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Sources, Structures, and Properties of Alginate

Alginate, also known as alginic acid, is a kind of natural linear anionic polysaccharide widely used in the field of biomedicine. Alginate is mainly distributed in the cell wall of brown algae and extracted in the form of acidification and sodium salt. In recent years, some researchers have extracted alginate through microbial fermentation of *Pseudomonas aeruginosa* [1].

Alginate is a straight-chain polymer composed of 1-4-linked β-D-mannuronic acid (M) or α-L-guluronic acid (G), which is interspersed with regions containing alternating M–G sequence [2]. The chemical structure of alginate from different sources is also quite different. Alginate from algae has high content of Poly G and excellent antibacterial activity, while alginate from microorganism has high content of Poly M, which can induce monocytes to produce inflammatory mediators such as interleukin-1, interleukin-6, and tumor necrosis factor. Na^+ in alginate guluronic acid (Poly G) can be exchanged with divalent cations to form physically cross-linked hydrogels, in which the cross-linking model with Ca^{2+} is called "eggs-box model" (side-by-side Poly G can form a pore conducive to Ca^{2+} cross-linking, and each Poly G binds to the corresponding two Poly G in an orderly manner) [3]. During the cross-linking process, the alginate droplets containing the required protein will be extruded from the gel frame to form alginate microspheres. Due to the mild cross-linking conditions and excellent mechanical properties, alginate hydrogel has become a research hotspot in the field of cell encapsulation and tissue engineering. Owing to the free hydroxyl and carboxyl groups, alginate also has excellent bio-adhesion. In addition, the pH sensitivity of alginate (which shrinks under low pH conditions) also has a great prospect in targeted drug delivery.

At present, the modification of alginate is mainly based on the following two aspects: Firstly, the alginate materials with different properties were obtained by adjusting the content of Poly M and Poly G in alginate. Secondly, the active sites of alginate (carboxyl group, hydroxyl group, 1-4 glycosidic, internal glycolic bonds) were modified to improve the properties of the derived materials.

Natural Polymers for Biomedical Applications, First Edition. Wenguo Cui and Lei Xiang.

1.1 Alginate-Based Hydrogel for Biomedical Application

1.1.1 Drug and Cell Delivery

Systematic administration of antibiotics is the main cause of widespread drug resistance throughout the body, and the development of a local targeted administration system is an effective way to solve this clinical problem. In order to solve many side effects of intravenous application of antibiotics, Czuban et al. [4] prepared tetrazine-modified alginate hydrogel. Based on the principle of the inverse electron-demand Diels–Alder chemistry, vancomycin and daptomycin loaded with hydrogel can be released at the site of infection, and this hydrogel can repeatedly achieve drug loading and local release, significantly reducing adverse reactions caused by the use of antibiotics.

Autologous tumor cell vaccine is an individualized therapeutic strategy to activate tumor-specific immune response. However, it has limited efficacy in "cold" solid tumors that lack tumor-infiltrating T cells and are insensitive to immunotherapy. Ke et al. constructed a dendritic cell (DC)-activated hydrogel system using bifunctional fusion membrane nanoparticles (FM-NPs) composed of autologous tumor cell membranes and *Mycobacterium leprae* membrane extract to provide tumor antigenic signals and to interact with granulocyte-macrophage colony-stimulating factor (GM-CSF). Nanoparticles (NPs) composed of autologous tumor cell membranes and *Mycobacterium leucocephala* membrane extracts were used to provide tumor antigen signaling and were co-loaded with GM-CSF in an alginate hydrogel. Rapid release of GM-CSF recruited DCs; FM-NPs continuously activated the maturation of DCs and provided tumor antigens. The hydrogel system could increase the infiltration of effector memory T cells and activate "cold" tumors to exert significant anti-tumor effects. This study provides a feasible strategy to overcome the bottleneck of the efficacy of autologous tumor vaccines in "cold" tumors and points out a new direction to improve the clinical efficacy [5] (Figure 1.1).

The adenosinergic axis limits the effectiveness of current tumor immunotherapy by inhibiting the activity of effector T cells. How to effectively remodel the adenosinergic axis has become a key target to improve the effect of anti-tumor immunotherapy. Zhao et al. constructed an injectable hydrogel system based on alginic acid, and used the synergistic effects of adenosine deaminase, docetaxel, and benzotricarboxylic acid to realize the conversion from immunosuppressive adenosine to immuno-strengthening inosine to remodel the adenosinergic axis and exert anti-tumor effects. Docetaxel and benzotricarboxylic acid synergistically induced a large release of ATP, which triggered a strong immune response; adenosine deaminase catalyzed the conversion of adenosine to inosine, which further enhanced the immune effect; and ultimately achieved the reversal of the negative feedback from adenosine to positive feedback from inosine. The hydrogel strategy reshaped the adenosinergic axis through cascade amplification of ATP-mediated anti-tumor immune response, which provided a new idea and means to enhance the effect of tumor immunotherapy [6].

In the postoperative treatment of breast cancer, high local recurrence rates and potential wound infections pose significant risks to patient survival. To overcome

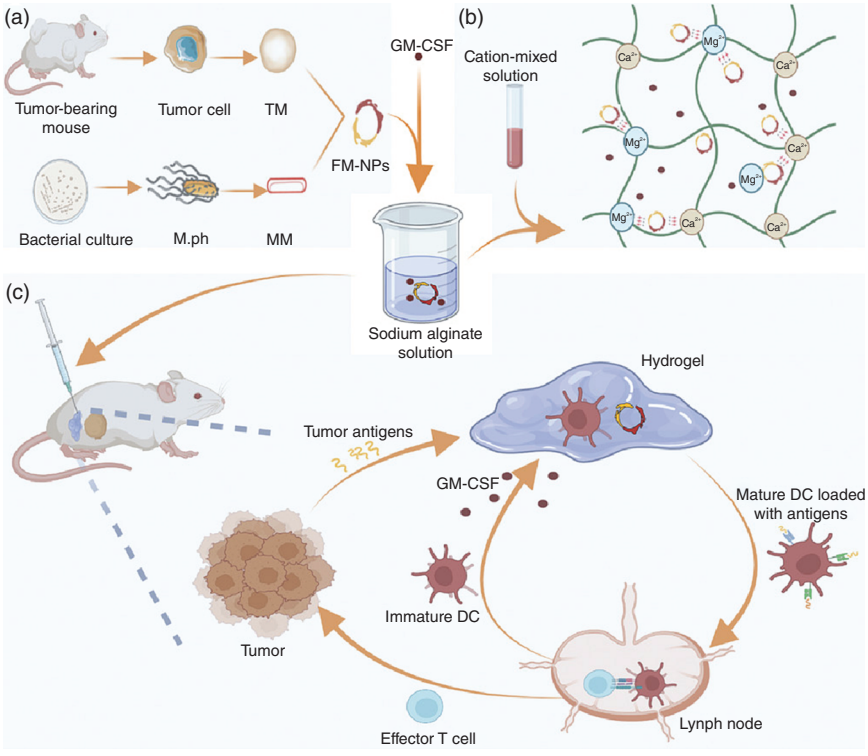

Figure 1.1 (a) Fusion membrane nanoparticles (FM-NPs) were prepared from autologous tumor cell membranes and *Bacillus* membrane extracts. (b) Sodium alginate solution was cross-linked with cationic solution to form hydrogels at room temperature. (c) FM-NPs and sodium alginate solution were used to form a hydrogel in vivo, which attracted dendritic cells and were activated by FM-NPs. Mature dendritic cells carrying tumor antigens stimulated the increase of effector memory T-cells, which exerted anti-tumor effects. Source: Ref. [5]/John Wiley & Sons.

these challenges, Wu et al. conducted a study on a nanocomposite dual-network (NDN) hydrogel. The hydrogel was constructed using polyethylene glycol acrylate (PEGDA) and alginate, embedded with 125i-labeled RGDY peptide-modified gold nanorods (125I-GNR-RGDY). This study formed hydrogels with a dual-network structure by near infrared (NIR) light-induced polymerization of PEGDA and endogenous Ca^{2+} cross-linking of alginate to construct a second network. This design enabled the hydrogel to exhibit stable photothermal effects and radiolabeling under NIR light irradiation. Photothermal therapy synergizes with brachytherapy by inhibiting DNA self-repair, promoting blood circulation, and improving the hypoxic microenvironment to enhance the therapeutic effect. This study provides a novel therapeutic approach by in situ injection of a precursor solution into the lumen of excised mouse cancerous breasts to form a rapidly gelatinizing hydrogel. By combining photothermal therapy and radiation therapy, this approach is expected to reduce the risk of local recurrence and decrease the likelihood of wound infection in postoperative breast cancer patients. This targeted therapeutic strategy offers new prospects for improving the outcome and survival of breast cancer patients [7].

The presence of immunosuppressive cells in the tumor microenvironment, especially tumor-associated macrophages (TAMs), poses a limitation on T-cell infiltration and activation, which in turn constrains the anticancer effect of immune checkpoint blockade. Li et al. developed a biocompatible alginate-based hydrogel that carries encapsulated nanoparticles loaded with pessitinib (PLX). The hydrogel gradually released PLX at the tumor site by blocking the colony-stimulating factor 1 receptor (CSF1R) in order to reduce the presence of TAMs. This strategy not only creates an environment conducive to promoting local and systemic delivery of anti-PD-1 antibodies (aPD-1), thereby inhibiting postoperative tumor recurrence, but also further contributes to T-cell infiltration of tumor tissue by reprogramming the tumor immunosuppressive microenvironment. In addition, the postoperative inflammatory environment triggers platelet activation, which promotes the release of aPD-1 and reactivates T cells by binding to the PD-1 receptor. It was noted that hydrogels can act as local reservoirs for sustained release of PLX-NP and P-aPD-1 to enhance the efficacy of tumor immunotherapy. The immunotherapeutic effect of systemic injection of P-aPD-1 could also be further enhanced by the hydrogel strategy of local depletion of TAMs, broadening the route of administration of immune checkpoint inhibitors. This study provides new ideas for regulating the tumor immune environment and improving the therapeutic effect [8].

Refractory keratitis and diabetic foot ulcers pose a great threat to human health due to drug-resistant bacterial infections and prolonged tissue hypoxia, and novel and effective therapeutic strategies are urgently needed. A self-oxygenated bilayer hydrogel was developed and prepared for the treatment of these diseases by Zhu et al. The inner hydrogel was composed of oxidized sodium alginate and carboxymethyl chitosan-containing photosensitizer PCN-224 and pH indicator bromothymol blue, while the outer hydrogel contained photosynthetic cyanobacteria. The inner hydrogel could sense the change of pH value to monitor the bacterial infection in real time and release PCN-224 in response to the infection for photodynamic bactericidal treatment; the cyanobacteria in the outer hydrogel continued to photosynthesize to produce oxygen to alleviate the hypoxic state of the tissues, enhance the effect of photodynamic therapy, and provide the necessary oxygen for the wound-healing process. In diabetic rat skin ulcer model and refractory keratitis animal model, the hydrogel could effectively sterilize, reduce inflammation, promote blood vessel regeneration and fibrous tissue formation, and significantly improve the therapeutic effect. The self-oxygenated bilayer hydrogel provides a novel strategy for the treatment of refractory ocular and skin diseases, and shows great application prospects and value [9].

Gan et al. used microfluidic electrospray technology to encapsulate mesenchymal stem-cell-derived exosomes in multilayered sodium alginate-gelatin (Gel) microcapsules for the targeted release of exosomes for protective delivery in the gastrointestinal tract and treatment of inflammatory bowel disease. In this study, inspired by the acid–base stability of Gel capsules, a novel multilayer microcapsule was prepared to encapsulate MSC-derived exosomes. The exosomes were first encapsulated in a core of sodium alginate gel microspheres using microfluidic electrospray technology and then coated with a Gel interlayer to protect them from degradation. The resistance of the microcapsules to gastric juices was enhanced by the use of a synthesized enteric

polymer outer coating. The results showed that the prepared microcapsules could effectively protect the stability and bioactivity of exosomes against gastrointestinal digestion, and enable the release of exosomes at the site of intestinal injury to perform the biological functions of immunomodulation and damage repair. Therefore, the exosome-encapsulated microcapsules provide a new strategy of effective protection and targeted release for various oral cell therapies [10].

Zhang et al. designed an injectable hydrogel to simultaneously modulate T-cell exhaustion and MHC I expression for enhanced T-cell-based cancer immunotherapy. The hydrogel utilized sodium-oxidized alginate-modified tumor cell membrane vesicles (O-TMV) as the gelling agent and contained axitinib, 4-1BB antibody, and PF-06446846 nanoparticles. After the immune response was triggered by the O-TMV antigen, the hydrogel demonstrated superior immunotherapeutic effects through multiple mechanisms. 4-1BB antibody promoted T-cell mitochondrial biogenesis, axitinib reversed T-cell exhaustion, and PF-06446846 amplified MHC I expression to improve T-cell recognition of tumor cells. O-TMV@ABP hydrogel effectively inhibited tumor growth through strong immune responses and long-term memory immune response effectively inhibits tumor growth, metastasis, and recurrence. This innovative strategy provides a new concept for T-cell-based cancer immunotherapy and demonstrates the strong potential of the hydrogel platform [11].

Stem cell injection therapy has significant efficacy in the treatment of many diseases such as diabetes, but simple stem cell injection has the problems of immune rejection and graft removal. In order to solve these problems, Delcassian et al. [12] loaded COOH-modified iron oxide nanoparticles and living islet cells into alginate hydrogel. On the one hand, the immune rejection of alginate hydrogel is significantly reduced; on the other hand, iron oxide nanoparticles can make the inhibitor move directionally in the magnetic field, which solves the problem of recovery after failed transplantation (Figure 1.2).

An adhesive and adjustable methacrylic-acid-modified alginate saline gel has been developed by Hasani-Sadrabadi et al. [13], which was coated with gingival mesenchymal stem cells and hydroxyapatite particles were introduced to induce bone regeneration, which effectively repaired mouse craniofacial bone defects. Whitehead Jacklyn et al. [14] reported that hydroxyapatite nanoparticles adsorbed BMP-2 were added to mesenchymal stromal cell spheres and then embedded in Arginine–Glycine–Aspartic acid (RGD)-modified alginate hydrogels. They found that the viscoelastic dynamic mechanical properties of alginate hydrogels obtained by ionic cross-linking significantly enhanced the therapeutic potential of MSCs spheres in bone formation and repair. Hung et al. [15] have prepared a peptide-modified viscous hydrogel which can double stimulate MSCs. On the one hand, QK peptides are used to functionalize alginate to promote the secretion of angiogenic factors; on the other hand, RGD modification promotes cell adhesion and proliferation.

Hasturk et al. prepared enzyme-cross-linked alginate and alginate/gelatin composite microspheres by a simple and economical centrifugation method for microencapsulation protection of mammalian cells. The composite microspheres were more structurally stable under ionic conditions and had better mechanical properties than the ionically cross-linked alginate microspheres only. It was shown

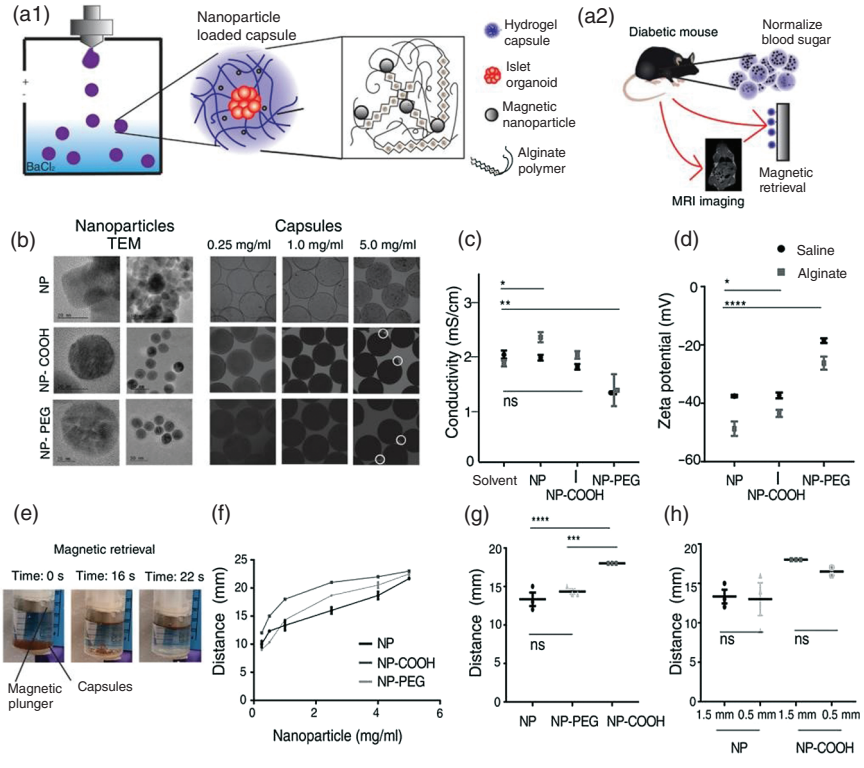

Figure 1.2 (a1) Schematic diagram of hydrogel vesicle formation containing nanoparticles; (a2) MRI and magnetic retrieval for diabetic transplantation. (b) TEM images of different nanoparticles and microscopic images of hydrogel capsules containing 0.25–5 mg/ml nanoparticles. (c,d) Conductivity and zeta potential of nanoparticles in saline or saline-sodium alginate. The conductivity and zeta potential of the nanoparticles were reduced compared to that of sodium alginate. (e–h) Magnetic recovery experiments were performed on hydrogels containing nanoparticles. As the concentration of nanoparticles increased, the traveling distance of the hydrogel increased; decreasing the capsule size decreased the traveling distance. Source: Ref. [12]/John Wiley & Sons.

that the human mesenchymal stem cells and neural progenitor cells encapsulated in the composite microspheres were effectively protected against various environmental factors, including extracellular toxins, acidosis, apoptotic factors, ultraviolet radiation, hypoxic conditions, and mechanical stresses. The microencapsulated cells maintained high viability, proliferation, and directed differentiation after extrusion through a 27-gauge needle. This demonstrated that the novel microencapsulation strategy has promising applications in cell injection delivery and three-dimensional bioprinting. Overall, the double-cross-linked composite microspheres provide a new, simple, and effective method for microenvironmental regulation and protection of mammalian cells [16].

Myocardial infarction (MI) is a major cause of sudden cardiac death, and platelet-rich fibrin is beneficial for restoring vascular regeneration in the infarcted area due to its richness in growth factors. Based on its biocompatibility and cost-effectiveness,

alginate hydrogel is an advantageous way to achieve targeted delivery of platelet-rich fibrin. Qian et al. achieved infiltration of M2 macrophages in the infarcted area through the construction of alginate hydrogel, which is beneficial for improving the degree of myocardial fibrosis, and this hydrogel can also provide strong mechanical support for the ventricular wall and improve cardiac function [17].

Localized stem cell delivery to the human locomotor system and major weight-bearing tissues requires high structural strength of the delivery platform. Panebianco et al. used degradable alginate microspheres to microencapsulate mesenchymal cells to protect the cells and maintain cell viability and phenotype upon release. The composite of cell-carrying microspheres with high-modulus cross-linked fibrin gels balanced biomechanical properties and cell biological activity. The composites showed better cell survival and matrix synthesis than fibrin gels alone. The biomechanical stability of the composites and their ability to promote extracellular matrix synthesis were verified in large animal in vitro experiments. The composites significantly improved biomechanical function and biological repair compared with discectomy alone [18].

Diabetic foot ulcer (DFU) is a serious complication of diabetes mellitus, and the local hypertonicity makes the wound prolonged, Theocharidis et al. thoroughly investigated the potential application of alginate dressings for the local delivery of macrophages and their secretory products for the treatment of DFU. By preparing alginate dressings with a microporous structure, they were able to achieve a uniform loading of primary macrophages and realized the loading of macrophages with different polarization states (M0, M1, M2a, M2c) onto the dressing and their migration into the wound. The experimental results demonstrated that the treatment of each macrophage subtype promoted DFU healing in db/db mice [19].

1.1.2 Cell and Organoid Culture

Different hydrogel micropatterns can be formed by adjusting the cross-linking density of various parts of the hydrogel. Jeon et al. [20] used chemical and optical double-cross-linking methods to give oxidized, methacrylated alginate and 8-arm poly amine hydrogel micropatterns of different sizes, and found that the size of these micropatterns can significantly affect the proliferation and differentiation behavior of cells. The larger the micropattern (25–100 μm), the more obvious the proliferation and osteoblast differentiation of human adipose-derived stem cells. In addition, Gonzalez-Pujana et al. [21] further studied the effect of hydrogel on controlling the gene expression and secretory behavior of human marrow mesenchymal stem cells (HMSCs). They prepared click functionalized sodium alginate and fibrous collagen composite hydrogels and loaded with interferon-γ and heparin-coated microspheres. In the hydrogel microenvironment, bone marrow mesenchymal stem cells increased the expression of key genes such as indoleamine 2-dioxygenase-1 and galactose lectin-9, and promoted the secretion of license response factor Gal-9. And HMSCs cultured in hydrogel can inhibit activated human T cells, which proves that this hydrogel can enhance the immunomodulatory properties of HMSCs. Liu et al. [22] developed a hydrogel microfluidic system based on the interfacial

complexation of inversely charged sodium alginate and chitosan, and used this capsule to culture human induced pluripotent stem cells (HiPSCs), and successfully formed islet organisms (containing islet-specific α and β-like cells, highly expressing pancreatic hormone-specific genes and proteins). It reveals the great potential of alginate hydrogel in the preparation of artificial organs (Figure 1.3).

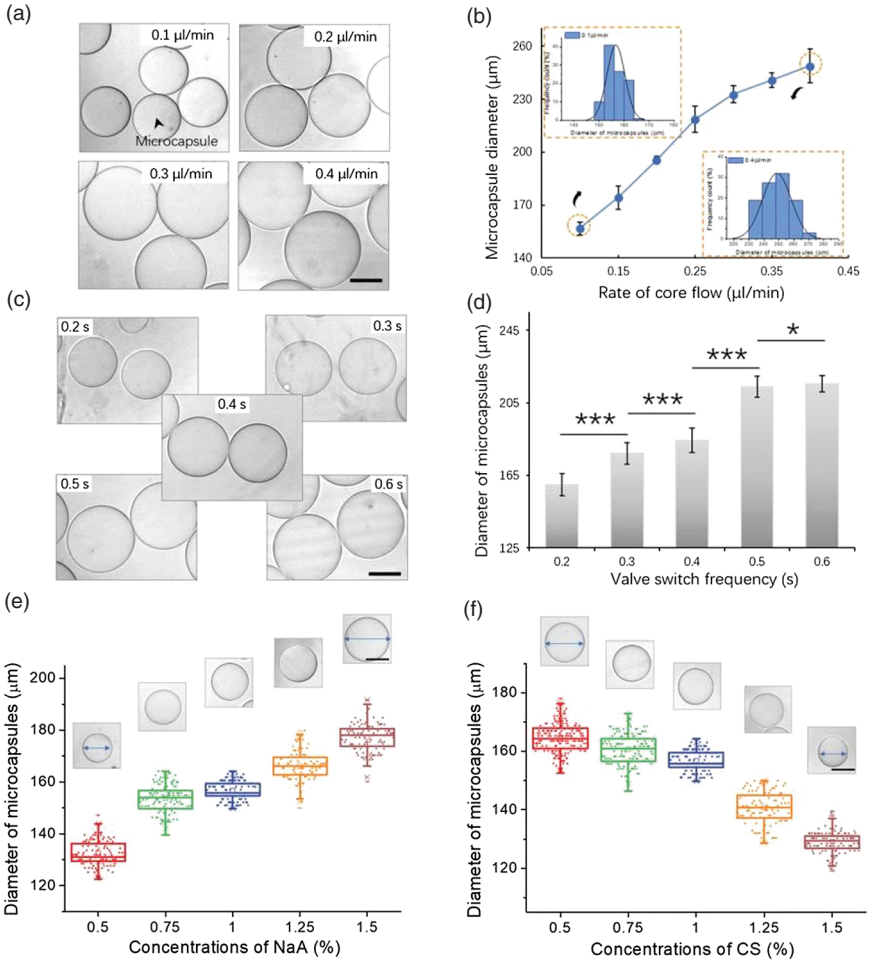

Figure 1.3 (a) Images of hydrogel vesicles generated at different core flow rates. The hydrogel vesicle size decreases with increasing core flow velocity. (b) At a fixed boundary flow rate, the hydrogel vesicle size is negatively correlated with the core flow rate. Increasing the core flow rate can reduce the size distribution of hydrogel vesicles. (c) Hydrogel vesicle images generated at different valve switching frequencies. The hydrogel vesicle size decreases with increasing frequency. (d,e) Hydrogel vesicles generated with different concentrations of sodium alginate. The size of the hydrogel vesicle decreases as the concentration of sodium alginate increases. (f) Hydrogel vesicles generated by using different concentrations of chitosan. The size of the hydrogel vesicles decreases as the concentration of chitosan increases. Source: Ref. [22]/John Wiley & Sons/CC BY 4.0.

1.1.3 Tissue Regeneration

The repair of cartilage-bone defects has always been a challenging task. Liao et al. [23] used alginate and calcium gluconate to cross-linking the upper hydrogel (composed of CSMA and NIPAM) and the lower hydrogel (composed of PECDA, AAm, and PEGDA) to form bidirectional hydrogels. The problem that the junction tissue is difficult to repair is perfectly solved by a two-way hydrogel design (the lower layer promotes bone regeneration and the upper layer promotes cartilage regeneration). Öztürk et al. [24] reported sulfated alginate hydrogel can induce the rapid proliferation of chondrocytes through gene-mediated FGF receptors and downstream signals, which opens up a new idea for cartilage repair. When the cartilage is so damaged that it is difficult to repair by its own tissue cells, the defect can only be repaired by artificial cartilage implantation (Figure 1.4).

Liao et al. [25] used the inter-transmission network structure formed by sodium alginate, fibrin, and polyacrylamide to prepare a hydrogel similar to that of natural cartilage with friction coefficient, and the implanted hydrogel is beneficial to

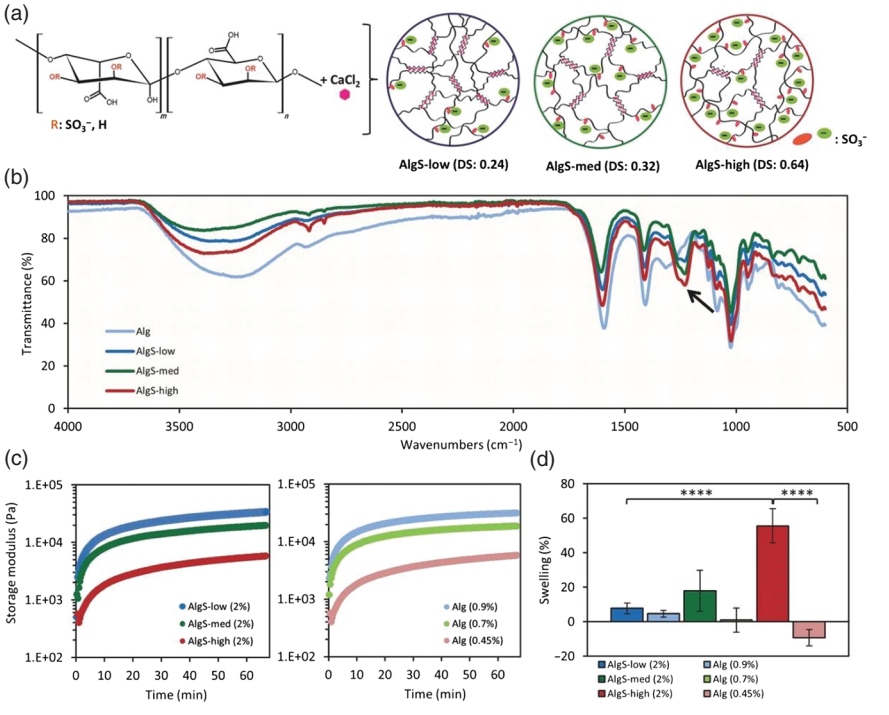

Figure 1.4 (a) Schematic of the chemical structure of sulfated sodium alginate. The degree of sulfation increases and cross-links with calcium ions to form an alginate sulfate hydrogel. (b) Infrared spectra of sodium alginate with different degrees of sulfation. The S=O characteristic peaks are enhanced by increasing the degree of sulfation. (c) Rheological profiles of alginate sulfate and sodium alginate hydrogels. The storage modulus of the hydrogels increases with the increase of the degree of sulfation. (d) Mass swelling percentage of the two hydrogels. The swelling percentage of alginate sulfate hydrogel is lower than that of sodium alginate hydrogel. Source: Ref. [24]/John Wiley & Sons.

cell infiltration and angiogenesis. It has great potential in artificial cartilage transplantation.

Dental tissue stem cells not only have great potential in promoting bone repair, but also play a great role in tendon regeneration. Moshaverinia et al. [26] prepared TGF β 3-RGD-coupled alginate microspheres as carriers to construct a co-transport system of periodontal ligament stem cells and gingival mesenchymal stem cells. A large number of regenerated tendon tissue deposits were found by injecting modified alginate microspheres into immunocompromised mice. Mredha et al. [27] study in-depth of alginate hydrogel and further confirmed its role in the preparation of artificial tendons. By adding calcium ions to the alginate solution to form a physical gel, and while drying the hydrogel, the tractive force in the length direction is applied at both ends of the hydrogel, so that the length of the hydrogel remains unchanged while the width and thickness of the hydrogel are reduced during the drying process. In this way, the hydrogel forms a hierarchical fiber structure, and further drying will induce the nanofibers to gather and form thicker fibers. Due to the stable supramolecular interaction between polymers, the re-swollen gel maintains the same structure as the natural tendon.

Alginate hydrogel also has a very important application in vascular and cardiac structural reconstruction. Campbell et al. [28] made the hydrogel achieve controllable degradation by adding alginate lyase to the alginate hydrogel, and the pore size of the hydrogel increased obviously with the addition of alginate lyase. The experimental results show that the migration of outgrowth endothelial cells is 10 times higher than that of non-degradable hydrogel. The ability of promoting angiogenesis was confirmed by chicken chorionic sac experiment. The effect of stem cell transplantation has been restricted by the reactive oxygen microenvironment caused by myocardial infarction. Hao et al. [29] use alginate saline to coagulate loaded fullerenol nanoparticles to remove free radicals and reactive oxygen species from the injured site, so as to improve the reactive oxygen microenvironment caused by myocardial infarction. By activating ERK and p38 pathway, inhibiting JNK pathway, inhibiting oxidative stress damage of brown adipose-derived stem cells, improving its survival ability in ROS microenvironment, the therapeutic effect of stem cell transplantation in the treatment of myocardial infarction was improved. Anker et al. [30] demonstrated the efficacy of injection of alginate hydrogel in the treatment of advanced chronic heart failure in a clinical controlled trial involving 78 patients. The results showed that the 6MWT distance and NYHA functional class of 40 cases in the alginate hydrogel group was significantly improved. Alginate hydrogel therapy is superior to standard drug therapy in improving exercise ability and relieving symptoms. Leor et al. [31] also confirmed that injection of cross-linked calcium alginate solution (which can cause liquid–gel phase transition after deposition in the infarcted myocardium) four days after infarction can effectively prevent ventricular enlargement and promote myocardial remodeling.

Stem-cell-based tissue engineering offers new therapeutic avenues for the treatment of various diseases and tissue regeneration; however, the infusion of exogenous stem cells still has many problems such as limited cell sources and potential rejection. Therefore, how to maximize the mobilization of residual stem cells around

the defect site may be the key to solving the problem of tissue regeneration. He et al. utilized the acoustic response of alginate hydrogel to load BMP-2, which has the ability to recruit stem cells, and used pulsed ultrasound to stimulate the intrinsic resonance of the hydrogel to achieve rapid degradation of the hydrogel and on-demand recruitment of endogenous stem cells [32].

Conventional implantable block hydrogels for cavernous injuries cannot meet the needs of repairing irregular lesions, so injectable hydrogels with shear-thinning properties have a more suitable application environment. Zheng et al. prepared an injectable hydrogel system based on bioglass, alginate, and filipin proteins. This hydrogel can achieve responsive degradation according to the ionic concentration of the biological environment, as well as anti-inflammatory and pro-regenerative effects through the MAPK signaling pathway [33].

Wang et al. synthesized conductive hydrogels using silver nanowires (AgNW) and methacrylic acid alginate (MAA) to efficiently promote wound healing by using electrical stimulation. Meanwhile, the incorporation of silver nanowires greatly improved the structural steeliness of the soft electronic material. By applying localized electrical stimulation to the wound area, rapid wound closure could be achieved within seven days, and the expression of growth factors in NIH-3T3 cells could be promoted, as well as the proliferation and migration of NIH-3T3 cells [34].

A novel dual-network sodium alginate-platelet-rich plasma hydrogel was designed and prepared for the promotion of wound healing by Wang et al. The hydrogel was prepared by a simple one-step thrombin activation process and had a three-dimensional network structure. The hydrogel can release growth factors that promote cell proliferation and vascular regeneration. Topical application of the hydrogel to rat wounds significantly increased the rate of wound closure. The dual-network hydrogel provides a simple and effective strategy to overcome the shortcomings of traditional wound dressings [35].

Tendons are composed of soft collagen, whose anisotropic structure and tendon-osteointegration allow for a strong attachment to bone. To mimic this property, Choi et al. developed a tough triple-network (TN) hydrogel realized by a dual network of imidazole-containing polyaspartic amide and alginate-polyacrylamide. The hydrogel exhibited high tensile modulus and strength on the bone surface while maintaining excellent bone adhesion without the need for chemical treatment of the bone surface. By introducing a third polymer, a bone–ligament–bone structure resembling natural ligaments was also successfully realized [36].

The treatment of skin wounds in joints and moving parts has always been a difficult problem. Due to frequent movement and flexion, wound closure using conventional sutures and skin adhesives not only causes additional trauma, but also anesthetic side effects and severe scarring. Liu et al. designed and prepared a novel hydrogel composed of THMA, PEGDA, and sodium alginate. This hydrogel utilized hydrogen bonding and chemical cross-linking to form an interpenetrating network structure, which gave it ultra-high elongation (>700%) and surface adhesion (7.5 kPa) for long-lasting apposition of high-frequency moving parts, and its transparent gel appearance facilitated the observation of wound recovery [37].

To address the problem that hydrogels are not directly absorbed and utilized by wounds, Yao et al. developed a novel histidine-based healing hydrogel. Histidine is a natural dietary essential amino acid that is highly beneficial for tissue formation. Through dynamic coordination and hydrogen bonding, histidine was cross-linked with zinc ions (Zn^{2+}) and sodium alginate (SA) to form a histidine–SA–Zn^{2+} (HSZH) hydrogel. The hydrogel exhibited good injectability, adhesion, biocompatibility, and antimicrobial properties. The HSZH hydrogel was cross-linked with double dynamic bonds, which accelerated the migration and angiogenesis of skin-associated cells in vitro. In vivo experiments demonstrated that the hydrogel significantly facilitated the healing of infected diabetic wounds, taking only about 13 days to fully repair the wounds, compared to the healing process in the control group, which took about 27 days. This study provides new ideas for the design of wound dressing materials, in which weakly cross-linked materials based on tissue-friendly micromolecules are more effective in promoting wound healing compared to highly cross-linked materials based on long-chain polymers [38] (Figure 1.5).

1.1.4 Other Applications

In addition, alginate hydrogel still has many applications in cancer targeted therapy, in vitro imaging, skin repair, and hemostasis. Wang et al. [39], through preparing sodium alginate-calcium hydrogel and loading platinum nanoparticle in its matrix, can degrade the hydrogel on demand and release chemotherapeutic drugs through repeated photothermal therapy, which can improve the therapeutic effect of cancer. Patrick et al. [40] made use of the natural metal chelation characteristics of alginate to creatively cross-link alginate hydrogel with radioactive metal cations In^{3+} and Zr^{4+}, realized the detection of alginate hydrogel in vitro, and demonstrated its in vivo nuclear imaging of longitudinal retention and clearance of alginate hydrogel in oral and nasal administration, stem cell transplantation, and heart tissue engineering. By preparing bio-glass and sodium alginate composite hydrogel, Zhu et al. [41] found that the hydrogel can polarize macrophages to M2 phenotype and up-regulate the expression of anti-inflammatory genes. In addition, it can also recruit fibroblasts and endothelial cells to accelerate the repair process of full-thickness skin. Ren et al. [42] reported coating alginate calcium chloride hydrogel on the needle can significantly reduce the incidence of puncture bleeding, and confirmed its function of in situ hemostasis after tissue puncture in vein, kidney, and liver puncture experiments, which provides a feasible scheme for improving the safety of clinical tissue biopsy.

A study by Jons et al. evaluated the rheological characteristics of calcium alginate and polymer–nanoparticle gels and correlated them with their ability to form drug reservoirs after subcutaneous administration in mice. The rheology of the tested gels included stiffness, viscoelasticity, yield stress, and creep behavior. By combining the rheological characteristics of the gels with the effects of in vivo administration, it was found that yield stress predicted the initial formation of reservoirs and creep predicted the persistence of reservoirs, with yield stresses >25 Pa resulting in the formation of solid reservoirs. This provides a predictive reference for the design

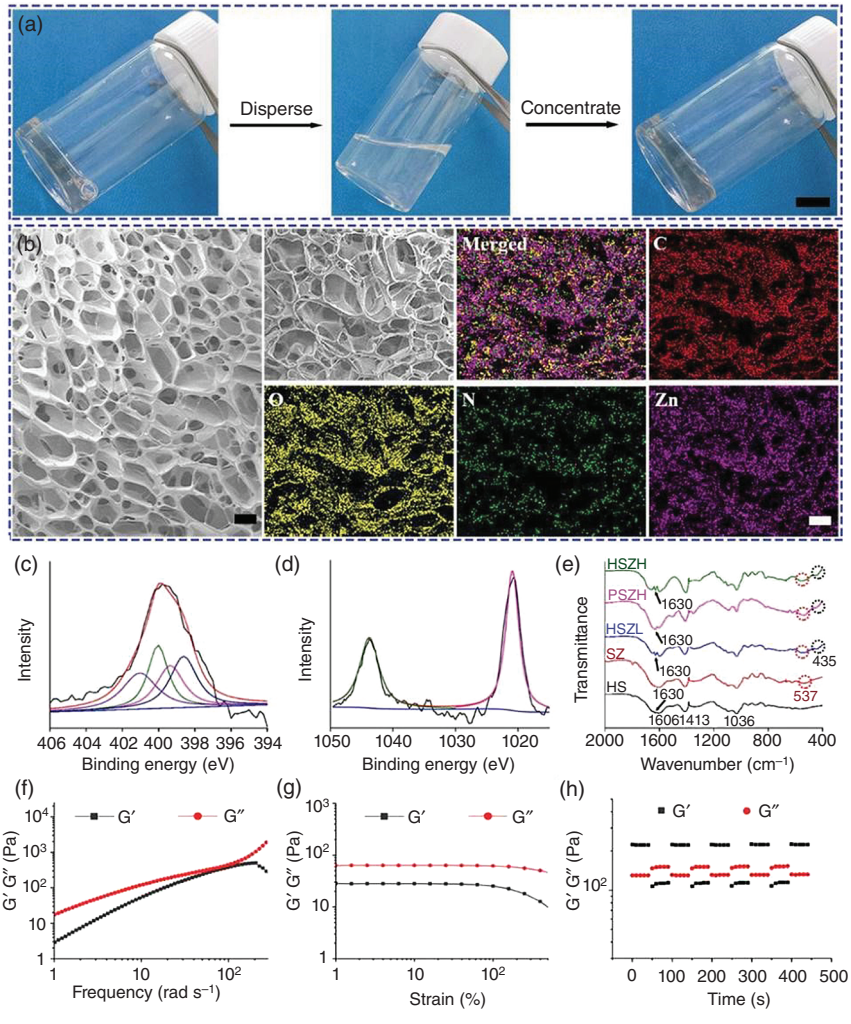

Figure 1.5 (a) The hydrogel is reversible and can be formed and dissolved repeatedly. (b) Scanning electron microscopy images of HSZH hydrogel showed a porous network structure, and elemental analysis confirmed the inclusion of Zn. (c) X-ray photoelectron spectroscopy analysis showed that the HSZH hydrogel contained nitrogen. (d) X-ray photoelectron spectroscopy analysis showed that the HSZH hydrogel contained Zn. (e) Infrared spectroscopy analyzed the characteristic peaks of different hydrogels. (f–h) Rheological tests show that HSZH hydrogels have frequency-dependent, strain-dependent, and step-strain oscillatory shear rheological properties. Source: Ref. [38]/John Wiley & Sons.

of hydrogel systems for sustained controlled release. Prospect: This study reveals the relationship between rheological characteristics and in vivo drug reservoir formation and durability, and provides guidance for the future design and optimization of the rheology of hydrogel controlled-release systems, which is expected to promote the advancement of related technologies and ultimately benefit patients [43] (Figure 1.6).

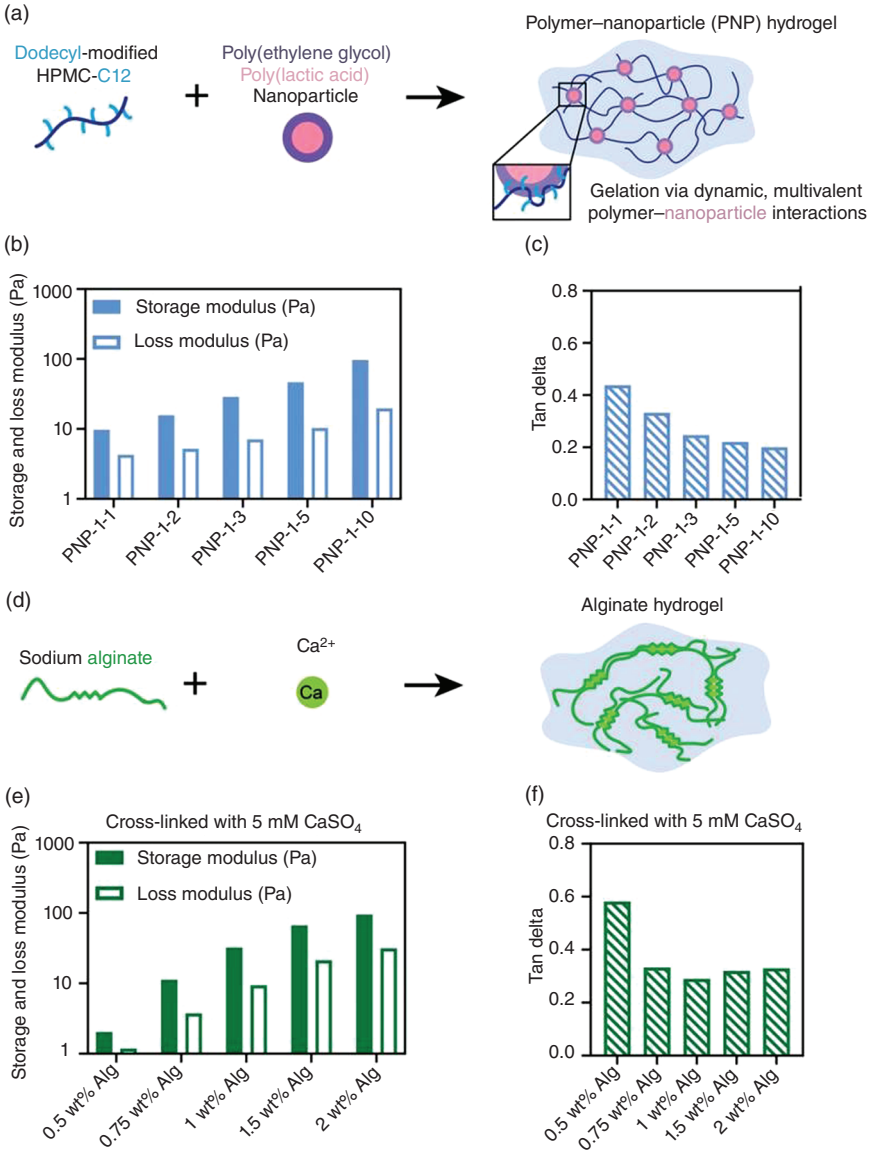

Figure 1.6 (a) A hydrogel preparation method formed from polymers and nanoparticles by non-covalent cross-linking. (b) Energy storage modulus and loss modulus of PNP hydrogel formulation. Increasing the nanoparticle content increases the energy storage modulus. (c) tanδ of PNP hydrogel formulation. Increasing nanoparticle content can decrease tanδ. (d) A hydrogel preparation method formed by cross-linking sodium alginate and calcium ions. (e) Energy storage modulus and loss modulus of sodium alginate hydrogel formulation. Increasing the calcium ion concentration increases the energy storage modulus. (f) tanδ of sodium alginate hydrogel formulation. Increasing calcium ion concentration can decrease tanδ [43].

Ji et al. reported a simple reconstruction process to prepare alginate hydrogels with ultra-strong, ultra-hard, and conductive properties, which can be widely used in artificial biological tissues, flexible electronic devices, and conductive membranes. Through anisotropic densification of the pregel and subsequent ionic rehydration ion cross-linking, the reconstructed hydrogels exhibit exceptional tensile strength (8–57 MPa) and elastic modulus (94–1290 MPa) depending on the type of cross-linked ions. Such hydrogels are able to accommodate sufficient cations (e.g. Li^+) without affecting their mechanical properties and exhibit good ionic conductivity, which is suitable for the preparation of gel electrolyte membranes. In addition, we demonstrate the incorporation of conducting polymers into hydrogel matrices to prepare ionic/conducting hydrogels with outstanding mechanical properties. Through simple surface de-crossing and re-crossing, such hydrogels have strong interfacial adhesion. In conclusion, we have developed mechanically enhanced hydrogels by a simple reconstruction method that forms tightly connected polymer networks. This simple process is suitable for large-scale production and can be expected to be used in practical manufacturing applications [44].

Microwave ablation (MWA) is a local tumor treatment strategy, but is often challenged by tumor recurrence. Therefore, the development of adjuvant biomaterials to enhance the effectiveness of MWA is relevant. The results showed that alginate-immobilized Ca^{2+} formed hydrogels under microwave exposure, exhibiting efficient heating and a restricted heating zone. High extracellular Ca^{2+} concentrations synergized with microwave subthermal therapy to induce immunogenic cell death by interfering with intracellular Ca^{2+} homeostasis. Thus, Ca^{2+}-remaining alginate hydrogel combined with MWA can effectively ablate different tumors in mice and rabbits while reducing surgical trauma. This treatment also triggered an anti-tumor immune response, especially when combined with interferon gene pathway activators, which inhibited the growth of untreated distant and recurrent tumors. This study highlights the potential of metal alginate hydrogels as biomaterials for microwave therapy and immunostimulation, with promising clinical applications [45].

1.2 Alginate-Based Electrospinning for Biomedical Application

1.2.1 Drug Delivery

Electrospinning can well simulate the structure of extracellular matrix, coupled with its unique porous structure, so it is widely used in the field of tissue engineering and drug delivery. However, alginate is easy to gelate; low content and impurities restrict its application in electrospinning technology. At present, most researchers improve its electrospinning properties by adding alginate into polymer solution. In addition, Asadi-Korayem et al. [46] further studied the effect of intermolecular hydrogen bonding on the properties of alginate electrospinning. According to the hard and soft acids and bases theory, they used Li^+ instead of Na^+, to further

strengthen the interaction between ions and reduce the hydrogen bond density, which significantly improved the electrospinning properties of alginate. Fujita et al. [47] successfully prepared alginate nanofibers by wrapping sodium alginate in a nanofiber shell, then gelating sodium alginate and removing the shell core electrospinning method, and immobilized fibronectin on the resulting alginate fibers to control the proliferation of cells along the fiber direction.

Kaassis et al. [48] blended polyoxymethylene, sodium alginate, and ibuprofen in the preparation of electrospun fibers. They found that ibuprofen microcrystals were present on the electrospun fibers prepared by this method, and the experimental drugs were pulsed in different pH environments. They can control the release amount and release interval of the fiber at different stages by adjusting the loading of sodium alginate and ibuprofen. De Silva et al. [49] loaded cefalexin onto cemented carbide nanotube to enhance the mechanical properties of alginate-based nanofiber scaffolds. The addition of cemented carbide nanotubes extended the drug release time from 24 hours to 7 days, and showed strong broad-spectrum antibacterial properties against Gram-positive and Gram-negative bacteria.

Dodero et al. [50] used electrospinning technology to prepare polycaprolactone (PCL)- and sodium alginate-embedded nano-ZnO bilayer fiber films. The outer layer of polycaprolactone has good hydrophobicity, while the inner layer of sodium alginate can promote tissue regeneration and remove wound exudates. In addition, they have verified the drug-carrying capacity of the fiber membrane, and the release form of the drug can be controlled by adjusting the concentration of the loaded drug. Mulholland et al. [51] used siRNA targeting FK506-binding protein-like proteins to promote angiogenesis. Chitosan-sodium alginate double-layer wound patches were prepared by electrospinning. They found that the blood vessel density in the treatment group increased by 326% compared with the control group, confirming the great role of siRNA targeting FK506-binding protein-like proteins in wound healing. Tang et al. [52] introduced honey into sodium alginate/polyvinyl alcohol (PVA) electrospun nanofiber membrane to give the fiber membrane stronger antioxidant activity and antibacterial activity. Similarly, Hajiali et al. [53] prepared wound dressings with both antibacterial and anti-inflammatory effects by adding essential oils. On the basis of preparing alginate electrospun films containing ZnO nanoparticles, Dodero et al. [54] further explored the effects of different cross-linking agents on the properties of fiber membranes. They found that compared with the most commonly used Ca^{2+}, Sr^{2+} has a higher affinity for alginate, can improve and accelerate tissue regeneration, and the fiber membrane cross-linked by Sr^{2+} is closer to human skin in mechanical properties. Electromagnetic action is also an emerging way to achieve targeted drug delivery, and Chen et al. [55] prepared magnetic induction fiber membrane by chelating alginate electrospun fiber with Fe^{2+}/Fe^{3+}, which showed targeted killing effect on tumor cells in alternating magnetic field.

Alloisio et al. successfully synthesized alginate chain-stabilized silver nanoparticles (Alg@AgNPs) in situ in polysaccharide solutions using wet chemistry as a powerful alternative to antimicrobial materials. This nanocomposite material combines the efficient and broad-spectrum biocidal properties of silver nanoparticles with the biocompatibility and environmental friendliness of natural polysaccharide

components. Nonwoven films with uniform nanostructures with fiber diameters between 100 and 150 nm, influenced by the size of the embedded metal nanoparticles (between 20 and 35 nm), were prepared by electrostatic spinning technique. Preliminary tests showed that these nanocomposite films exhibited a biocidal effect against Gram-negative *Escherichia coli* (*E. coli*) that could occur within one day and was observed even when the content of AgNPs in the polysaccharide fibers was well below nanomolar levels [56].

Among numerous tissue engineering applications, electrospun fibers are considered as a material with good potential to be used as wound dressings, which help to promote the healing process and maintain the regeneration of damaged skin. However, conventional electrospun fibers have some drawbacks in wound repair, such as the lack of antibacterial, anti-inflammatory, and angiogenesis-promoting properties. Lashkari et al. prepared bilayer scaffolds consisting of PCL/Gel nanofibers and collagen alginate (Col/Alg) hydrogel and implanted adipose-derived stem cells (ADSCs) into the nanofibers and the bilayer scaffold. The double-layer scaffold with implanted ADSCs and nanofibers was used to dress the whole level of trauma on the back of rats. Histopathological assessment was performed using H&E staining at 14 and 21 days postoperatively. The results showed that the double-layered scaffolds and nanofibers implanted with ADSCs significantly enhanced re-epithelialization, angiogenesis, and collagen remodeling of the wounds compared with the control group [57].

Electrospun fibers have potential applications in wound healing but need to possess antimicrobial, anti-inflammatory, and pro-angiogenic properties. Wang et al. improved their spinning properties by synthesizing sodium oxide selenobacterium alginate (OSA) and prepared composite fibrous membranes by mixing it with zinc oxide nanoparticles (ZnO-NPs). In vitro and in vivo studies showed that these membranes exhibited good biocompatibility, antimicrobial effect, and positive impact on wound healing. Such nanofiber membranes have a wide range of potential applications for wound healing [58]. Hu et al. developed a composite wound dressing consisting of alginate and PCL nanofibers. This dressing combines the moist environment of alginate, the cell adhesion enhancement of PCL, and the long-lasting antimicrobial properties of nanosilver. Meanwhile, plasmid DNA containing the platelet-derived growth factor-B (PDGF-B) gene was adsorbed onto the alginate fibers via carrier nanoparticles, enabling intracellular transfection on the wound and promoting wound healing. The release of calcium ions from the alginate fibers helped accelerate hemostasis. It was demonstrated that this composite dressing significantly improved the wound closure speed and promoted collagen formation, which had multifaceted healing-promoting advantages [59].

1.2.2 Tissue Regeneration

A composite scaffold composed of methacrylate alginate fiber and polycaprolactone fiber by electrospinning has been fabricated by Apsite et al. [60]. This scaffold can spontaneously curl into various forms in water and induce myoblasts to further differentiate into muscle tubes along the axial arrangement. It provides

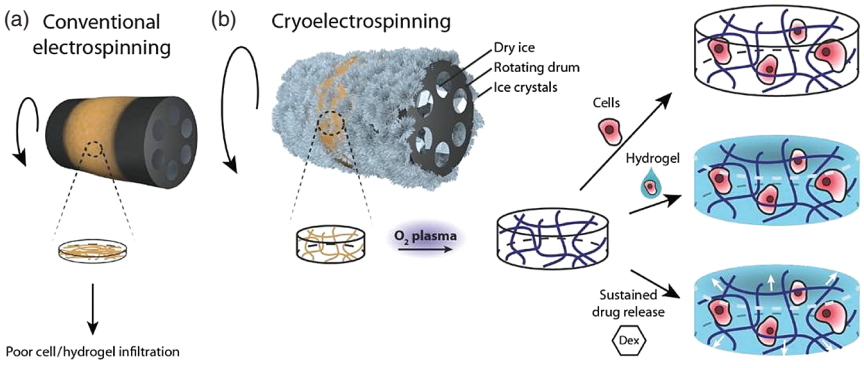

Figure 1.7 (a) Conventional rotary mandrel electrospinning forms a dense polymer network that prevents cell and hydrogel penetration. (b) Electrospinning on a −78 °C mandrel induces ice crystal co-deposition, which sublimates to form a super porous biocompatible membrane. Plasma treatment reduces the intrinsic hydrophobicity of PCL, making the skeleton permeable to cells or hydrogels. Source: Ref. [62]/John Wiley & Sons.

a feasible method for the preparation of artificial muscle. Yeo et al. [61] used 3D printing and electrospinning techniques to fabricate alginate-polycaprolactone scaffolds with uniaxial arrangement of micron and nanometer patterns. Due to the synergistic effect of different patterns, the expression of different muscle-derived genes in myoblasts was significantly increased. For simulating the components of natural extracellular matrix, Formica et al. [62] infiltrated chondrocytes/alginate solution into polycaprolactone fiber network, and then physically cross-linked to make cell-loaded electrospinning scaffolds. This scaffold can significantly induce chondrocytes to produce matrix rich in glycosaminoglycan and type II collagen and promote cartilage repair (Figure 1.7).

Hazeri et al. [63] further explored the effects of alginate sulfated PVA/alginate nanofibers with different concentrations (10, 20, and 30 wt%) on nerve regeneration. They found that nanofiber scaffolds containing 30 wt% alginate were suitable for the growth of bone marrow mesenchymal stem cells and induced bone marrow mesenchymal stem cells to differentiate into nerves over a period of up to 14 days.

Ductal carcinoma in situ (DCIS) is a breast cancer type, and Sadeghi et al. developed a three-layer tubular 3D scaffold based on a complex tissue environment for use in DCIS models and chemo-photothermal therapy studies. The scaffold consists of an intermediate layer of PLA/PCL nanofibers, and the outer and inner layers are dopamine alginate/PVA nanofibers, which provide a suitable environment for cell growth. The scaffolds were prepared by electrospinning to mimic the structure of natural extracellular matrix, and the inner and outer layers were added with dopamine nanoparticles with photothermal properties. MDA-MB-231 breast cancer cells were cultured on the scaffolds, and the synergistic effects of cell growth promotion and chemo-photothermal therapy were confirmed by in vitro experiments [64].

To enhance bone regeneration, Joo et al. prepared a sodium alginate-cast polycaprolactone-gelatin-β-calcium triphosphate bilayer membrane using an electrospinning process. This porous membrane degraded gradually in vivo, showing

appropriate hydrophilicity and degradability. The electrospun membrane provided a favorable growth environment, while the alginate membrane inhibited cell attachment and was a nontoxic material. After implantation, the bilayer membrane promoted bone formation and effectively inhibited fibrous tissue infiltration. Immunocytochemical analysis showed that the bilayer membrane directed more proteins, controlled bone mineralization, and improved the guiding properties of tissue-engineered bone grafts [65].

Inflammation induced by myocardial infarction (MI) can lead to necrosis of cardiomyocytes. Hydrogen hydrosulfide (H_2S) is considered an important gas signaling molecule with various biological effects such as anti-inflammatory, antioxidant, and angiogenesis promotion. However, the feasibility of using H_2S directly to treat MI is limited by its short residence time and drastic side effects. To alleviate this problem, Li et al. investigated and developed a composite scaffold (AAB) that gradually releases H_2S from a patch made of modified alginate and albumin by electrospinning. The patch was exposed to an 808 nm laser, and the thermal energy generated by the black phosphorous nanosheets altered the molecular structure, enabling precise attachment to the myocardium. The AAB alleviated inflammation by reducing ROS levels and enhancing M2 macrophages. This engineered cardiac patch is expected to alleviate inflammation and promote angiogenesis after MI, thus helping to restore cardiac function and providing a new avenue for MI treatment [66] (Figure 1.8).

Wound healing is a complex biological process involving a series of cell signaling pathways and molecular interactions. In this process, microRNAs (miRNAs), as a class of short non-coding RNAs, play an important regulatory role. Bombin et al. found that down-regulation of microRNA-31 (miR-31) and microRNA-132 (miR-132) in wound healing was associated with delayed healing. Therefore, by effectively delivering these two microRNAs, it is expected to play an active role in promoting tissue repair. In order to fully utilize the efficacy of microRNAs, the delivery challenge must be addressed. They propose to employ RALA peptide nanocomplexes, which can encapsulate miR-31 and miR-132 into nanoparticles, to achieve efficient and safe intracellular delivery of these two microRNAs. By mixing the delivery of miR-31 and miR-132, the study aims to reduce potential toxicity while exploiting the synergistic effect of the two with a view to maximizing the therapeutic effect of promoting wound healing. As demonstrated by wound-healing experiments in mice, this technology significantly accelerated the rate of wound closure, increased epidermal thickness and blood vessel counts, demonstrating the potential for innovative therapeutic applications [67]. Chen et al. developed electrospun composite nanofibers of fucoidan derivatives using electrospinning technology to enhance the electrospun properties of fucoidan by chemically modifying the molecular flexibility of oxidized fucoidan derivatives (RAOA). These fibers were able to load the hydrophobic anti-inflammatory drug ibuprofen with the assistance of PVA. The synthesized RAOA was characterized by FT-IR, 1H NMR, and fluorescence photometry. The effects of surface tension, conductivity, and rheological properties of the RAOA/PVA blend solution on the electrospinning performance and fiber morphology were investigated. The drug loading and release mechanism of ibuprofen from RAOA/PVA electrospun

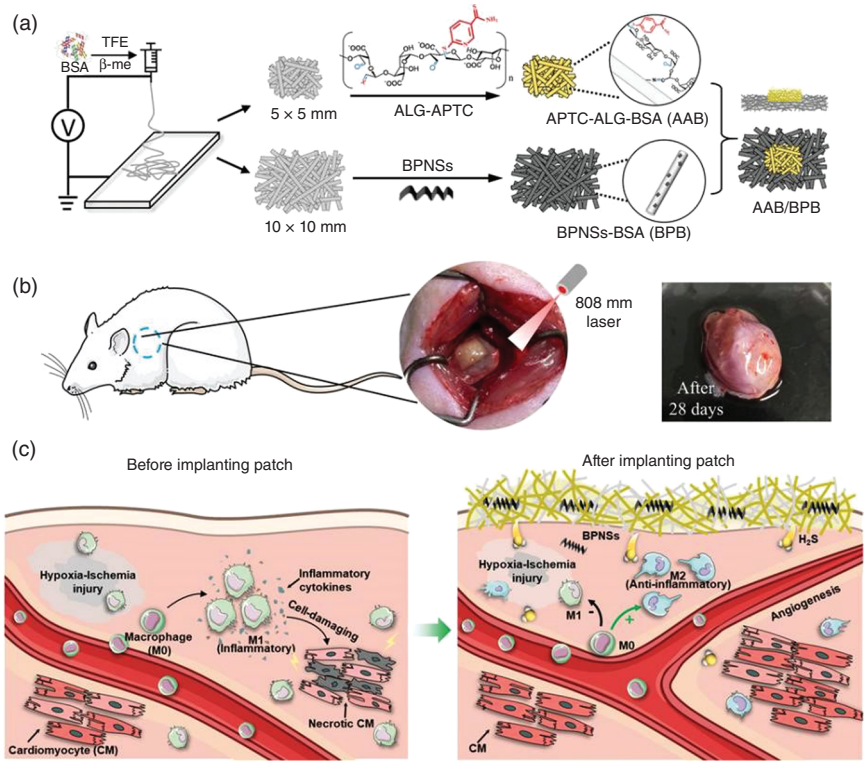

Figure 1.8 (a) Cardiac patch preparation process. (b) Application process: the patch is implanted into the infarcted area to cover and replace the dead tissue. (c) Mechanism of action: The implanted adMSC-secreted factor promotes neovascularization, and the hydrogel degrades to release Philadelphia chromatin-positive cells, which interact with host cells to improve cardiac function. Source: Ref. [66]/John Wiley & Sons/CC BY 4.0.

composite nanofibers was further analyzed by simulated drug release experiments. The RAOA/PVA electrospun composite nanofibers exhibited high encapsulation efficiency and sustainable-release properties, and low cytotoxicity to L929 cells. These electrospun nanofibers combine good self-assembly properties, colloidal interfacial activity, sustainable-release properties, and cytocompatibility, showing potential as functional wound dressings in biomedical applications [68]. Biodegradable and biocompatible scaffolds based on PVA/alginate mixtures were prepared by electrospinning followed by cross-linking with calcium chloride by Soto-Quintero et al. The morphology and degradation rate of the scaffolds were found to be tunable by varying the SA concentration (i.e. 3.5%, 4.0%, and 5.0% SA.) The highest SA content on the PVA/alginate scaffolds showed the highest degradation rate during the 100-day test period. In addition, these scaffolds experienced shrinkage and possible microstructural changes after degradation. The degradation of the scaffolds was studied by electrochemical impedance spectroscopy, and the lowest resistance and highest capacitance behaviors were found to correlate with high degradation rates, i.e. greater mass loss. Increasing the surface roughness of the scaffolds

induced proper biocompatibility, as observed in 4.0% PVA/alginate scaffolds. Thus, HaCat cells in aqueous solution had a direct effect on the topology and stability (controlled degradation) of the scaffolds. These scaffolds have great potential in the field of tissue engineering, especially in cell regeneration [69].

Achieving functionally intact skin regeneration has long been a challenging task. As a multilayered and complex organ, the skin undergoes a continuous healing process influenced by multiple mechanisms. Critical nutrient, oxygen, and biochemical signals can direct specific cellular behaviors that ultimately contribute to the formation of high-quality tissue. Such biomolecular exchanges can be modulated through scaffold engineering, which is one of the frontiers in the field of skin substitution and equivalents. Molina et al. prepared a novel 3D fibrous scaffold consisting of PCL/sodium fucoxanthate (CA) aimed at inducing keratogenesis through the action of calcium loss. The scaffolds were prepared by electrospinning using a PCL/sodium fucoidan solution that was treated by immersion in a calcium chloride solution in order to replace the sodium ions attached to the fucoidan with calcium ions. This treatment not only provided ionic substitution but also induced cross-linking of the fibers. The in vitro performance of the scaffolds was investigated by growing and staining fibroblasts and keratin-forming cells on the scaffolds and using differentiation markers to detect the evolution of basal, spiny, and granular keratin-forming cells. The findings reveal the potential of PCL/CA scaffolds for tissue engineering and suggest that calcium loss in the scaffolds may contribute to the formation of a suitable biological environment that promotes the attachment, proliferation, and differentiation of major skin cells [70].

Ashraf et al. prepared collagen (Col)/sodium alginate (SA)/polyethylene oxide (PEO)/exocytidyl polysaccharide (EPS) nanofibers skin substitutes generated by *Rhodotorula mucilaginosa* sp. GUMS16 using biaxial electrostatic spinning technique. Among them, collagen is a natural scaffold, sodium alginate absorbs excess wound fluid, and GUMS16-generated EPS acts as an antioxidant. In this study, collagen and sodium alginate nanofibers containing different amounts of exocytopolysaccharide were successfully prepared by biaxial electrostatic spinning. The study aimed to improve the mechanical properties and cytocompatibility. The results showed uniform morphology, high hydrophilicity, cytocompatibility, and non-toxicity. Col-SA/PEO + EPS2% nanofibers showed superior mechanical properties and cellular behaviors and are expected to be potential cytocompatible scaffolds for various tissue engineering applications [71].

Building gene activation matrices (GAMs) by combining gene delivery with functional scaffolds is a promising strategy for tissue engineering. He et al. used nonviral DNA delivery nanocomplexes modified with PLGA/PEI nanoparticles carrying pVEGF plasmid DNA immobilized on electrospun fucoidan nanofibrous scaffolds to form GAMs. This innovative system exhibited low cytotoxicity, high transfection efficiency, and sustained gene release of VEGF was achieved in vitro. In a rat skin wound model, GAM accelerated wound healing, promoted re-epithelialization, attenuated inflammatory response, and enhanced neoangiogenesis. This study demonstrates the potential of the GAM system in tissue engineering [72]. Bioactive scaffolds for the treatment of large bone defects remain a great challenge in

clinical applications. GAM, as a combination of gene therapy and tissue-engineered scaffolds, offers a promising strategy for the restoration of structure and function of damaged or dysfunctional tissues. He et al. developed a gene-activated bionic composite scaffold consisting of an outer sheath of electrospun polycaprolactone fibers and a hydrogel core of alginate carrying plasmid DNA encoding for bone-forming protein 2 (pBMP2) and vascular endothelial growth factor (pVEGF)) in an alginate hydrogel core. A low cytotoxic and efficient peptide-modified polymer nanocarrier was used as a nonviral DNA delivery vehicle. The obtained GAM enabled spatiotemporal release of pVEGF and pBMP2 and promoted osteogenic differentiation of preosteogenic osteoblasts in vitro. Through in vivo evaluation in a rat critical-size iliac defect model, the dual gene delivery system was demonstrated to significantly accelerate bone healing through activation of angiogenesis and osteogenesis. These results indicate that the developed fiber–hydrogel composite scaffolds with dual gene-activated nucleus-sheath structures have significant effects in the regeneration of critical-size bone defects, and confirm the potential of cell-free scaffold-based gene therapy in tissue engineering [73].

1.3 Alginate-Based 3D Printing for Biomedical Application

1.3.1 Alginate-Based Bio-Ink and Printing Strategies Improvement

3D printing technology can prepare various forms of biomaterials according to the actual needs, so it is widely used in the field of biomedicine. The improvement of 3D printing technology can be divided into two categories, one is to improve the properties of biological ink, the other is to improve printing strategies and methods.

Biological ink with natural polymer as the main component has poor stability in vivo, while synthetic polymer can cause immune rejection and have a certain toxic effect on cell proliferation. These problems make it a great challenge to reshape soft tissue through 3D printing technology. Van Belleghem et al. [74] innovatively combined natural polymers with synthetic polymers to prepare polyethylene glycol (PEG) covalently linked with naturally derived and physically cross-linked alginate double-network biological inks and biodegradable GelMA biological inks. The combined use of two kinds of biological ink can reduce the biological toxicity and ensure the structural stability at the same time. Jia et al. [75] improved the rheological properties and mechanical strength of biological ink by adding four-arm polyethylene glycol-tetraacrylate (PEGTA) to gelatin methacryloyl (GelMA) and sodium alginate biological ink. Firstly, Ca^{2+} was pre-cross-linked with alginate to construct the basic framework, and then the whole structure was permanently shaped by the photocross-linking of GelMA and 4-arm PEGTA components. Four-arm PEGTA has a number of active cross-linking sites to significantly improve the stability of the structure, coupled with the use of three-layer coaxial nozzles, they successfully prepared a perfusion vascular structure. Pataky et al. [76] invented a new 3D printing strategy using the rapid cross-linking of alginate with Ca^{2+}. They

used the hydrated gelatin matrix as the Ca^{2+} repository and used the drop-by-drop printing method to make the alginate biological ink accumulate like a wall to finely control the shape of the printing structure. Kirillova et al. [77] used 4D bio-printing technology to prepare vascular structure. They first modified alginate with methacrylate group to make it have the ability of photocross-linking, then printed alginate film on glass, and then curled the film spontaneously to form vascular poplar structure. The vascular poplar structure with a diameter of 20–150 μm can be prepared by controlling the thickness of the film.

The rapid prototyping performance of 3D printing bioinks is crucial for printing high-resolution fine structures, and Jeon et al. successfully achieved the preparation of complex pendant structures without external support by modifying alginate materials with methacrylated materials that utilize shear-thinning and calcium cross-linking properties. Thanks to its excellent biocompatibility, this bioink can be used for stem cell loading and organoid preparation [78].

In tissue engineering, the preparation of cellular scaffolds containing hollow microchannels that resemble blood vessels is critical for building thick cellular tissues. However, existing techniques have limited success in producing hollow channels with diameters smaller than a few hundred micrometers, and Bolívar-Monsalve et al. prepared hollow microchannels with cellular widths in a single step by co-extruding a methacryloyl-alginate hydrogel suspended from C2C12 of murine myoblasts and a sacrificial material, hydroxyethylcellulose, using a printhead with Kenics static mixing elements, and vascularized skeletal muscle-like fibrous scaffolds. Compared with solid scaffolds, the viability and metabolic activity of C2C12 myoblasts were higher in the hollow scaffolds. In addition, the hollow channels alleviated hypoxia, promoted the expression of Ki67, induced 82% of the cells to orient themselves, and facilitated the rapid elongation, differentiation, and maturation of the C2C12 cells into myogenic fibers [79].

The gradual weakening of bioelectrical stimulation around the wound leads to the downregulation of the wound-healing cascade and disorders in collagen fiber deposition, which is the main cause of delayed wound healing and scar tissue formation. The ZnO nanoparticle-modified PVDF/SA piezoelectric hydrogel scaffolds (ZPFSA), which were prepared by three-dimensional printing by Liang et al., were designed to solve the problem of gradual weakening of bioelectrical stimulation in the course of prolonged wound healing, diminishing during prolonged wound repair. The scaffold has a dual piezoresponse model that mimics and amplifies endogenous bioelectricity to promote wound healing and prevent scar formation. ZPFSA significantly accelerated wound healing within two weeks and prevented scar formation by modulating cell migration and angiogenic cascade responses [80].

1.3.2 Attempts at Bionic Matrix Ink

Extracellular matrix (ECM) has attracted much attention in promoting tissue regeneration, but its slow gel kinetics limits its application in 3D printing. In order to solve this problem, De Santis et al. [81] turned their attention to alginate which can be rapidly cross-linked with divalent cations. The tubes and branching structures

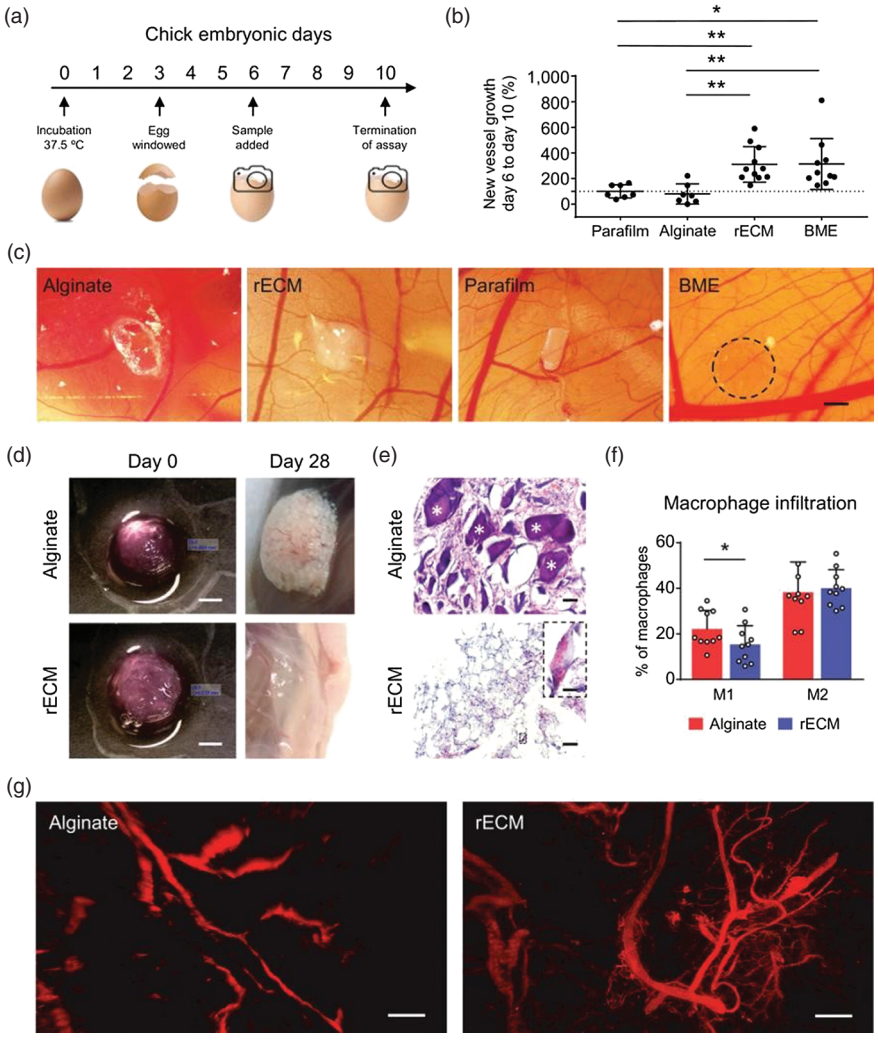

Figure 1.9 (a) Overview of CAM experiments. (b) Quantitative comparison of vascular growth induced by different hydrogels. rECM hydrogel induces better vascular growth than sodium alginate hydrogel. (c) Schematic diagram of CAM samples. rECM hydrogel is surrounded by abundant blood vessels. (d) Shape changes of different hydrogels implanted subcutaneously. (e) HE staining after 28 days of implantation. rECM hydrogel showed a few non-protein fragments, and both hydrogels showed neovascularization. (f) Inflammatory cell infiltration was lower in the early stage of rECM hydrogel implantation. (g) Spontaneous fluorescence showed better vascular regeneration in rECM hydrogel. Source: Ref. [81]/ John Wiley & Sons/CC BY 4.0.

prepared by the mixed biological ink system enhanced by alginate and extracellular matrix have been proved to promote cell proliferation and differentiation and angiogenesis in vivo (Figure 1.9).

Xie et al. [82] explored the potential application of 3D printing technology in drug screening in vitro. They successfully constructed a liver cancer model in a 3D structure made from a mixture of gelatin and sodium alginate. In the process of long-term culture in vitro, its tumorigenic potential and histological characteristics were preserved, which provided a platform for screening anticancer drugs in vitro.

Ma et al. constructed a multi-scale hierarchical bioactive calcium silicate nanowire/alginate composite hydrogel scaffold for tendon-to-bone interface tissue engineering. Three-dimensional printing technology was combined with mechanical stretching methods to introduce biomimetic reinforcement structures from nano- to micrometer- to micro-scale in this composite hydrogel, which significantly improved the mechanical properties. Experiments showed that the biochemical and topographical characteristics of the composite hydrogel provided a favorable microenvironment for rabbit bone marrow mesenchymal stem cells and tendon stem cells to promote their directional alignment and induced differentiation. The composite scaffold significantly promoted the regeneration of tendon–bone tissue in vivo, especially in the fibrocartilage transition zone. Therefore, this multi-scale structural design provides an innovative strategy for the engineering of functionalized tendon–bone tissues [83].

Zhu et al. prepared a multifunctional nanocomposite bioink for extrusion bioprinting using amine-functionalized copper (Cu), oxidized alginate, gelatin, and mesoporous bioactive glass nanoparticles (ACuMBGNs). This ink has good rheological properties and structural stability. Rapid cell spreading and high survival were supported by the reversible dynamic microenvironment and cell adhesion ligands introduced by aminated particles. Osteogenic differentiation and angiogenesis of mouse bone marrow stromal stem cells (BMSCs) were promoted in the bioprinted scaffolds without additional growth factors. This nanocomposite biomaterial is expected to be a superior platform for bioprinting complex three-dimensional matrix environments for superior cell support [84].

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