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Review

Tissue-Engineered Injectable Gelatin−Methacryloyl Hydrogel-Based Adjunctive Therapy for Intervertebral Disc Degeneration

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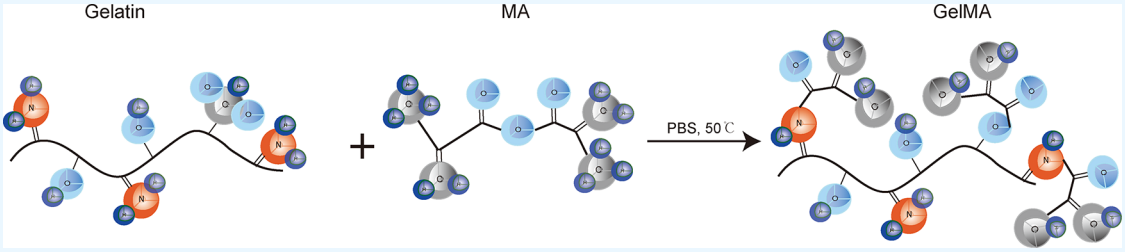

ABSTRACT: Gelatin−methacryloyl (GelMA) hydrogels are photosensitive with good biocompatibility and adjustable mechanical properties. The GelMA hydrogel composite system is a prospective therapeutic material based on a tissue engineering platform for treating intervertebral disc (IVD) degeneration (IVDD). The potential application value of the GelMA hydrogel composite system in the treatment of IVDD mainly includes three aspects: first, optimization of the current clinical treatment methods, including conservative treatment and surgical treatment; second, regeneration of IVD cells to reverse or repair IVDD; and finally, IVDD instead of injury plays a biomechanical role. In this paper, we summarized and analyzed the preparation of GelMA hydrogels and their excellent biological characteristics as carriers and comprehensively demonstrated the research status and prospects of GelMA hydrogel composite systems in IVDD treatment. In addition, the challenges facing the application of GelMA hydrogel composite systems and the progress of research on new hydrogels modified by GelMA hydrogels are presented. Hopefully, this study will provide theoretical guidance for the future application of GelMA hydrogel composite systems in IVDD.

1. INTRODUCTION

Intervertebral disc (IVD) degeneration (IVDD) is the leading cause of chronic low back pain (LBP) and disability in middle-aged and elderly individuals.[1] According to statistics, approximately 540 million people worldwide suffer from IVDD disease, which has become a serious global public health problem.[2,3] Studies have reported several factors that can lead to pathological changes in IVDs,[4,5] such as annulus fibrosus (AF), nucleus pulposus (NP), or cartilaginous end plate (CEP) changes in the structure and extracellular matrix (ECM) reduction,[2] resulting in back, lumbosacral, or hip pain (Figure. 1). However, both conservative and surgical treatments are symptom-based and have potential complications, and neither restore normal biological function in degenerative IVDs.[6,7] Although stem cell and biological factor transplantation showed potential application, the transplantation scheme cannot fill the target degenerative IVD areas, and there is a risk of cell and biological factor loss and injection leading to infection.[8]

GelMA hydrogels are photosensitive materials with good molecular adhesion, immunogenicity, histocompatibility, and nontoxic and adjustable mechanical properties. They are an excellent carrier for cell and biological factors.[9] Simple GelMA hydrogels cannot be used to treat IVDD diseases, but GelMA hydrogels encapsulating stem cells or biological factors will help optimize the traditional clinical treatment mode. The effect of topical medication can be achieved by minimally invasive transplantation, reducing the adverse side effects of drug therapy and surgical treatment.[10] Alternatively, forcing the regeneration of IVD cells reverses or repairs degenerated IVDs, such as NP cells (NPCs)[11] and extracellular matrix (ECM) regeneration.[12] Biological IVDs can also be made available by 3D printing modified GelMA hydrogel complexes to replace degenerated IVDs.[13] Thus, GelMA hydrogels mainly act as biomaterial carriers and scaffolds and can promote the differentiation and production of corresponding cells and biological factors.[14] A summary of the frontier advances of GelMA hydrogels in IVDD facilitates the development of novel adjuvant therapies.

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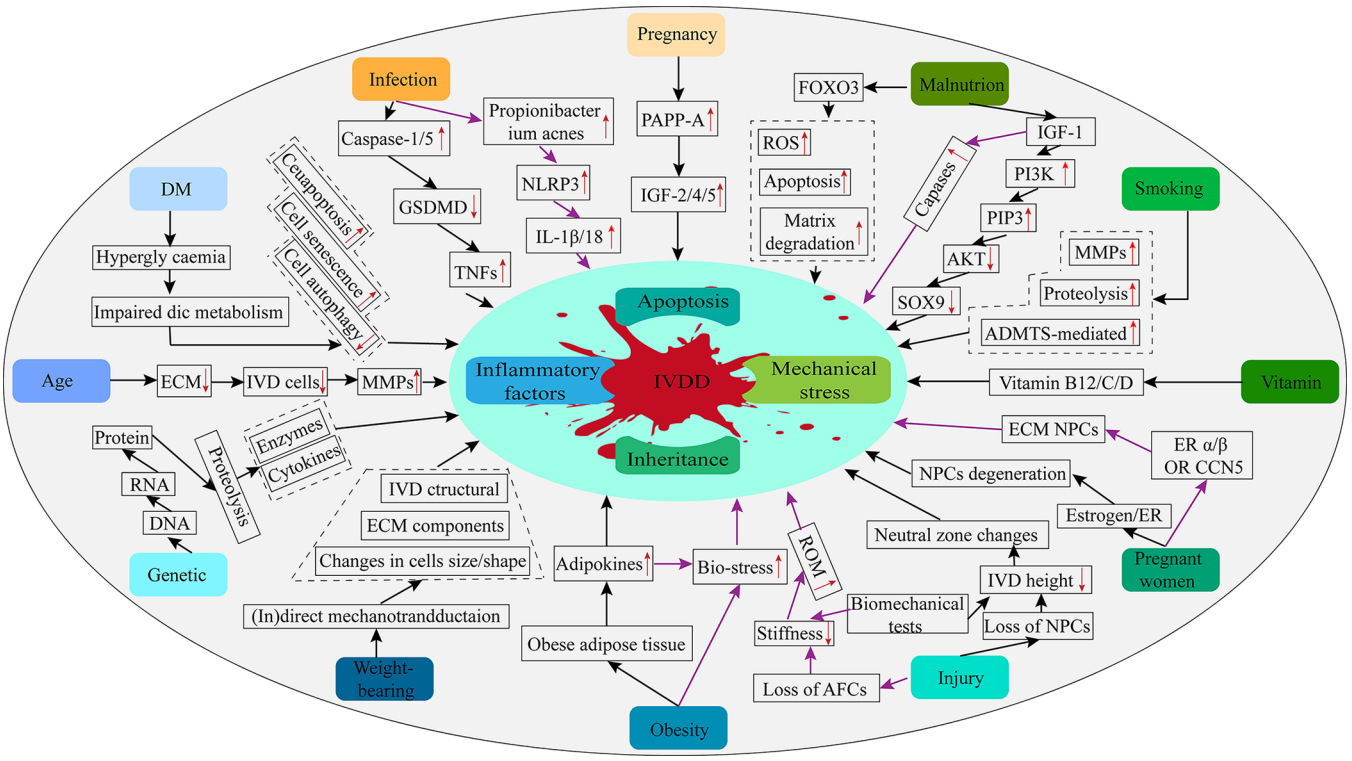

Figure 1. IVDD pathogenesis. IVDD has various etiologies, such as trauma, aging, infection, and genetics, which ultimately lead to the disease through intrinsic mechanisms, such as apoptosis, inflammation, mechanical stress, and genetics.

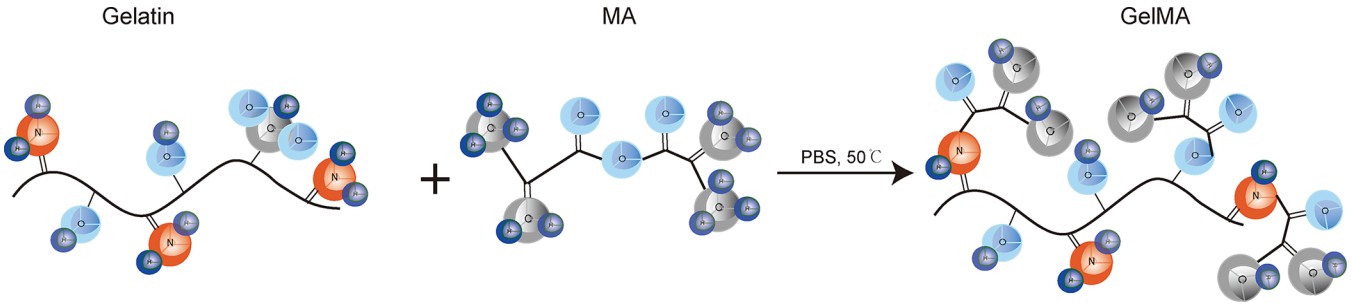

Figure 2. Schematic diagram of the synthesis and assembly of GelMA hydrogels.

2. GELMA HYDROGEL

GelMA hydrogel is a cross-linking network mixture of gelatin and methacrylic anhydride (MA) polymerized in phosphate buffer at 50 °C and is a photosensitive injectable biohydrogel material (Figure. 2).[15] Gelatin is the hydrolysis product of collagen, and it is also the ECM's main component. It contains many arginine glycine aspartate sequences that promote cell adhesion and matrix metalloproteinase (MMP) target sequences suitable for cell reconstruction.[16,17] Moreover, introducing MA substituents can make the GelMA hydrogel photo-cross-linked with a photoinitiator and illumination.[18] The polymerization of gelatin with MA can be carried out under mild conditions (room temperature, neutral pH, water environment, etc.). The reaction time and space can be controlled to obtain GelMA hydrogels with different mechanical properties.[19]

GelMA hydrogels exhibited stable thermal sensitivity, mechanical adjustment, low immunogenicity, biodegradability, and nontoxic properties on the experimental platform.[20−22] GelMA hydrogels are very similar to natural ECM, which can provide a suitable microenvironment and corresponding biological matrix for cell life activities, thereby promoting cell growth,[23] proliferation,[24] differentiation,[25] and migration.[26] Further research found that the cells cultivated in GelMA hydrogel have tighter signal transmission, and the contact between the cells and the ECM is more sufficient.[27] Compared with other hydrogels, GelMA hydrogels can induce mesenchymal stem cells (MSCs) to produce more ECM, proteoglycans, collagen II, and amino glycans.[28] This simulates the physiological activity of cells in vivo, can clearly explain the transportation of cell oxygen, nutrients, and metabolic waste, accurately reproduces the spatial organization and microenvironment of cells in vivo, and has strong repeatability.[27] At the same time, the research results of GelMA hydrogels on the cell level laid the foundation for GelMA hydrogels to simulate biological tissue structures and build biomimetic organs, such as IVDs.[15]

GelMA hydrogel transplantation and regenerative therapy are currently the focus of medical research. When GelMA hydrogels wrap biological factors to treat diseases, targeted and actionable biologic factors can be selected.[29] Currently,

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Table 1. Summary of GelMA Hydrogel-Loaded Cytokines and Cells

biological factor	target	type	organization	function	ref
ADMSCs and GDF-5	rats	GelMA hydrogel	IVD	maintain NP tissue integrity and accelerate ECM synthesis	12
hDPSCs and hUVECs	cells	GelMA hydrogel	dental pulp tissue	lead to ameloblast and odontoblast differentiation	30
hBMSCs	cells	GelMA bioink incorporating MSNCaPDex	bone tissue	stem cell osteogenic differentiation	31
BMSCs	cells	polycaprolactone−GelMA−USPIO	bile ducts	construct biologically active artificial bile ducts	32
human amniotic mesenchymal stromal cells and SDF-1α	rats	imidazole group-modified GelMA	brain tissue	promote stem cell differentiation and repair focal brain injury	33
human cardiac progenitor cells and ECM	rats	GelMA hydrogel	myocardial tissue	repair damaged myocardium	34
NPCs	cells	photocross-link GelMA hydrogel	IVD	promote NPC regeneration	6

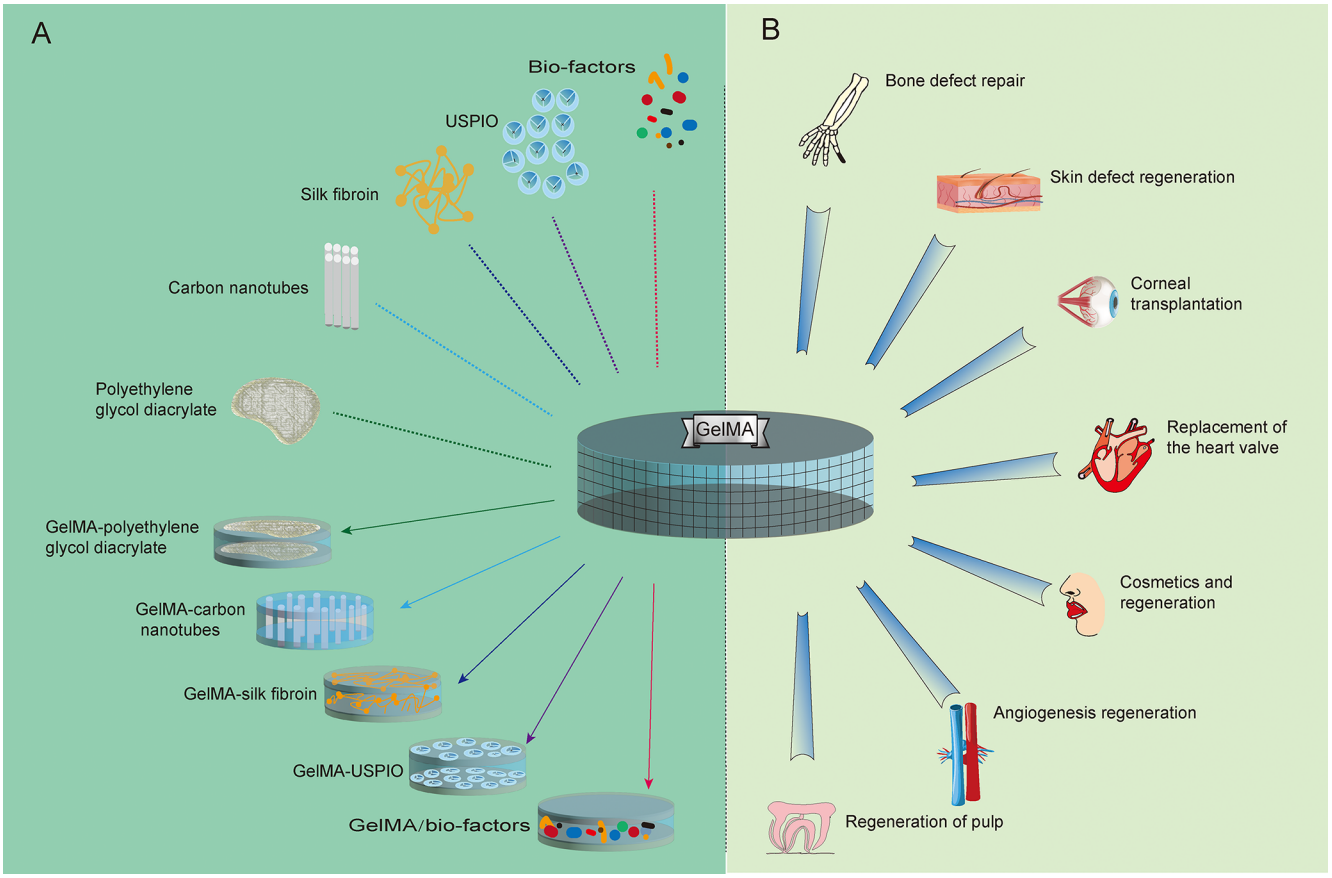

Figure 3. GelMA hydrogels treat clinical diseases by loading various bioactive molecules or substances. (A) GelMA hydrogel-loaded biological factors and cells, etc. (B) Clinical use of GelMA hydrogels.

cells,[6,30−34] proteins,[12,35−43] and drugs[14,44−52] are available for transplantation using GelMA hydrogels as carriers for treating clinical diseases. This also includes stromal-cell derived factor-1 (SDF-1α),[33] octacalcium phosphate,[53] cellulose nanofibrils,[54] decellularized liver matrix (DLM),[55] platelets,[56] 6-deoxy-amino cellulose derivatives,[57] and gelatin−hydroxyapatite (HA).[58] These small molecules can be encapsulated by GelMA hydrogels and transplanted into the lesion area (Table 1). Compared with traditional gelatin, such as Laponite cross-linked poly *N*-isopropylacrylamide-*co*-*N*,*N*′-dimethyl acrylamide hydrogel (NPgel),[59] GelMA hydrogel preserves excellent biocompatibility, low antigenicity, solubility, and bioactivity of gelatin, such as adhesion and proliferation of cells.[60] More importantly, the introduction of MA substituents gives the GelMA hydrogel fast polymerization in the presence of UV light and photoinitiators and produces GelMA hydrogel with stable physical properties through covalent cross-linking.[61] This allows clinicians to fine-tune the biological properties of GelMA hydrogels by changing the amount of MA added to the reaction mixture, rate, pH, and light time.

In the study of GelMA hydrogel-encapsulated biological factor transplantation for IVDD, the biological factors were mainly MSCs, bone morphogenetic protein (BMP), growth differentiation factor (GDF), and aspirin, which showed great potential in cell regeneration and bone and cartilage repair (Figure. 3A).[62−64] GelMA hydrogels can act as filling agents and scaffold materials for tissue defect repair.[65] This solves the problem of insufficient traditional materials to a certain extent.

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In short, it is expected to provide a new treatment plan to eliminate inflammatory reactions, shorten the fusion time, and promote bone fusion.

3. THERAPEUTIC MECHANISM BASED ON THE GELMA HYDROGEL SYSTEM

3.1. Local Delivery of the GelMA Hydrogel Composite System. The inflammatory response plays a vital role in the occurrence and development of IVDD. Inflammation inhibition helps maintain IVD microenvironment homeostasis.[66] If the inflammatory response cannot be controlled effectively, it will lead to clinical symptoms such as pain and aggravate the pathological process.[67] In addition, the inflammatory reaction is also considered to be the main factor affecting effective interbody fusion after IVD fusion surgery, which may lead to local severe tissue defects and tissue degeneration and even affect the postoperative interbody fusion process, resulting in operation failure.[14] Therefore, it is necessary to inhibit the inflammatory response occurrence and development. Moreover, because IVD tissues are nonvascular, oral or intravenous anti-inflammatory drugs take longer to eliminate the inflammatory response after IVD surgery.[14] They may even induce digestive or cardiovascular disease in severe cases.[68]

Previous studies have shown that local administration of anti-inflammatory drugs can not only completely block the inflammatory cycle but also significantly reduce the damage to the digestive system caused by drugs, reduce the dosage, and shorten the medication time cycle.[69,70] Studies have confirmed that after IVD resection, the inflammatory process lasts 14 days,[71] and tumor necrosis factor (TNF)-α and interleukin (IL)-1β or IL-6 play an essential role in releasing inflammatory mediators in the acute stage of inflammation.[72,73] Recently, Liu et al.[14] found through in vivo and in vitro experiments that the inflammatory response after IVDD could be treated by aspirin transplantation encapsulated by GelMA hydrogel. Inflammatory cytokines (such as IL-1β, IL-6, and TNF-α) in diseased IVD tissues were detected. MMP-3, disintegrin, and metalloprotease expression with thrombospondin (ADAMTS)-4 and ADAMTS-5 were inhibited. In addition, aspirin release from GelMA hydrogels is controllable and can completely block the inflammatory cycle after spinal fusion.[74,75] Aspirin composite GelMA hydrogel transplantation effectively alleviated local inflammation after spinal fusion. At the same time, it also lays a foundation for other anti-inflammatory drugs, such as celecoxib, to be used in local medication.[76,77]

Celecoxib is another widely used anti-inflammatory drug among nonsteroidal anti-inflammatory drugs (NSAIDs). Celecoxib-loaded polyesterimide microspheres can play an anti-inflammatory role in canine IVDD by injection, relieve pain, and delay the progression of IVDD to a certain extent.[77] This microsphere system is another drug release form effective in loading the GelMA hydrogel system. The GelMA hydrogel system encapsulates an anti-inflammatory drug microsphere system, which has obvious advantages in topical administration, such as accurately delivering anti-inflammatory drugs to inflammatory foci, ensuring effective drug concentration, shortening medication time, and reducing complications.[74] GelMA hydrogels are allowing the application of an increasing number of medications to the precise treatment of diseases. GelMA hydrogel system transplantation to eliminate the IVD tissue inflammatory response promises to become a new method to treat IVDD diseases.[29] Controllability and operability are also very important for restoring the mechanical stability of the spine.

3.2. The GelMA Hydrogel Composite System Promotes MSC Osteogenic Differentiation. MSCs can differentiate into various cells, such as osteoblasts, cartilage, fat, nerves, and myoblasts, under certain conditions. These cells have simple acquisition, good differentiation, low immunogenicity, and specific immune regulation.[78,79] They may be a candidate for bone regeneration and fusion therapy.[80] GelMA hydrogels are perfect scaffolds for tissue engineering applications. MSCs loaded into GelMA hydrogel can be induced to differentiate into osteoblasts, which theoretically can help promote bone growth and can thus accelerate the treatment of spinal intervertebral bone fusion. In recent years, TGFs, BMPs, and insulin-like growth factors have been increasingly used in the clinical treatment of orthopedic diseases due to their significant bone-forming effects.[81] TGF-β, in particular, has been found to form subunit complexes by activating the Smad pathway, acting as a transcription factor to initiate the translation process and affecting cell growth, differentiation, apoptosis, and immune regulation.[82] It promotes MSC differentiation into osteoblasts, regulates osteoblast growth, and promotes bone tissue healing.[83]

Recent studies have found that GelMA hydrogels can enhance the ability of MSCs to transform into osteoblasts and effectively control TGF release.[42,74] Aldanan et al.[42] found that GelMA/AlgH hydrogel loaded with 14-3-3ε protein enhanced the ability to induce osteoblast differentiation of human MSCs. Sun et al.[35] also confirmed that BMP-4-loaded GelMA hydrogels significantly increased the osteogenic differentiation of MSCs compared with ordinary flat plate medium. Therefore, GelMA hydrogel is a suitable carrier of transplanted cells and has excellent adhesion to various protein factors, laying the foundation for GelMA hydrogel-loaded growth factors to induce MSC osteogenic differentiation and promote bone growth.

However, Xu et al.[84] found that low levels of TGF-β1 can promote bone regeneration, while excessive TGF-β1 is not conducive to bone healing. While TGF-β2 can directly stimulate osteoprogenitor cell proliferation, it indirectly promotes osteoprogenitor cell differentiation into osteoblasts. Therefore, when TGF is used for treating diseases, it is necessary to strictly control its dose and avoid side effects caused by improper use. The above current research results are theoretically applicable for transplantation to accelerate the fusion between vertebral bodies after spinal fusion surgery, which provides a new idea for IVDD surgical treatment.

3.3. Promoting IVD Cell Regeneration. Regardless of whether the body is in motion or at rest, IVD will bear mechanical pressure from the vertical and horizontal directions and gradually cause some IVD cells to lose their normal biological functions. When the number of normal IVD cells is insufficient to meet the spine's usual stress, IVDD will occur.[85] When IVD tissue is exposed to abnormal mechanical stress, AF degeneration will lead to disordered NP colloidal components. Many NPCs lose their regular biological activity, the whole IVD structure loses balance, and IVDD development is more rapid.[86−88] Scholars agree that supplementing normal MSCs and NPCs in degenerative IVDs through minimally invasive methods inhibits IVDD development.[89−91] However, current studies have found that abnormal external mechanical force destroys IVD structure and scaffold function, and ECM secretion is lower than that in normal IVD tissue. Many cells

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Table 2. Summary of GelMA Hydrogels Loaded with Biological Factors

biological factor	target	type	organization	function	ref
small extracellular vesicles	rats	GelMA/nanoclay hydrogel	cartilage tissue	stimulate chondrogenesis and heal cartilage defects	37
hUVECs-exo	rats	GelMA hydrogel	skin tissue	accelerate wound healing	38
collagen	cells	GelMA hydrogel	vascular tissue	promote angiogenesis	36
aspirin	rabbits	aspirin−Lips−GelMA	IVD	regulation of inflammation after discectomy	14
exosomes and ECM	rabbits	GelMA hydrogel 3D stent	bone and cartilage tissue	promise osteochondral defects regeneration	39
octacalcium phosphate	cells	GelMA hydrogel 3D stent	bone and vascular tissue	promote osteogenesis and angiogenesis	53
riboflavin	cells	GelMA hydrogel	bone tissue	promote bone regeneration	40
iNSCs	mice	GelMA hydrogel	spinal cord	repair injured spinal cord	41
cellulose nanofibrils	cells	GelMA hydrogel	soft tissue	promote wound healing	54
decellularized liver matrix	cells	GelMA hydrogel	liver tissue	elevate liver functions	55
platelet	cells	GelMA hydrogel	bone and cartilage tissue	promote bone and cartilage regeneration	56
6-deoxy-aminocellulose derivatives	cells and rats	GelMA hydrogel	skin and soft tissue	accelerate wound healing	57
angiogenic growth factor	cells	GelMA−chitosan nanoparticles composite hydrogel	vascular tissue	deliver vascular growth factors and promote angiogenesis	45
14-3-3ε protein	cells	GelMA hydrogel	bone tissue	promote osteoblast differentiation and osteogenesis	42
TNP-470	cells	GelMA hydrogel	vascular tissue	inhibit tumor angiogenesis	46
sinomenium	mice	GelMA hydrogels and chitosan microspheres	bone and joint tissue	delay surgery-induced osteoarthritis	47
ciprofloxacin	cells	injectable and photo-cross-linkable GelMA	oral tissue	ablation for oral infection	48,49
puerarin	rabbits	photo-cross-link GelMA hydrogel	pelvic tissue	anti-inflammatory and promoting tissue regeneration	50
calcium peroxide	cells	GelMA hydrogel 4D	cartilage tissue	promote chondrocyte regeneration	51
hydroxyapatite	rats	HANW reinforced photo-cross-linkable GelMA	bone tissue	filling bone defect	58
metformin	cells	MSNs-laden GelMA	bone tissue	promote osteoblast proliferation	52
VEGF	cells	CBMA−GelMA	vascular tissue	promote angiogenesis	43

are lost during cell transplantation. The human body has the apparent immune rejection of some biological materials wrapping cells.[92,93] It is difficult for successfully transplanted cells to survive and reverse further IVDD deterioration. Fortunately, GelMA hydrogel products can be used as cell carriers to help improve IVDD treatment strategies.

GelMA hydrogels are the latest tissue engineering material. Encapsulated stem cell transplantation is a therapeutic strategy for IVD regeneration. The latest research showed that GelMA hydrogels do not cause transplant immune rejection in NP tissue. They can ensure that encapsulated MSCs, such as human MSCs (hMSCs),[59] adipose-derived mesenchymal stem cells (ADMSCs),[12] and BMSCs,[94] complete the fundamental growth, proliferation, differentiation, and migration process, and maintain the biological activity of MSCs.[95,96] Further studies have shown that the high water content and fluid pressure characteristics of GelMA hydrogels contribute to the nutritional supply and oxygen exchange of target cells.[13,97] They provide a suitable microenvironment for the growth of MSCs and NPCs, thus ensuring the value of transplantation.

There are few studies on GelMA hydrogel transplantation for IVDD. Nevertheless, its essence is similar to that of NPgel and other hydrogel transplantations for IVDD, and its biological performance is better. NPgel can simulate the matrix deposition of natural NP tissue and induce hMSCs to differentiate into NPCs, and injecting NPgel into IVD tissue can play the role of a scaffold and restore the mechanical function of the IVD.[98,99] Further studies found that hMSCs expressed the NP matrix components type II collagen and proteoglycan even in the presence of cytokines and free Ca^{2+}. NPgel-encapsulated human MSCs could differentiate into NPCs.[59] Future studies on GelMA hydrogels may achieve better transplantation results.

In addition, ADMSCs are widely used in composite GelMA hydrogels for clinical experimental research because of their wide range of sources, easy access, and multidirectional differentiation.[100] Studies have confirmed that the ADMSC−GDF/5−GelMA hydrogel system was transplanted into the degenerate rat IVD model by a minimally invasive method. ADMSCs showed good NPC differentiation ability under the directional induction of GDF-5, and much newly generated ECM was detected in NP tissues.[12] In addition, the GelMA hydrogel maintained the integrity of the degenerative IVD to some extent and delayed or even reversed the further development of IVDD.

GelMA hydrogel-encapsulated NPCs transplanted into degenerated IVD tissue may be a direct and effective way to reverse IVDD. In the late stage of IVDD, NPCs are deficient in nutrition and challenging to regenerate, limiting endogenous NP repair.[101] First, the high water content and porous structure of GelMA hydrogels facilitate oxygen and nutrient penetration and promote ECM production, which can provide an excellent local microenvironment for NPC growth.[6,102] Second, recent studies found that different concentrations of GelMA hydrogel could directly affect NPC biological activity after transplantation. Five percent GelMA hydrogels were most suitable for NPC transplantation, which was higher or lower than that which affected NPC survival after transplantation.[103]

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More importantly, in late IVDD, 5% GelMA hydrogel can bear the mechanical pressure of some spinal cords, which is conducive to maintaining NPC morphology and typical physiological structure after transplantation.[104] Therefore, GelMA hydrogel composite target cell transplantation is expected to cure IVDD at the cellular and molecular levels.

3.4. 3D-Printed IVD Structure. The effect of current surgical treatment on local stress and spine activity cannot be ignored. With the continuous development of 3D printing technology, based on the unique biological characteristics of GelMA hydrogels, GelMA hydrogel bionic IVDs can replace the IVD after surgical excision. The 3D-GelMA hydrogel IVD is expected to be used as a spinal fusion material without affecting spine stability and can better carry external mechanical pressure. For IVD diseases with impaired NP and AP, the significance of single simulated NP transplantation is low, and simulating the overall cell and IVD matrix structure remains a severe challenge (Table 2).

4. GELMA HYDROGEL CLINICAL VALUE

After more than 10 years of rapid development, GelMA hydrogels have been widely used in single-cell culture, cell signal transduction, and gene delivery. They stimulate the microenvironment of cell growth and build an economical and efficient 3D cell culture platform for cell culture in vitro.[105,106] The clinical application of GelMA hydrogels in combination with other hydrogel materials is becoming increasingly widespread, and the mechanical properties of the hydrogel are modified by adding various agents to meet clinical needs; for example, GelMA/poly(methyl methacrylate)/polydopamine with mild photothermal therapy hydrogel can perform excellent bone repair.[107] At present, GelMA hydrogels have been used in the exploration of various clinical diseases, such as bone defect repair,[108] skin defect regeneration,[38,109] cosmetics and regeneration,[19] corneal transplantation,[110,111] replacement of the heart valve,[34] pulp regeneration,[112] and angiogenesis regeneration.[53] The application of GelMA hydrogels from cell experiments and animal experiments to clinical trials and finally to treating clinical diseases is shown in Figure. 3B.

However, GelMA hydrogel products alone cannot meet the stress requirements of particular tissues and organs of biological organisms, such as the spine, lower limb bones, and blood vessels. Therefore, modified hydrocoagulants based on GelMA hydrogels have emerged when needed, such as three-dimensional-printed thermo/photo-cross-linked methacrylated chitosan-gelatin hydrogel,[113] injectable stress relaxation gelatin-based hydrogels with a positive surface,[114] GelMA-silk fibroin,[115] and polycaprolactone-GelMA-ultrasmall superparamagnetic iron oxide (USPIO).[32] These modified GelMA hydrogels show better mechanical performance and better match the mechanical properties of particular parts of the body.

5. LIMITATIONS AND PROSPECTS

Due to some shortcomings of GelMA hydrogels and the immaturity of current clinical technology, their clinical applications are limited to a certain extent. These limitations mainly include the following: (1) The adhesion, proliferation, and growth of cells are significantly affected by the concentration and amount of GelMA hydrogel, which is not conducive to cocultivation of multiple types of cells in the same matrix. (2) The degradation rate of in vitro pure GelMA hydrogel is relatively fast, which cannot meet the requirements of cell growth and proliferation. (3) In the 3D printing process of biomimetic organs, because the photosensitive GelMA hydrogel is strictly affected by the light control time, the strength of the single photo-cross-linked GelMA hydrogel material is poor, which is not conducive to 3D organ printing. (4) The characteristics of GelMA hydrogel for variable-biomolecule transplantation may cause rejection in the body. (5) The clinical application of GelMA hydrogel lacks uniform standards. (6) The current research on GelMA hydrogel is mainly limited to low-level organisms such as cells, rats, and rabbits and lacks experiments on high-level animals.

6. CONCLUSIONS

In summary, GelMA hydrogels and their modified structures/combinations will flourish in medicine. Especially for IVDD patients, its application prospects are broad. GelMA hydrogels can optimize the current clinical conservative and surgical treatment plan and improve the therapeutic effect from the current application value. Overall, GelMA hydrogels can reverse or repair IVDs at the etiological level through regeneration technology.

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Author Contributions

#These authors contributed equally.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Kirnaz, S.; Capadona, C.; Wong, T.; Goldberg, J. L.; Medary, B.; Sommer, F.; Mcgrath, L. B., Jr.; Härtl, R. Fundamentals of Intervertebral Disc Degeneration. *World Neurosurg* **2022**, *157*, 264−273.

(2) Hartvigsen, J.; Hancock, M. J.; Kongsted, A.; Louw, Q.; Ferreira, M. L.; Genevay, S.; Hoy, D.; Karppinen, J.; Pransky, G.; Sieper, J.; et al. What low back pain is and why we need to pay attention. *Lancet* **2018**, *391*, 2356−2367.

(3) Kassebaum, N. J.; Smith, A. G. C.; Bernabé, E.; Fleming, T. D.; Reynolds, A. E.; Vos, T.; Murray, C. J. L.; Marcenes, W.; et al. Global, Regional, and National Prevalence, Incidence, and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990−2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors. *J. Dent Res.* **2017**, *96*, 380−387.
(4) Wilson Zingg, R.; Kendall, R. Obesity, Vascular Disease, and Lumbar Disk Degeneration: Associations of Comorbidities in Low Back Pain. *Pm r* **2017**, *9*, 398−402.
(5) Cannata, F.; Vadalà, G.; Ambrosio, L.; Fallucca, S.; Napoli, N.; Papalia, R.; Pozzilli, P.; Denaro, V. Intervertebral disc degeneration: A focus on obesity and type 2 diabetes. *Diabetes Metab Res. Rev.* **2020**, *36*, No. e3224.
(6) Xu, P.; Guan, J.; Chen, Y.; Xiao, H.; Yang, T.; Sun, H.; Wu, N.; Zhang, C.; Mao, Y. Stiffness of photocrosslinkable gelatin hydrogel influences nucleus pulposus cell propertiesin vitro. *J. Cell Mol. Med.* **2021**, *25*, 880−891.
(7) Eisenstein, S. M.; Balain, B.; Roberts, S. Current Treatment Options for Intervertebral Disc Pathologies. *Cartilage* **2020**, *11*, 143−151.
(8) Chambers, A. W.; Lacy, K. W.; Liow, M. H. L.; Manalo, J. P. M.; Freiberg, A. A.; Kwon, Y. M. Multiple Hip Intra-Articular Steroid Injections Increase Risk of Periprosthetic Joint Infection Compared With Single Injections. *J. Arthroplasty* **2017**, *32*, 1980−1983.
(9) Kumar, H.; Sakthivel, K.; Mohamed, M. G. A.; Boras, E.; Shin, S. R.; Kim, K. Designing Gelatin Methacryloyl (GelMA)-Based Bioinks for Visible Light Stereolithographic 3D Biofabrication. *Macromol. Biosci* **2021**, *21*, No. e2000317.
(10) Xu, J.; Liu, J.; Gan, Y.; Dai, K.; Zhao, J.; Huang, M.; Huang, Y.; Zhuang, Y.; Zhang, X. High-Dose TGF-β1 Impairs Mesenchymal Stem Cell-Mediated Bone Regeneration via Bmp2 Inhibition. *J. Bone Miner Res.* **2020**, *35*, 167−180.
(11) Krouwels, A.; Melchels, F. P. W.; Van Rijen, M. H. P.; Öner, F. C.; Dhert, W. J. A.; Tryfonidou, M. A.; Creemers, L. B. Comparing Hydrogels for Human Nucleus Pulposus Regeneration: Role of Osmolarity During Expansion. *Tissue Eng. Part C Methods* **2018**, *24*, 222−232.
(12) Xu, H.; Sun, M.; Wang, C.; Xia, K.; Xiao, S.; Wang, Y.; Ying, L.; Yu, C.; Yang, Q.; He, Y. GDF5-GelMA injectable microspheres laden with adipose-derived stem cells for disc degeneration repair. *Biofabrication* **2021**, *13*, 015010.
(13) Curvello, R.; Raghuwanshi, V. S.; Garnier, G. Engineering nanocellulose hydrogels for biomedical applications. *Adv. Colloid Interface Sci.* **2019**, *267*, 47−61.
(14) Liu, Y.; Du, J.; Peng, P.; Cheng, R.; Lin, J.; Xu, C.; Yang, H.; Cui, W.; Mao, H.; Li, Y.; et al. Regulation of the inflammatory cycle by a controllable release hydrogel for eliminating postoperative inflammation after discectomy. *Bioact Mater.* **2021**, *6*, 146−157.
(15) Yue, K.; Trujillo-De Santiago, G.; Alvarez, M. M.; Tamayol, A.; Annabi, N.; Khademhosseini, A. Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. *Biomaterials* **2015**, *73*, 254−271.
(16) He, W.; Wang, H.; Zhang, X.; Mao, T.; Lu, Y.; Gu, Y.; Ju, D.; Qi, L.; Wang, Q.; Dong, C. Construction of a decellularized spinal cord matrix/GelMA composite scaffold and its effects on neuronal differentiation of neural stem cells. *J. Biomater Sci. Polym. Ed* **2022**, *33*, 2124.
(17) Xu, H.; Wang, J.; Wu, D.; Qin, D. A hybrid hydrogel encapsulating human umbilical cord mesenchymal stem cells enhances diabetic wound healing. *J. Mater. Sci. Mater. Med.* **2022**, *33*, 60.
(18) Wang, Y.; Ma, M.; Wang, J.; Zhang, W.; Lu, W.; Gao, Y.; Zhang, B.; Guo, Y. Development of a Photo-Crosslinking, Biodegradable GelMA/PEGDA Hydrogel for Guided Bone Regeneration Materials. *Materials (Basel)* **2018**, *11*, 1345.
(19) Nichol, J. W.; Koshy, S. T.; Bae, H.; Hwang, C. M.; Yamanlar, S.; Khademhosseini, A. Cell-laden microengineered gelatin methacrylate hydrogels. *Biomaterials* **2010**, *31*, 5536−5544.
(20) Xiao, S.; Zhao, T.; Wang, J.; Wang, C.; Du, J.; Ying, L.; Lin, J.; Zhang, C.; Hu, W.; Wang, L.; et al. Gelatin Methacrylate (GelMA)-Based Hydrogels for Cell Transplantation: an Effective Strategy for Tissue Engineering. *Stem Cell Rev. Rep* **2019**, *15*, 664−679.
(21) Sheikhi, A.; De Rutte, J.; Haghniaz, R.; Akouissi, O.; Sohrabi, A.; Di Carlo, D.; Khademhosseini, A. Microfluidic-enabled bottom-up hydrogels from annealable naturally-derived protein microbeads. *Biomaterials* **2019**, *192*, 560−568.
(22) Xie, M.; Gao, Q.; Qiu, J.; Fu, J.; Chen, Z.; He, Y. 3D biofabrication of microfiber-laden minispheroids: a facile 3D cell co-culturing system. *Biomater Sci.* **2020**, *8*, 109−117.
(23) Alge, D. L.; Anseth, K. S. Bioactive hydrogels: Lighting the way. *Nat. Mater.* **2013**, *12*, 950−952.
(24) Kirsch, M.; Birnstein, L.; Pepelanova, I.; Handke, W.; Rach, J.; Seltsam, A.; Scheper, T.; Lavrentieva, A. Gelatin-Methacryloyl (GelMA) Formulated with Human Platelet Lysate Supports Mesenchymal Stem Cell Proliferation and Differentiation and Enhances the Hydrogel's Mechanical Properties. *Bioengineering (Basel)* **2019**, *6*, 76.
(25) Luo, Y.; Shoichet, M. S. A photolabile hydrogel for guided three-dimensional cell growth and migration. *Nat. Mater.* **2004**, *3*, 249−253.
(26) West, J. L. Protein-patterned hydrogels: Customized cell microenvironments. *Nat. Mater.* **2011**, *10*, 727−729.
(27) Kim, M. J.; Chi, B. H.; Yoo, J. J.; Ju, Y. M.; Whang, Y. M.; Chang, I. H. Structure establishment of three-dimensional (3D) cell culture printing model for bladder cancer. *PLoS One* **2019**, *14*, No. e0223689.
(28) Naqvi, S. M.; Buckley, C. T. Differential response of encapsulated nucleus pulposus and bone marrow stem cells in isolation and coculture in alginate and chitosan hydrogels. *Tissue Eng. Part A* **2015**, *21*, 288−299.
(29) Elkhoury, K.; Sanchez-Gonzalez, L.; Lavrador, P.; Almeida, R.; Gaspar, V.; Kahn, C.; Cleymand, F.; Arab-Tehrany, E.; Mano, J. F. Gelatin Methacryloyl (GelMA) Nanocomposite Hydrogels Embedding Bioactive Naringin Liposomes. *Polymers (Basel)* **2020**, *12*, 2944.
(30) Khayat, A.; Monteiro, N.; Smith, E. E.; Pagni, S.; Zhang, W.; Khademhosseini, A.; Yelick, P. C. GelMA-Encapsulated hDPSCs and HUVECs for Dental Pulp Regeneration. *J. Dent Res.* **2017**, *96*, 192−199.
(31) Tavares, M. T.; Gaspar, V. M.; Monteiro, M. V.; Farinha, J. P. S.; Baleizao, C.; Mano, J. GelMA/bioactive silica nanocomposite bioinks for stem cell osteogenic differentiation. *Biofabrication* **2021**, *13*, 035012.
(32) Li, H.; Yin, Y.; Xiang, Y.; Liu, H.; Guo, R. A novel 3D printing PCL/GelMA scaffold containing USPIO for MRI-guided bile duct repair. *Biomed Mater.* **2020**, *15*, 045004.
(33) Zheng, Y.; Wu, G.; Chen, L.; Zhang, Y.; Luo, Y.; Zheng, Y.; Hu, F.; Forouzanfar, T.; Lin, H.; Liu, B. Neuro-regenerative imidazole-functionalized GelMA hydrogel loaded with hAMSC and SDF-1α promote stem cell differentiation and repair focal brain injury. *Bioact Mater.* **2021**, *6*, 627−637.
(34) Bejleri, D.; Streeter, B. W.; Nachlas, A. L. Y.; Brown, M. E.; Gaetani, R.; Christman, K. L.; Davis, M. E. A Bioprinted Cardiac Patch Composed of Cardiac-Specific Extracellular Matrix and Progenitor Cells for Heart Repair. *Adv. Healthc Mater.* **2018**, *7*, No. e1800672.
(35) Sun, X.; Ma, Z.; Zhao, X.; Jin, W.; Zhang, C.; Ma, J.; Qiang, L.; Wang, W.; Deng, Q.; Yang, H.; et al. Three-dimensional bioprinting of multicell-laden scaffolds containing bone morphogenic protein-4 for promoting M2 macrophage polarization and accelerating bone defect repair in diabetes mellitus. *Bioact Mater.* **2021**, *6*, 757−769.
(36) Stratesteffen, H.; Köpf, M.; Kreimendahl, F.; Blaeser, A.; Jockenhoevel, S.; Fischer, H. GelMA-collagen blends enable drop-on-demand 3D printablility and promote angiogenesis. *Biofabrication* **2017**, *9*, 045002.
(37) Hu, H.; Dong, L.; Bu, Z.; Shen, Y.; Luo, J.; Zhang, H.; Zhao, S.; Lv, F.; Liu, Z. miR-23a-3p-abundant small extracellular vesicles

https://doi.org/10.1021/acsomega.3c00211
ACS Omega 2023, 8, 13509−13518

released from Gelma/nanoclay hydrogel for cartilage regeneration. *J. Extracell Vesicles* **2020**, *9*, 1778883.
(38) Zhao, D.; Yu, Z.; Li, Y.; Wang, Y.; Li, Q.; Han, D. GelMA combined with sustained release of HUVECs derived exosomes for promoting cutaneous wound healing and facilitating skin regeneration. *J. Mol. Histol* **2020**, *51*, 251−263.
(39) Chen, P.; Zheng, L.; Wang, Y.; Tao, M.; Xie, Z.; Xia, C.; Gu, C.; Chen, J.; Qiu, P.; Mei, S.; et al. Desktop-stereolithography 3D printing of a radially oriented extracellular matrix/mesenchymal stem cell exosome bioink for osteochondral defect regeneration. *Theranostics* **2019**, *9*, 2439−2459.
(40) Goto, R.; Nishida, E.; Kobayashi, S.; Aino, M.; Ohno, T.; Iwamura, Y.; Kikuchi, T.; Hayashi, J. I.; Yamamoto, G.; Asakura, M. Gelatin Methacryloyl-Riboflavin (GelMA-RF) Hydrogels for Bone Regeneration. *Int. J. Mol. Sci.* **2021**, *22*, 1635.
(41) Fan, L.; Liu, C.; Chen, X.; Zou, Y.; Zhou, Z.; Lin, C.; Tan, G.; Zhou, L.; Ning, C.; Wang, Q. Directing Induced Pluripotent Stem Cell Derived Neural Stem Cell Fate with a Three-Dimensional Biomimetic Hydrogel for Spinal Cord Injury Repair. *ACS Appl. Mater. Interfaces* **2018**, *10*, 17742−17755.
(42) Aldana, A. A.; Uhart, M.; Abraham, G. A.; Bustos, D. M.; Boccaccini, A. R. 14−3-3ε protein-loaded 3D hydrogels favor osteogenesis. *J. Mater. Sci. Mater. Med.* **2020**, *31*, 105.
(43) Lai, T. C.; Yu, J.; Tsai, W. B. Gelatin methacrylate/carboxybetaine methacrylate hydrogels with tunable crosslinking for controlled drug release. *J. Mater. Chem. B* **2016**, *4*, 2304−2313.
(44) Luo, Z.; Sun, W.; Fang, J.; Lee, K.; Li, S.; Gu, Z.; Dokmeci, M. R.; Khademhosseini, A. Biodegradable Gelatin Methacryloyl Microneedles for Transdermal Drug Delivery. *Adv. Healthc Mater.* **2019**, *8*, No. e1801054.
(45) Modaresifar, K.; Hadjizadeh, A.; Niknejad, H. Design and fabrication of GelMA/chitosan nanoparticles composite hydrogel for angiogenic growth factor delivery. *Artif Cells Nanomed Biotechnol* **2018**, *46* (8), 1799−1808.
(46) Nguyen, D. T.; Fan, Y.; Akay, Y. M.; Akay, M. TNP-470 Reduces Glioblastoma Angiogenesis in Three Dimensional GelMA Microwell Platform. *IEEE Trans Nanobioscience* **2016**, *15*, 683−688.
(47) Chen, P.; Xia, C.; Mei, S.; Wang, J.; Shan, Z.; Lin, X.; Fan, S. Intra-articular delivery of sinomenium encapsulated by chitosan microspheres and photo-crosslinked GelMA hydrogel ameliorates osteoarthritis by effectively regulating autophagy. *Biomaterials* **2016**, *81*, 1−13.
(48) Ribeiro, J. S.; Daghrery, A.; Dubey, N.; Li, C.; Mei, L.; Fenno, J. C.; Schwendeman, A.; Aytac, Z.; Bottino, M. C. Hybrid Antimicrobial Hydrogel as Injectable Therapeutics for Oral Infection Ablation. *Biomacromolecules* **2020**, *21*, 3945−3956.
(49) Ribeiro, J. S.; Bordini, E. a. F.; Ferreira, J. A.; Mei, L.; Dubey, N.; Fenno, J. C.; Piva, E.; Lund, R. G.; Schwendeman, A.; Bottino, M. C. Injectable MMP-Responsive Nanotube-Modified Gelatin Hydrogel for Dental Infection Ablation. *ACS Appl. Mater. Interfaces* **2020**, *12*, 16006−16017.
(50) Qin, M.; Jin, J.; Saiding, Q.; Xiang, Y.; Wang, Y.; Sousa, F.; Sarmento, B.; Cui, W.; Chen, X. In situ inflammatory-regulated drug-loaded hydrogels for promoting pelvic floor repair. *J. Controlled Release* **2020**, *322*, 375−389.
(51) Montesdeoca, C. Y. C.; Afewerki, S.; Stocco, T. D.; Corat, M. a. F.; De Paula, M. M. M.; Marciano, F. R.; Lobo, A. O. Oxygen-generating smart hydrogels supporting chondrocytes survival in oxygen-free environments. *Colloids Surf. B Biointerfaces* **2020**, *194*, 111192.
(52) Qu, L.; Dubey, N.; Ribeiro, J. S.; Bordini, E. a. F.; Ferreira, J. A.; Xu, J.; Castilho, R. M.; Bottino, M. C. Metformin-loaded nanospheres-laden photocrosslinkable gelatin hydrogel for bone tissue engineering. *J. Mech Behav Biomed Mater.* **2021**, *116*, 104293.
(53) Anada, T.; Pan, C. C.; Stahl, A. M.; Mori, S.; Fukuda, J.; Suzuki, O.; Yang, Y. Vascularized Bone-Mimetic Hydrogel Constructs by 3D Bioprinting to Promote Osteogenesis and Angiogenesis. *Int. J. Mol. Sci.* **2019**, *20*, 1096.
(54) Xu, W.; Molino, B. Z.; Cheng, F.; Molino, P. J.; Yue, Z.; Su, D.; Wang, X.; Willför, S.; Xu, C.; Wallace, G. G. On Low-Concentration Inks Formulated by Nanocellulose Assisted with Gelatin Methacrylate (GelMA) for 3D Printing toward Wound Healing Application. *ACS Appl. Mater. Interfaces* **2019**, *11*, 8838−8848.
(55) Wu, G.; Wu, D.; Lo, J.; Wang, Y.; Wu, J.; Lu, S.; Xu, H.; Zhao, X.; He, Y.; Li, J.; et al. A bioartificial liver support system integrated with a DLM/GelMA-based bioengineered whole liver for prevention of hepatic encephalopathy via enhanced ammonia reduction. *Biomater Sci.* **2020**, *8*, 2814−2824.
(56) Jiang, G.; Li, S.; Yu, K.; He, B.; Hong, J.; Xu, T.; Meng, J.; Ye, C.; Chen, Y.; Shi, Z.; et al. A 3D-printed PRP-GelMA hydrogel promotes osteochondral regeneration through M2 macrophage polarization in a rabbit model. *Acta Biomater* **2021**, *128*, 150−162.
(57) Nazir, F.; Ashraf, I.; Iqbal, M.; Ahmad, T.; Anjum, S. 6-deoxy-aminocellulose derivatives embedded soft gelatin methacryloyl (GelMA) hydrogels for improved wound healing applications: In vitro and in vivo studies. *Int. J. Biol. Macromol.* **2021**, *185*, 419−433.
(58) Zhang, Y.; Leng, H.; Du, Z.; Huang, Y.; Liu, X.; Zhao, Z.; Zhang, X.; Cai, Q.; Yang, X. Efficient regeneration of rat calvarial defect with gelatin-hydroxyapatite composite cryogel. *Biomed Mater.* **2020**, *15*, 065005.
(59) Vickers, L.; Thorpe, A. A.; Snuggs, J.; Sammon, C.; Le Maitre, C. L. Mesenchymal stem cell therapies for intervertebral disc degeneration: Consideration of the degenerate niche. *JOR Spine* **2019**, *2*, No. e1055.
(60) Marques, C. F.; Diogo, G. S.; Pina, S.; Oliveira, J. M.; Silva, T. H.; Reis, R. L. Collagen-based bioinks for hard tissue engineering applications: a comprehensive review. *J. Mater. Sci. Mater. Med.* **2019**, *30*, 32.
(61) Bettinger, C. J.; Bruggeman, J. P.; Borenstein, J. T.; Langer, R. S. Amino alcohol-based degradable poly(ester amide) elastomers. *Biomaterials* **2008**, *29*, 2315−2325.
(62) Qiao, Y.; Liu, X.; Zhou, X.; Zhang, H.; Zhang, W.; Xiao, W.; Pan, G.; Cui, W.; Santos, H. A.; Shi, Q. Gelatin Templated Polypeptide Co-Cross-Linked Hydrogel for Bone Regeneration. *Adv. Healthc Mater.* **2020**, *9*, No. e1901239.
(63) Subbiah, R.; Balbinot, G. S.; Athirasala, A.; Collares, F. M.; Sereda, G.; Bertassoni, L. E. Nanoscale mineralization of cell-laden methacrylated gelatin hydrogels using calcium carbonate-calcium citrate core-shell microparticles. *J. Mater. Chem. B* **2021**, *9*, 9583−9593.
(64) Huang, L.; Zhang, Z.; Guo, M.; Pan, C.; Huang, Z.; Jin, J.; Li, Y.; Hou, X.; Li, W. Biomimetic Hydrogels Loaded with Nanofibers Mediate Sustained Release of pDNA and Promote In Situ Bone Regeneration. *Macromol. Biosci* **2021**, *21*, No. e2000393.
(65) Xin, T.; Gu, Y.; Cheng, R.; Tang, J.; Sun, Z.; Cui, W.; Chen, L. Inorganic Strengthened Hydrogel Membrane as Regenerative Periosteum. *ACS Appl. Mater. Interfaces* **2017**, *9*, 41168−41180.
(66) Xing, H.; Zhang, Z.; Mao, Q.; Wang, C.; Zhou, Y.; Zhou, X.; Ying, L.; Xu, H.; Hu, S.; Zhang, N. Injectable exosome-functionalized extracellular matrix hydrogel for metabolism balance and pyroptosis regulation in intervertebral disc degeneration. *J. Nanobiotechnology* **2021**, *19*, 264.
(67) Tang, K.; Su, W.; Huang, C.; Wu, Y.; Wu, X.; Lu, H. Notoginsenoside R1 suppresses inflammatory response and the pyroptosis of nucleus pulposus cells via inactivating NF-κB/NLRP3 pathways. *Int. Immunopharmacol* **2021**, *101*, 107866.
(68) Roberts, S.; Evans, H.; Trivedi, J.; Menage, J. Histology and pathology of the human intervertebral disc. *J. Bone Joint Surg Am.* **2006**, *88 Suppl 2*, 10−14.
(69) Bernthal, N. M.; Hart, C. M.; Sheth, K. R.; Bergese, S. D.; Ho, H. S.; Apfel, C. C.; Stoicea, N.; Rojhani, A.; Jahr, J. S. Local and Intra-articular Administration of Nonsteroidal Anti-inflammatory Drugs for Pain Management in Orthopedic Surgery. *Am. J. Ther* **2020**, *29* (2), e219−e228.
(70) Liang, J. P.; Accolla, R. P.; Jiang, K.; Li, Y.; Stabler, C. L. Controlled Release of Anti-Inflammatory and Proangiogenic Factors

https://doi.org/10.1021/acsomega.3c00211
ACS Omega 2023, 8, 13509−13518

from Macroporous Scaffolds. *Tissue Eng. Part A* **2021**, *27*, 1275−1289.
(71) Molinos, M.; Almeida, C. R.; Caldeira, J.; Cunha, C.; Gonçalves, R. M.; Barbosa, M. A. Inflammation in intervertebral disc degeneration and regeneration. *J. R Soc. Interface* **2015**, *12*, 20141191.
(72) Molinos, M.; Almeida, C. R.; Caldeira, J.; Cunha, C.; Gonçalves, R. M.; Barbosa, M. A. Inflammation in intervertebral disc degeneration and regeneration. *J. R Soc. Interface* **2015**, *12*, 20150429.
(73) Dudek, M.; Yang, N.; Ruckshanthi, J. P.; Williams, J.; Borysiewicz, E.; Wang, P.; Adamson, A.; Li, J.; Bateman, J. F.; White, M. R.; et al. The intervertebral disc contains intrinsic circadian clocks that are regulated by age and cytokines and linked to degeneration. *Ann. Rheum Dis* **2017**, *76*, 576−584.
(74) Wu, W.; Dai, Y.; Liu, H.; Cheng, R.; Ni, Q.; Ye, T.; Cui, W. Local release of gemcitabine via in situ UV-crosslinked lipid-strengthened hydrogel for inhibiting osteosarcoma. *Drug Deliv* **2018**, *25*, 1642−1651.
(75) Zhao, X.; Sun, X.; Yildirimer, L.; Lang, Q.; Lin, Z. Y. W.; Zheng, R.; Zhang, Y.; Cui, W.; Annabi, N.; Khademhosseini, A. Cell infiltrative hydrogel fibrous scaffolds for accelerated wound healing. *Acta Biomater* **2017**, *49*, 66−77.
(76) Puljak, L.; Marin, A.; Vrdoljak, D.; Markotic, F.; Utrobicic, A.; Tugwell, P. Celecoxib for osteoarthritis. *Cochrane Database Syst. Rev.* **2017**, *5* (5), Cd009865.
(77) Wiersema, T.; Tellegen, A. R.; Beukers, M.; Van Stralen, M.; Wouters, E.; Van De Vooren, M.; Woike, N.; Mihov, G.; Thies, J. C.; Creemers, L. B. Prospective Evaluation of Local Sustained Release of Celecoxib in Dogs with Low Back Pain. *Pharmaceutics* **2021**, *13*, 1178.
(78) Shim, E. K.; Lee, J. S.; Kim, D. E.; Kim, S. K.; Jung, B. J.; Choi, E. Y.; Kim, C. S. Autogenous Mesenchymal Stem Cells from the Vertebral Body Enhance Intervertebral Disc Regeneration via Paracrine Interaction: An in Vitro Pilot Study. *Cell Transplant* **2016**, *25*, 1819−1832.
(79) Tian, Y.; Yuan, W.; Li, J.; Wang, H.; Hunt, M. G.; Liu, C.; Shapiro, I. M.; Risbud, M. V. TGFbeta regulates Galectin-3 expression through canonical Smad3 signaling pathway in nucleus pulposus cells: implications in intervertebral disc degeneration. *Matrix Biol.* **2016**, *50*, 39−52.
(80) Noriega, D. C.; Ardura, F.; Hernandez-Ramajo, R.; Martin-Ferrero, M. A.; Sanchez-Lite, I.; Toribio, B.; Alberca, M.; Garcia, V.; Moraleda, J. M.; Sanchez, A.; et al. Intervertebral Disc Repair by Allogeneic Mesenchymal Bone Marrow Cells: A Randomized Controlled Trial. *Transplantation* **2017**, *101*, 1945−1951.
(81) Lo, K. W.; Ulery, B. D.; Ashe, K. M.; Laurencin, C. T. Studies of bone morphogenetic protein-based surgical repair. *Adv. Drug Deliv Rev.* **2012**, *64*, 1277−1291.
(82) Bian, Z.; Sun, J. Development of a KLD-12 polypeptide/TGF-beta1-tissue scaffold promoting the differentiation of mesenchymal stem cell into nucleus pulposus-like cells for treatment of intervertebral disc degeneration. *Int. J. Clin Exp Pathol* **2015**, *8*, 1093−1103.
(83) Tao, Y.; Zhou, X.; Liang, C.; Li, H.; Han, B.; Li, F.; Chen, Q. TGF-beta3 and IGF-1 synergy ameliorates nucleus pulposus mesenchymal stem cell differentiation towards the nucleus pulposus cell type through MAPK/ERK signaling. *Growth Factors* **2015**, *33*, 326−336.
(84) Xu, J.; Liu, J.; Gan, Y.; Dai, K.; Zhao, J.; Huang, M.; Huang, Y.; Zhuang, Y.; Zhang, X. High-Dose TGF-beta1 Impairs Mesenchymal Stem Cell-Mediated Bone Regeneration via Bmp2 Inhibition. *J. Bone Miner Res.* **2020**, *35*, 167−180.
(85) Bowden, J. A.; Bowden, A. E.; Wang, H.; Hager, R. L.; Lecheminant, J. D.; Mitchell, U. H. In vivo correlates between daily physical activity and intervertebral disc health. *J. Orthop Res.* **2018**, *36*, 1313−1323.
(86) Zhang, K.; Xue, C.; Lu, N.; Ren, P.; Peng, H.; Wang, Y.; Wang, Y. Mechanical loading mediates human nucleus pulposus cell viability and extracellular matrix metabolism by activating of NF-κB. *Exp Ther Med.* **2019**, *18*, 1587−1594.
(87) Liang, H.; Chen, S.; Huang, D.; Deng, X.; Ma, K.; Shao, Z. Effect of Compression Loading on Human Nucleus Pulposus-Derived Mesenchymal Stem Cells. *Stem Cells Int.* **2018**, *2018*, 1481243.
(88) Molladavoodi, S.; Mcmorran, J.; Gregory, D. Mechanobiology of annulus fibrosus and nucleus pulposus cells in intervertebral discs. *Cell Tissue Res.* **2020**, *379*, 429−444.
(89) Zhang, X. B.; Chen, X. Y.; Qi, J.; Zhou, H. Y.; Zhao, X. B.; Hu, Y. C.; Zhang, R. H.; Yu, D. C.; Gao, X. D.; Wang, K. P.; et al. New Hope for Intervertebral Disc Degeneration: Bone Marrow Mesenchymal Stem Cells and Exosomes Derived from Bone Marrow Mesenchymal Stem Cell Transplantation. *Curr. Gene Ther* **2022**, *22*, 291−302.
(90) Krut, Z.; Pelled, G.; Gazit, D.; Gazit, Z. Stem Cells and Exosomes: New Therapies for Intervertebral Disc Degeneration. *Cells* **2021**, *10*, 2241.
(91) Yamada, K.; Iwasaki, N.; Sudo, H. Biomaterials and Cell-Based Regenerative Therapies for Intervertebral Disc Degeneration with a Focus on Biological and Biomechanical Functional Repair: Targeting Treatments for Disc Herniation. *Cells* **2022**, *11*, 602.
(92) Risbud, M. V.; Schaer, T. P.; Shapiro, I. M. Toward an understanding of the role of notochordal cells in the adult intervertebral disc: from discord to accord. *Dev. Dyn.* **2010**, *239*, 2141−2148.
(93) Desmoulin, G. T.; Pradhan, V.; Milner, T. E. Mechanical Aspects of Intervertebral Disc Injury and Implications on Biomechanics. *Spine (Phila Pa 1976)* **2020**, *45*, E457−e464.
(94) Wang, F.; Nan, L. P.; Zhou, S. F.; Liu, Y.; Wang, Z. Y.; Wang, J. C.; Feng, X. M.; Zhang, L. Injectable Hydrogel Combined with Nucleus Pulposus-Derived Mesenchymal Stem Cells for the Treatment of Degenerative Intervertebral Disc in Rats. *Stem Cells Int.* **2019**, *2019*, 8496025.
(95) Lu, Z. F.; Zandieh Doulabi, B.; Wuisman, P. I.; Bank, R. A.; Helder, M. N. Influence of collagen type II and nucleus pulposus cells on aggregation and differentiation of adipose tissue-derived stem cells. *J. Cell Mol. Med.* **2008**, *12*, 2812−2822.
(96) Mohammadian, M.; Abasi, E.; Akbarzadeh, A. Mesenchymal stem cell-based gene therapy: A promising therapeutic strategy. *Artif Cells Nanomed Biotechnol* **2016**, *44*, 1206−1211.
(97) Silva-Correia, J.; Gloria, A.; Oliveira, M. B.; Mano, J. F.; Oliveira, J. M.; Ambrosio, L.; Reis, R. L. Rheological and mechanical properties of acellular and cell-laden methacrylated gellan gum hydrogels. *J. Biomed Mater. Res. A* **2013**, *101*, 3438−3446.
(98) Thorpe, A. A.; Dougill, G.; Vickers, L.; Reeves, N. D.; Sammon, C.; Cooper, G.; Le Maitre, C. L. Thermally triggered hydrogel injection into bovine intervertebral disc tissue explants induces differentiation of mesenchymal stem cells and restores mechanical function. *Acta Biomater* **2017**, *54*, 212−226.
(99) Thorpe, A. A.; Boyes, V. L.; Sammon, C.; Le Maitre, C. L. Thermally triggered injectable hydrogel, which induces mesenchymal stem cell differentiation to nucleus pulposus cells: Potential for regeneration of the intervertebral disc. *Acta Biomater* **2016**, *36*, 99−111.
(100) Huang, Q.; Zou, Y.; Arno, M. C.; Chen, S.; Wang, T.; Gao, J.; Dove, A. P.; Du, J. Hydrogel scaffolds for differentiation of adipose-derived stem cells. *Chem. Soc. Rev.* **2017**, *46*, 6255−6275.
(101) Wong, J.; Sampson, S. L.; Bell-Briones, H.; Ouyang, A.; Lazar, A. A.; Lotz, J. C.; Fields, A. J. Nutrient supply and nucleus pulposus cell function: effects of the transport properties of the cartilage endplate and potential implications for intradiscal biologic therapy. *Osteoarthritis Cartilage* **2019**, *27*, 956−964.
(102) Da Silva, K.; Kumar, P.; Van Vuuren, S. F.; Pillay, V.; Choonara, Y. E. Three-Dimensional Printability of an ECM-Based Gelatin Methacryloyl (GelMA) Biomaterial for Potential Neuro-regeneration. *ACS Omega* **2021**, *6*, 21368−21383.
(103) Lavrentieva, A.; Fleischhammer, T.; Enders, A.; Pirmahboub, H.; Bahnemann, J.; Pepelanova, I. Fabrication of Stiffness Gradients of

https://doi.org/10.1021/acsomega.3c00211
ACS Omega 2023, 8, 13509−13518

GelMA Hydrogels Using a 3D Printed Micromixer. *Macromol. Biosci* **2020**, *20*, No. e2000107.
(104) Xu, P.; Guan, J.; Chen, Y.; Xiao, H.; Yang, T.; Sun, H.; Wu, N.; Zhang, C.; Mao, Y. Stiffness of photocrosslinkable gelatin hydrogel influences nucleus pulposus cell propertiesin vitro. *J. Cell Mol. Med.* **2021**, *25*, 880.
(105) Antunes, J.; Gaspar, V. M.; Ferreira, L.; Monteiro, M.; Henrique, R.; Jerónimo, C.; Mano, J. F. In-air production of 3D co-culture tumor spheroid hydrogels for expedited drug screening. *Acta Biomater* **2019**, *94*, 392−409.
(106) Kaemmerer, E.; Melchels, F. P.; Holzapfel, B. M.; Meckel, T.; Hutmacher, D. W.; Loessner, D. Gelatine methacrylamide-based hydrogels: an alternative three-dimensional cancer cell culture system. *Acta Biomater* **2014**, *10*, 2551−2562.
(107) Wu, Y.; Zhang, X.; Tan, B.; Shan, Y.; Zhao, X.; Liao, J. Near-infrared light control of GelMA/PMMA/PDA hydrogel with mild photothermal therapy for skull regeneration. *Biomater Adv.* **2022**, *133*, 112641.
(108) Yu, Y.; Wang, Y.; Zhang, W.; Wang, H.; Li, J.; Pan, L.; Han, F.; Li, B. Biomimetic periosteum-bone substitute composed of preosteoblast-derived matrix and hydrogel for large segmental bone defect repair. *Acta Biomater* **2020**, *113*, 317−327.
(109) Rehman, S. R. U.; Augustine, R.; Zahid, A. A.; Ahmed, R.; Tariq, M.; Hasan, A. Reduced Graphene Oxide Incorporated GelMA Hydrogel Promotes Angiogenesis For Wound Healing Applications. *Int. J. Nanomedicine* **2019**, *14*, 9603−9617.
(110) Uyanıklar, M.; Günal, G.; Tevlek, A.; Hosseinian, P.; Aydin, H. M. Hybrid Cornea: Cell Laden Hydrogel Incorporated Decellularized Matrix. *ACS Biomater Sci. Eng.* **2020**, *6*, 122−133.
(111) Kilic Bektas, C.; Hasirci, V. Cell loaded 3D bioprinted GelMA hydrogels for corneal stroma engineering. *Biomater Sci.* **2020**, *8*, 438−449.
(112) Nemeth, C. L.; Janebodin, K.; Yuan, A. E.; Dennis, J. E.; Reyes, M.; Kim, D. H. Enhanced chondrogenic differentiation of dental pulp stem cells using nanopatterned PEG-GelMA-HA hydrogels. *Tissue Eng. Part A* **2014**, *20*, 2817−2829.
(113) Osi, A. R.; Zhang, H.; Chen, J.; Zhou, Y.; Wang, R.; Fu, J.; Müller-Buschbaum, P.; Zhong, Q. Three-Dimensional-Printable Thermo/Photo-Cross-Linked Methacrylated Chitosan-Gelatin Hydrogel Composites for Tissue Engineering. *ACS Appl. Mater. Interfaces* **2021**, *13*, 22902−22913.
(114) Wang, K. Y.; Jin, X. Y.; Ma, Y. H.; Cai, W. J.; Xiao, W. Y.; Li, Z. W.; Qi, X.; Ding, J. Injectable stress relaxation gelatin-based hydrogels with positive surface charge for adsorption of aggrecan and facile cartilage tissue regeneration. *J. Nanobiotechnology* **2021**, *19*, 214.
(115) Xiao, W.; Li, J.; Qu, X.; Wang, L.; Tan, Y.; Li, K.; Li, H.; Yue, X.; Li, B.; Liao, X. Cell-laden interpenetrating network hydrogels formed from methacrylated gelatin and silk fibroin via a combination of sonication and photocrosslinking approaches. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2019**, *99*, 57−67.

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