have become a core research direction in minimally invasive tissue repair due to their excellent injectability, biocompatibility and in-situ gelation capability, with their crosslinking mechanism directly determining material structural properties and clinical efficacy. Current studies are largely confined to optimizing single crosslinking mechanisms, lacking systematic comparative perspective of the three mainstream types (physical, chemical and enzymatic crosslinking). Moreover, the precise matching principles between crosslinking mechanisms and repair scenarios remain unclear, leading to trial-and-error-based material selection and hindering clinical translation. This paper first systematically reviews the reaction principles and core characteristics of the three mechanisms, and comprehensively compares their advantages and disadvantages from three dimensions: reaction characteristics, core mechanical/biological properties, and large-scale clinical translation potential. It then deeply analyzes their adaptability differences in three typical minimally invasive scenarios (skin, cartilage and arterial hemostasis repair) based on the "demand priority-performance matching-operational feasibility" framework. The results reveal significant differentiated adaptability of the three mechanisms, clarifying their performance boundaries and applicable scopes, establishing a systematic material selection framework, and providing theoretical reference for the targeted design and precise clinical application of injectable hydrogels.

Keywords: injectable hydrogels, crosslinking mechanism, minimally invasive tissue repair

1. Introduction

In the biomedical materials field, it is essential to develop materials with good deliverability, mechanical support and biocompatibility for repairing tissue defects. Generally, the traditional biomedical materials have some disadvantages like high surgical invasion, poor adaptability to irregular defect areas and mechanical inconsistency, failing to meet the requirements of accurate delivery and tissue regeneration. Therefore, injectable hydrogels, polymer materials with three-dimensional network structures, have received much attention in the minimally invasive tissue repair due to their excellent injectability, biocompatibility and in-situ gelation ability [1]. These hydrogels can reach damaged sites minimally invasively and form a gel in situ to offer a stable micro-

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environment for cell adhesion, proliferation and extracellular matrix deposition, thus effectively solving the problems of traditional repair materials. However, the key to their suitability for different minimally invasive repair situations depends on their internal cross-linking mechanism.

Injectable hydrogels have some special advantages in the treatment of minimal invasively damaged tissues, but their complete properties are not intrinsic and are mainly decided by the fabrication methods. The crosslinking mechanism is an important factor affecting the structure and properties of injectable hydrogels, which directly influence the injectability, mechanical strength, degradation behavior and biocompatibility, and are essential for their therapeutic effects [2]. Various crosslinking mechanisms show great differences in preparation conditions, network structures and performance characteristics, and are suitable for different repair scenarios. However, the exact corresponding relationship between different crosslinking mechanisms and specific minimally invasive repaired tissues has not been systematically studied, hindering the establishment of definite material selection standards [3]. At present, the main crosslinking techniques for injectable hydrogels can be classified into three categories: physical crosslinking, chemical crosslinking and enzymatic crosslinking. Physical crosslinking has simple preparation conditions and good biocompatibility, but its mechanical strength is low and the structural stability is poor, therefore it is only applicable for short-term and superficial tissue repair. Chemical crosslinking produces stable networks through covalent bonds, possesses excellent mechanical properties, structural stability and controllable degradation, but it needs harsh crosslinking conditions and may cause certain cytotoxicity. Enzymatic crosslinking has precise reactions, simple conditions and high biocompatibility, which can rapidly and controllably gel under physiological conditions, but it is restricted by high costs and limited application fields.

Despite the advances in the study of crosslinking mechanisms of injectable hydrogels, there are still two main unsolved research problems which emphasize the urgent necessity of this review. Firstly, most of the present research concentrates on the improvement and practical use of one kind of crosslinking mechanism [4], without a comprehensive evaluation system and a unified comparative viewpoint for the three common crosslinking mechanisms. The difficulty in establishing consistent evaluation criteria among different studies also aggravates this obstacle, preventing academic discussions and the practical application of the related findings. Secondly, the corresponding rules between crosslinking mechanisms and the minimally invasive repair situations are not clear. Although the advantages of different crosslinking methods have been recognized, it is still a major gap that accurately matches the crosslinking mechanisms with the repair demands of tissues in the complicated and dynamic physiological microenvironments during minimally invasive surgery. This ambiguity leads to scattered research results and the lack of specific selection guidelines for various tissue repair needs, thus hindering rational material selection in clinical practice and severely limiting the clinical application of injectable hydrogels.

Therefore, this paper concentrates on the three main crosslinking methods of injectable hydrogels, comprehensively evaluates their merits and demerits and their suitability for common minimally invasive tissue repair situations, and summarizes specific material choosing schemes. It aims to fill these research gaps, overcome barriers to rational material selection and clinical application, and give scientific theoretical support for the reasonable design, performance improvement and clinical application of injectable hydrogels. We expect that this paper can provide clear directions for material selection and research and development in the field of minimally invasive tissue repair, promote the specialized design and enhancement of performance of crosslinked hydrogel materials, and speed up the transition of high-efficiency medical hydrogels from fundamental research to clinical practice.

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2. Three main crosslinking mechanisms of injectable hydrogels

At present, the common crosslinking methods are divided into three types: physical crosslinking, chemical crosslinking and enzymatic crosslinking. Although enzymatic crosslinking belongs to covalent crosslinking, it is considered as an independent method in this review due to its unique bio-catalytic property and clinical value. This part introduces their reaction principles and representative systems, providing a basis for subsequent performance comparison.

2.1. Physical crosslinking

Physically crosslinked injectable hydrogels depend on non-covalent bonds like intermolecular hydrophobic interactions, hydrogen bonds and electrostatic interactions to build network structures [5, 6]. These hydrogels show significant shear-thinning property and good self-healing ability, which are advantageous for minimal invasive injection and in-situ defect adjustment [7]. They do not need chemical cross-linking agents, having mild preparation conditions and reversible cross-linking procedures, thus enabling the preservation of the material's biological activity. Some typical examples are temperature-sensitive hydrogels (such as PNIPAM-based hydrogels and chitosan/sodium glycerophosphate composite hydrogels), electrostatic composite cross-linked hydrogels (for instance, alginate/chitosan systems) and host-guest inclusion cross-linked hydrogels. The gelling behaviour can be controlled by the external environmental factors or the concentrations of components [8-10].

2.2. Chemical crosslinking

Chemical crosslinked injectable hydrogels form three-dimensional networks via covalent linkage. The common methods are crosslinking with chemical crosslinkers (such as genipin and EDC/NHS system) and photochemical crosslinking (such as GelMA and HAMA hydrogels) [11]. The strong covalent bonds give them good structural stability, mechanical strength and controllable degradation, which allows them to offer prolonged mechanical support for tissue repair.

In the recent years, the research on chemical crosslinking systems has mainly focused on the control of pre-gelation kinetics of hydrogels. For example, the borax-mediated boronate ester crosslinking method can regulate the gelation time from seconds to minutes without altering the basic physical properties of hydrogels [12, 13]. Thus, the material can be utilized in different situations such as emergency hemostasis and minimal invasive sealing [14].

2.3. Enzymatic crosslinking

Enzymatically crosslinked injectable hydrogels use enzymes as biocatalysts to achieve covalent crosslinking through specific reactions between polymer precursors. They combine the favorable biocompatibility of physical crosslinking with the superior structural stability of chemical crosslinking [15, 16]. Moreover, they can realize rapid and precise reactions under physiological conditions without involving toxic reagents or harsh external stimuli, thus rendering this crosslinking strategy highly competitive in clinical translation. The mainstream systems include the HRP/H_2O_2 catalytic system (e.g., HA-Tyr, Gel-Tyr hydrogels) and the transglutaminase (TG enzyme) catalytic system [17]. The former can achieve precise regulation of gelation time and mechanical properties by adjusting component concentrations, while the latter requires no auxiliary reagents and has excellent biocompatibility.

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Relevant research optimizations have further expanded their application boundaries. For instance, eutectogels based on natural deep eutectic systems (NADES) have improved enzyme activity and stability, realizing long-term controlled drug release; interpenetrating network hydrogels combining TG enzyme crosslinking and Schiff base reaction have demonstrated multifunctional advantages in arterial hemostasis and anti-adhesion [18]. Notably, some enzymatic composite systems introduce dynamic covalent bonds as auxiliary cross-linking sites. Among them, the Schiff base reaction belongs to dynamic chemical cross-linking rather than enzymatic reaction, which only serves as an auxiliary approach to strengthen the structure and function, while the main cross-linking process of the system is still dominated by enzyme catalysis [19, 20].

3. Advantages and disadvantages comparison of three types of crosslinking mechanisms

This section systematically compares the three mechanisms from three dimensions: reaction characteristics, core mechanical/biological properties, and clinical translation potential, providing a basis for subsequent scenario adaptability analysis.

3.1. Overview of core dimension comparison

Based on the intrinsic characteristics of the three types of crosslinking mechanisms and existing research results, the key comparison dimensions are summarized as follows. The quantitative data refer to the typical ranges reported in relevant literatures, striving to objectively reflect their performance differences:

Table 1. Core performance comparison of three crosslinking mechanisms for injectable hydrogels

Comparison item	Physical crosslinking	Chemical crosslinking	Enzymatic crosslinking
Crosslinking bond type	Non-covalent bonds	Stable covalent bonds	Enzyme-catalyzed covalent bonds
Gelation time	Narrow adjustable range, mostly minute-level (1–30 min) [7]	Wide adjustable range (seconds to hours)	Precisely tunable (seconds to minutes, 10 s–5 min)
Storage modulus G'	Typically < 1 kPa [21]	> 10 kPa (up to 10–20 kPa) [22]	1–8 kPa [19]
Compressive modulus	Typically < 10 kPa [21]	50–300 kPa [12]	10–80 kPa [15]
Crosslink density	Low and heterogeneous	High and uniformly controllable	Moderate and relatively homogeneous
Crosslink density	Low and heterogeneous	High and uniformly controllable	Moderate and relatively homogeneous

3.2. Interpretation of key advantages and disadvantages

3.2.1. Comparison of reaction characteristics and operational feasibility

The core advantage of physical crosslinking lies in its mild reaction conditions and simple operation. It does not require the introduction of toxic chemical crosslinkers or reliance on professional equipment. Some systems can trigger gelation through physiological signals such as body temperature and in vivo pH, which is naturally compatible with the operational requirements of

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minimally invasive delivery. Additionally, its simple preparation process and controllable cost are conducive to promotion in primary care settings. However, conventional physical crosslinking systems exhibit a narrow adjustable range of gelation rate, mostly at the minute level, making them difficult to adapt to emergency minimally invasive scenarios requiring second-scale rapid response such as arterial hemostasis.

Chemical crosslinking features strong controllability of reaction conditions and a wide coverage of gelation rates (seconds to hours) [23]. Suitable crosslinking methods can be selected according to minimally invasive repair needs—for example, photochemical crosslinking can achieve second-level rapid gelation, while chemical crosslinker-mediated crosslinking can adapt to different repair rhythms through concentration regulation. Nevertheless, this type of crosslinking often requires exogenous crosslinkers or light irradiation triggering. Some crosslinkers (such as glutaraldehyde) have risks of cytotoxic residues, and photochemical crosslinking also requires auxiliary professional light equipment, which increases the complexity and cost of minimally invasive operations.

Enzymatic crosslinking combines the advantages of mild reaction and rapid controllability. It can complete crosslinking under physiological conditions without toxic reagents, demonstrating prominent operational convenience and biosafety. Furthermore, the gelation rate can be precisely regulated via enzyme and substrate concentrations (seconds to minutes), enabling flexible adaptation to the time requirements of different minimally invasive scenarios. However, its reaction characteristics are highly dependent on enzyme activity, which is susceptible to environmental factors such as in vivo temperature, pH, and ion concentration, resulting in insufficient stability. Additionally, the high cost of enzyme preparation and purification restricts its large-scale application.

3.2.2. Comparison of core performance advantages and disadvantages

Regarding mechanical strength and structural stability, chemical crosslinking forms three-dimensional networks through covalent bonds and presents the most remarkable comprehensive mechanical performance and structural stability, accompanied by high storage modulus G', compressive modulus and crosslink density. The storage modulus G' can exceed 10 kPa and the compressive modulus ranges from 50 to 300 kPa, which can effectively satisfy the demand for tissue support. Meanwhile, it exhibits excellent resistance to swelling, shearing and structural collapse in vivo, thereby providing long-term stable mechanical support for defective tissues. As such, it represents the sole mechanism among the three categories that can satisfy the mechanical requirements of moderate to severe minimally invasive tissue repair. However, the high crosslink density required for such mechanical performance tends to increase the viscosity of the pre-gel precursor solution, which partially compromises the injectable flowability and increases the injection resistance. Enzymatic crosslinking exhibits moderate storage modulus, compressive modulus and structural stability, with crosslink density and network uniformity between those of physical and chemical crosslinking. The typical storage modulus G' is 1–8 kPa and the compressive modulus is about 10–80 kPa, which can be further enhanced by composite systems and basically satisfy the supporting needs of most minimally invasive repair scenarios. Due to the weak non-covalent interactions, physical crosslinking possesses the lowest storage modulus, compressive modulus, crosslink density and swelling resistance. The typical storage modulus G' is generally less than 1 kPa and the compressive modulus is mostly below 10 kPa. The network is prone to structural collapse induced by dilution and swelling of physiological fluids in vivo, making it unsuitable for long-term or moderate to severe minimally invasive tissue repair.

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Physical crosslinking and enzymatic crosslinking both demonstrate favorable biocompatibility profiles. Physical crosslinking does not require exogenous toxic reagents, which can maximize the retention of the bioactivity of natural polymers without residual toxicity risks. Enzymatic crosslinking uses enzymes as biocatalysts, and enzymes can be naturally degraded in vivo without producing toxic residues. Meanwhile, its mild reaction causes little damage to surrounding normal tissues [24]. The biocompatibility of chemical crosslinking is greatly affected by the crosslinking method. Some crosslinkers (such as glutaraldehyde) and photoinitiators have cytotoxicity. Although low-toxic crosslinking systems (such as genipin and EDC/NHS) have been gradually promoted, it is still necessary to balance performance and safety.

Regarding performance tunability, both chemical crosslinking and enzymatic crosslinking present distinct superiorities. Chemical crosslinking can precisely adjust the crosslinking density, pore size, mechanical strength, and degradation rate of hydrogels by regulating parameters such as crosslinker concentration, light intensity, and reaction time, adapting to diverse minimally invasive repair needs. Enzymatic crosslinking can achieve performance customization by changing enzyme concentration and substrate ratio, with simple regulation logic and a wide adaptation range. The performance tunability of physical crosslinking is relatively limited, mainly relying on the adjustment of polymer component ratios, with low regulation precision for key parameters such as crosslinking density and pore size, making it difficult to match the personalized needs of complex minimally invasive scenarios.

3.2.3. Comparison of large-scale and clinical translation potential

Physical crosslinking has the lowest difficulty in large-scale preparation, with a simple process and controllable cost, and no reliance on special raw materials or equipment. Moreover, most matrix materials for physically crosslinked hydrogels (e.g., hyaluronic acid, gelatin) are classified as Generally Recognized as Safe (GRAS) materials by the U.S. FDA, with clear regulatory approval pathways and minimal regulatory barriers, thus possessing strong clinical translation and popularization potential. However, its inherent disadvantages of insufficient mechanical support and poor structural stability limit its application scope in moderate to severe minimally invasive repair, and it can only adapt to short-term superficial scenarios.

The difficulty in large-scale preparation of chemical crosslinking is medium. Some systems (such as EDC/NHS-mediated crosslinking) have mature processes and controllable costs, but photochemical crosslinking and high-toxic crosslinker systems are restricted by equipment requirements and safety, requiring comprehensive toxicological evaluations of crosslinker residues, degradation products and photoinitiator cytotoxicity, which prolongs the approval cycle and increases the translation threshold. Overall, its clinical translation potential mainly depends on the selection of crosslinking systems. Low-toxic and simple-to-operate chemical crosslinking systems (such as genipin crosslinking and simple photochemical crosslinking) have good translation prospects, especially in the field of moderate to severe minimally invasive repair with urgent demand.

The core bottleneck of clinical translation of enzymatic crosslinking lies in large-scale preparation. The high cost of enzyme preparation and purification, as well as the difficulty in ensuring the consistency of enzyme activity during mass production, lead to high product quality control difficulty and cost. In particular, the batch-to-batch variation in enzyme activity will cause significant fluctuations in the gelation rate and mechanical properties of the final product. Such inconsistency fails to meet the strict consistency requirements of pharmaceutical regulatory authorities and further increases the difficulty of registration and approval, restricting its clinical

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popularization [4]. Nevertheless, the prominent advantages of this type of crosslinking in biocompatibility and mild reaction endow it with irreplaceable value in high-end scenarios such as cell loading and minimally invasive repair of sensitive parts, still possessing important translational research significance.

4. Adaptability comparison of different crosslinking mechanisms in common minimally invasive tissue repair scenarios

Based on the systematic review and performance comparison of the three mainstream crosslinking mechanisms (physical crosslinking, chemical crosslinking, and enzymatic crosslinking) in the previous sections, this chapter selects three typical minimally invasive tissue repair scenarios with extensive clinical application, clear requirements, and representativeness. By correlating the intrinsic properties of crosslinking mechanisms with practical clinical demands, this chapter systematically analyzes the applicability and underlying mechanisms of each mechanism in different scenarios, and highlights scenario-driven matching rules. Grounded in real clinical application scenarios, this chapter provides targeted theoretical support for clinical material selection and targeted material design, and promotes the translational research of minimally invasive tissue repair.

4.1. Adaptability comparison in minimally invasive skin repair scenarios

Minimally invasive skin repair is the most common clinical minimally invasive scenario, including superficial traumatic wounds, superficial burns, minimally invasive postoperative facial repair, chronic superficial ulcers, etc. The core needs vary according to the type of wound: irregular and deep cavity wounds (such as mild trauma and superficial second-degree burns) focus on convenient operation, low cost, and high biocompatibility, without the need for long-term mechanical support; long-term refractory wounds (such as deep burns and chronic ulcers) require a certain degree of mechanical strength and structural stability to avoid wound contraction and infection; repair of sensitive areas (such as minimally invasive facial and periorbital repair) has extremely high requirements for biocompatibility, requiring avoidance of irritation and toxic residues.

Physical crosslinking mechanism is most suitable for minimally invasive repair of short-term small wounds [21]. It does not require toxic chemical crosslinkers and has mild preparation conditions. Some systems can trigger in-situ gelation through body temperature, which is easy to operate and does not require professional equipment, adapting to the minimally invasive operation needs of outpatient clinics and primary medical institutions. At the same time, its low preparation cost and high scalability can meet the core needs of short-term small wounds for rapid coverage, temporary protection and accelerated initial healing. In addition, physical crosslinking hydrogels can load antibacterial drugs for local sustained release, further reducing the risk of wound infection, and have no toxic residues, which can maximize the retention of the natural activity of skin tissue, conforming to the simple, safe and economical repair requirements for short-term small wounds.

Chemical crosslinking mechanism is more suitable for minimally invasive repair of long-term refractory skin wounds. Long-term refractory wounds have a long repair cycle, requiring hydrogels to provide continuous mechanical support to prevent wound contraction and deformation, and resist the erosion of exudate [25]. The three-dimensional network constructed by chemical crosslinking through covalent bonds has strong anti-swelling and anti-collapse capabilities, and can stably exist at the wound site for a long time, providing a stable microenvironment for skin cell adhesion and proliferation. In addition, chemical crosslinking has strong performance tunability. The degradation rate of hydrogels can be precisely adjusted by regulating crosslinker concentration and reaction

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conditions to match the rhythm of skin tissue regeneration. For example, GelMA photochemically crosslinked hydrogels can adjust mechanical strength and degradation rate by regulating light parameters, which have been extensively studied in minimally invasive repair of deep burns and can effectively promote granulation tissue growth and epithelialization.

Enzymatic crosslinking mechanism is mainly suitable for minimally invasive repair of sensitive skin areas (such as minimally invasive postoperative facial repair and periorbital wounds). The skin tissue in sensitive areas is delicate and extremely sensitive to toxic stimuli [26, 27]. Enzymatic crosslinking uses enzymes as biocatalysts, which can achieve mild and rapid crosslinking under physiological conditions without toxic reagents and severe external stimulation. Enzymes can be naturally degraded in the body without any toxic residues, which can maximize the protection of the activity of sensitive skin tissue and avoid complications such as Erythema and localized inflammation. For instance, HA-Tyr hydrogels optimized under mild conditions exhibit favorable biocompatibility and rapid gelation behavior in a low-concentration HRP/H_2O_2 system. Moreover, these hydrogels can load growth factors for precise delivery, effectively accelerating wound healing in minimally invasive postoperative facial repair without causing significant damage to adjacent normal skin tissues.

4.2. Adaptability comparison in minimally invasive cartilage repair scenarios

Minimally invasive cartilage repair is a current research hotspot in minimally invasive tissue repair, mainly targeting articular cartilage defects such as knee and hip joints. The core needs of such scenarios are: high mechanical strength (mimicking the extracellular matrix microenvironment for chondrocyte development), good biocompatibility (promoting chondrocyte adhesion and proliferation), long-term structural stability and controllable degradation (matching the cartilage regeneration cycle), and adaptation to minimally invasive injection delivery to avoid trauma caused by open surgical procedures. The differences in adaptability of the three types of crosslinking mechanisms mainly focus on the balance between mechanical strength and biocompatibility.

Chemical crosslinking mechanism is a highly favorable candidate for minimally invasive cartilage repair. Articular cartilage needs to bear a certain mechanical load during movement, requiring repair materials to have high mechanical strength and wear resistance [28]. The three-dimensional network constructed by chemical crosslinking through covalent bonds can effectively resist the pressure and friction during joint movement and provide stable mechanical support for the growth and proliferation of chondrocytes. At the same time, chemical crosslinking has strong performance tunability. The pore size, crosslinking density and degradation rate of hydrogels can be precisely adjusted by regulating crosslinker concentration, light parameters, etc., to match the regeneration rhythm of cartilage tissue (usually 3-6 months). For example, genipin-crosslinked chitosan hydrogels and photocrosslinked GelMA hydrogels have attracted significant attention in minimally invasive cartilage repair. Their low toxicity, high strength and controllable degradation characteristics can effectively promote the repair and regeneration of cartilage defects. In addition, chemical crosslinking hydrogels can load chondrocytes or growth factors to further improve the repair effect, adapting to the clinical needs of minimally invasive injection repair of cartilage.

Enzymatic crosslinking mechanism serves as a highly valuable adjunct for minimally invasive cartilage repair, suitable for cartilage defect scenarios with high requirements for biocompatibility (such as defects at the junction of cartilage and soft tissue, and cartilage repair in young patients). Enzymatic crosslinking has both mild reaction characteristics and medium mechanical strength, excellent biocompatibility, can achieve rapid gelation under physiological conditions without toxic reagents, can maximize the protection of chondrocyte activity, avoid damage to cells by chemical

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crosslinkers, and is especially suitable for loading chondrocytes for minimally invasive delivery, providing a suitable microenvironment for cell growth [29]. For example, TG enzyme-catalyzed gelatin hydrogels have excellent biocompatibility without auxiliary reagents, can load chondrocytes to achieve in-situ gelation, and show good application prospects in the repair of mild to moderate cartilage defects; HRP/H_2O_2-catalyzed HA-Tyr hydrogels can adjust mechanical strength by regulating enzyme concentration, adapting to the needs of different degrees of cartilage defects.

The adaptability of physical crosslinking mechanism in minimally invasive cartilage repair is poor. Cartilage repair requires long-term mechanical support, while physical crosslinking relies on weak non-covalent interactions, which is difficult to bear the mechanical load during joint movement, prone to swelling and collapse, and unable to provide a stable growth microenvironment for chondrocytes. In addition, its degradation rate is relatively fast, which is difficult to match the long-term regeneration cycle of cartilage tissue. Therefore, physical crosslinking hydrogels can only be used as auxiliary materials for minimally invasive cartilage repair, for temporary filling and protection of mild cartilage defects, and cannot alone meet the core needs of cartilage repair, with limited application in this scenario.

4.3. Adaptability comparison in minimally invasive arterial hemostasis repair scenarios

Minimally invasive arterial hemostasis repair is an urgent clinical minimally invasive scenario, mainly targeting arterial puncture, minimally invasive vascular surgery, traumatic arterial bleeding, etc. The core needs are: rapid gelation (second to minute level, missing the hemostasis window is likely to lead to massive bleeding), good mechanical strength (resisting arterial systolic blood pressure up to 120–160 mmHg to prevent rebleeding after hemostasis), high biocompatibility (avoiding damage to vascular endothelial cells and reducing the risk of thrombosis), and adaptation to minimally invasive injection delivery to achieve precise hemostasis. The differences in adaptability of the three types of crosslinking mechanisms mainly focus on the balance between gelation rate and mechanical strength.

Enzymatic crosslinking mechanism is the optimal adaptation type for minimally invasive arterial hemostasis repair. Arterial bleeding has an acute onset and large blood loss, requiring hydrogels to complete in-situ gelation within seconds to minutes to form a stable hemostatic plug and resist arterial blood pressure impact. Enzymatic crosslinking can achieve precise gelation from seconds to minutes by regulating enzyme and substrate concentrations [18]. For example, TG enzyme-catalyzed interpenetrating network hydrogels can complete gelation within 10 seconds to quickly block arterial bleeding sites. At the same time, its excellent biocompatibility allows enzymes to be naturally degraded in the body, without damaging vascular endothelial cells or causing thrombosis, reducing the risk of postoperative complications. In addition, some enzymatic crosslinking systems can achieve integrated hemostasis and anti-adhesion. For example, TG enzyme and Schiff base composite hydrogels can effectively prevent adhesion between blood vessels and surrounding tissues while completing arterial hemostasis, avoiding secondary minimally invasive intervention and further improving clinical repair effects.

Chemical crosslinking mechanism can be used as a supplement for minimally invasive arterial hemostasis repair, suitable for sub-scenarios with high requirements for hemostasis stability and no need for rapid response (such as auxiliary hemostasis after minimally invasive vascular surgery). Chemical crosslinking has high mechanical strength and strong structural stability, and the formed hemostatic plug can effectively resist arterial blood pressure impact to prevent rebleeding. In addition, it has strong performance tunability, and the gelation rate can be adjusted (from seconds to hours) by regulating crosslinker concentration. For example, borax-mediated amidation crosslinked

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hydrogels can achieve second-level rapid gelation, adapting to the needs of emergency hemostasis. However, chemical crosslinking has obvious shortcomings: some crosslinkers have cytotoxicity, which is easy to damage vascular endothelial cells and increase the risk of thrombosis; photochemical crosslinking is easily hindered by deep bleeding sites, where blood can significantly shield light and cause severe light attenuation, making it difficult to achieve effective crosslinking in deep hemorrhagic regions. Therefore, it is only suitable for non-emergency minimally invasive arterial hemostasis scenarios with relatively low safety requirements.

The adaptability of physical crosslinking mechanism in minimally invasive arterial hemostasis repair is poor. Arterial hemostasis requires rapid gelation, while the gelation rate of physical crosslinking is mostly at the minute level with a narrow adjustable range, which is difficult to adapt to the window demand of emergency hemostasis [7]. At the same time, its low mechanical strength and poor structural stability make the formed gel easy to break under the impact of arterial blood pressure, unable to achieve effective hemostasis, and prone to swelling and collapse, leading to hemostasis failure. In addition, physical crosslinking hydrogels have weak anti-swelling ability and are easy to be diluted by arterial blood, further reducing the hemostasis effect. Therefore, they are rarely used in minimally invasive arterial hemostasis scenarios and can only be used as temporary auxiliary hemostatic materials for mild arterial oozing.

5. Conclusion

This review focuses on the three mainstream crosslinking mechanisms of injectable hydrogels, namely physical, chemical and enzymatic crosslinking. It systematically summarizes their core characteristics, advantages and disadvantages, and analyzes their adaptability to three typical clinical minimally invasive repair scenarios, thereby establishing targeted material selection strategies. Mechanistically, the three mechanisms differ significantly in reaction principles and key properties: physical crosslinking based on non-covalent interactions offers mild conditions, good biocompatibility and low cost, but is limited by insufficient mechanical strength and poor structural stability [30]. Chemical crosslinking via covalent bonds provides outstanding mechanical properties, long-term stability and controllable degradation, yet some systems involve potential cytotoxicity and complex operation. Enzymatic crosslinking achieves rapid and precise gelation under physiological conditions with excellent biocompatibility, but is restricted by high cost and unstable enzyme activity.

For scenario adaptability, this review establishes a material selection logic centered on demand priority, performance matching and operational feasibility. Physical crosslinking is preferred for short-term superficial minimally invasive skin repair, owing to its good safety, simple operation and low cost. Chemical crosslinking is the most suitable choice for minimally invasive cartilage repair, as its high mechanical strength and long-term stability match the mechanical load and regeneration cycle of cartilage tissue. Enzymatic crosslinking presents unique advantages in minimally invasive arterial hemostasis, meeting urgent hemostasis needs and reducing complication risks. Through systematic comparison and scenario-oriented analysis, this review clarifies their performance boundaries and applicable scopes, providing a scientific basis for the rational design, precise clinical application and accelerated translation of injectable hydrogels.

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