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Kazuhiko Ishihara

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Advances in bioinspired polymer hydrogel systems with biomedical functionalities

Kazuhiko Ishihara

Division of Materials and Manufacturing Science, Graduate School of Engineering, Osaka University
2-1, Yamadaoka, Suita, Osaka 565-0871, Japan

Email: k-ishihara@mat.eng.osaka-u.ac.jp
ORCID: 0000-0002-4196-5488

ABSTRACT: The concepts of bioinspiration and biomimetics that seek to elucidate the morphology and functions of living organisms and specific reactions within cells, and extraction of important elements from these concepts to design functional molecules and high-performance materials are becoming more and more widespread. This review summarizes the progress in research on hydrogels inspired by the stimuli-responsiveness of cell functions. For application to a self-regulated release system of insulin to regulate blood glucose levels, various polymer hydrogels have been designed using bioactive molecules such as enzymes and lectins to sense glucose concentrations. In addition, as a fully synthetic glucose-responsive hydrogel, a complex of a polymer having phenylboronic acid groups that form reversible bonds with sugars and a multivalent hydroxyl group polymer has been researched. This reversible hydrogel system can be further developed to act as an extracellular matrix in which cells can preferably reside. The proliferation and differentiation of encapsulated cells in hydrogels are controlled by reversible changes in the hydrogel properties in response to sugar. Another advantage is that cells can be safely retrieved by adding sugar to dissociate the hydrogel. These bioinspired polymer hydrogels can serve as important materials for the development of new medical technologies,

such as the controlled release of bioactive molecules, regulated cell culture environmental matrices, and applications in layered and three-dimensional cell culture systems to create organized tissue structures.

Keywords: Stimuli-responsive hydrogel; cytocompatibility, cell encapsulation; drug delivery system, tissue engineering

1. Introduction

This review explores the relationship between the structural design and function of synthetic polymer hydrogels inspired by biological systems. This includes the research performed by Professor Alan Hoffman at the University of Washington, USA, whose group has conducted many pioneering studies since the 1980s [1-3].

The ability to continuously and reversibly control the properties of a material using external stimuli or the concentration of chemical species present in the system has enabled a major revolution in materials science, where previously materials were understood to have fixed shapes and properties [4-6]. Initially, morphological changes of polymers in solution were conceived based on phenomena such as the helix–coil transition of polypeptides, and bioinspired or biomimetic research was conducted to realize these changes using photochromic molecules [7-9]. The human visual system employs structural changes in the protein rhodopsin, which binds to the retinal to sense light; this structural change was reproduced in an artificial system. It was further extended to photoresponsive polymer film systems, making it possible to realize reversible control of the hydrophilicity of the surface by changing the wavelength of irradiated light [10,11]. The application of hydrophobic chromatography supports has allowed antibiotics, proteins, and cells to be separated in a system using water as the sole solvent [12,13]. This is a new concept of separation in a single-solvent system that operates by changing the polarity of the solid phase rather than changing the liquid phase polarity to dissolve the adsorbed substance. In addition,, the control of hydrogel water content and viscoelasticity using photochromic molecules has also been reported [14-16]. Subsequently, the properties of liquid crystals and other materials were incorporated into photoresponsive polymers, resulting in greater changes in material properties, such as improved photoresponsivity and changes in surface morphology [17-19]. Owing to the major achievements of these photoresponsive polymers, they have been put into practical use as the basic technology for devices that efficiently select and separate specific cells [20].

An active line of research is the investigation of stimuli-responsive polymers, including

polymer hydrogel systems that change their properties in response to physical factors other than light (such as temperature, magnetic fields, and electric fields) and polymer systems that respond to changes in pH or the concentration of chemical species (such as ions) in a medium. Expanding the dimensions from the one-dimensional molecular level functional expression to two-dimensional surface functionalization and even research into three-dimensional (3D) gels effectively realize high performance as a material. Hoffman and his colleagues have carried out research using N, N-diisopropyl acrylamide (NIPAAm)-based polymers known to undergo phase transitions in response to temperature. First, poly(NIPAAm) (PNIPAAm) was grafted to antibodies and enzymes control their activity by a change in temperature [21]. Next, PNIPAAm was grafted onto the polydimethylsiloxane surface by photoinduced polymerization to create a temperature-responsive substrate. The surface could capture PNIPAAm-grafted nanobeads below phase transition temperature but decrease the adsorption ability above the temperature [22]. They have also reported much research on the properties of PNIPAAm-based hydrogels [23]. Many reviews have also been published on these topics; referring to them for additional details is recommended [24-26].

Bioinspired or biomimetic approaches, in which the functions of biological tissues and cells are elucidated, and the necessary elements are extracted and reproduced in artificial systems, are expected to create new functional materials and devices (Figure 1). Bioinspired and biomimetic materials have been actively researched in recent years, and some have already been put to practical use in devices and surface treatments [27-31]. Focusing on cellular functions, there is a process by which necessary molecules are taken in from outside the cell as raw materials for intracellular reactions or as signals to control reactions. Stimuli-responsive systems that reproduce this series of processes in an artificial system can realize dynamic changes in material properties, leading to changes in the diffusion, selectivity, and reactivity of the material [32-34]. This will allow dynamic cellular response systems to be artificially reproduced. Here, regarding the functions of cells and the extracellular matrix (ECM), this system summarizes the insulin secretion system that responds to blood glucose levels, and the corresponding artificial ECM system can reversibly control its characteristics using sugars to change the function of the immobilized cells.

The stimuli-responsiveness of polymer hydrogels is based on volume changes that induce changes in their water content. In this case, the balance between the hydrophilicity and hydrophobicity of the polymers that form the hydrogel can be changed by stimuli, or exchange reactions can change the cross-linking density with specific chemical species. Phase transitions that dramatically change the volume of the hydrogel have also been found, and the solute

permeability of the hydrogel can be controlled to either the “on” or “off” state in the vicinity of these phase transitions [35-37]. The early phase of research on hydrogel systems that respond to chemical species in the body often focused on the function of pancreatic β-cells, which release insulin in response to fluctuations in blood glucose levels [38]. It is well-known that insulin administration is effective for treating patients with diabetes. Patients with diabetes are required to receive insulin via injection. However,, excessive insulin administration can induce hypoglycemia and endanger the life of the patient. Therefore, a device combining a blood glucose sensor with an insulin pump was developed. The blood glucose sensor comprises an enzyme electrode that can selectively measure blood glucose and glucose oxidase (GOD) combined with a redox electrode [39,40]. In addition, to administer insulin solution subcutaneously from an insulin pump, the injection needle must be remained in a subcutaneous location. The tissues around these electrodes and the injection needle undergo a foreign body recognition reaction, causing inflammation and further tissue encapsulation [41,42]. In addition, the possibility of infection at the puncture site cannot be negated. This poses a major problem that prevents these devices from being used for long periods. Therefore, a polymer system that autonomously detects changes in glucose concentration and releases insulin was devised. Of course, these only present basic operating principles and material design concepts; however, it is expected that progress will be made toward practical applications as technologies that can ensure biocompatibility, operational stability, safety, etc. are developed. Furthermore, the polymer molecular designs that will emerge from research into this process may be applicable to other fields.

With recent advances in cell engineering, the use of induced pluripotent stem cells (iPS cells) and embryonic stem cells (ES cells) have attracted increasing attention. Furthermore, research on organ-level functional expression through 3D organization is also progressing [43-45]. From an engineering perspective, technologies for securing cell sources, quality assurance, and functional verification are required. To meet these requirements, there is strong demand for materials that can support cell cultures, differentiation induction, and tissue formation. The ability to create an artificial ECM using stimuli-responsive hydrogels, immobilize cells inside it, and safely recover the cells after their function has been expressed would provide significant benefits for future cell engineering and tissue regenerative medicine.

As mentioned above, the reversible reactions such as the photoisomerization of molecules related to vision and the folding of protein structures involved in biological activities are typical examples of biomimetic molecular stimuli-responsive functions. By linking this to changes in higher-dimensional material functions. This review first explains polymer hydrogels that can

change the permeability/diffusivity of insulin, regarding the concentration of sugar compounds contained in the system as a trigger for changing the material properties. Then, a system that controls the function of cells encapsulated in the hydrogel gels in a cell culture environment is introduced, using sugar-responsive hydrogels as an artificial ECM. These findings are expected to provide important information for understanding the relationship between the structure and function of new bioinspired stimuli-responsive polymer hydrogels.

2. Bioinspired molecule-responsive polymer hydrogel systems

2.1 Enzyme-based responsive hydrogel systems

Hydrogel structures are extremely important for biological tissues, and their main characteristics include solute permeability, flexibility, and mobility in the hydrated state. Research has been conducted since the 1980s to reproduce these features in synthetic polymer hydrogels [46,47]. In particular, considerable research has focused on polymer hydrogel systems whose properties change in response to changes in the concentration of specific molecules occurring in the body.

Early research focused on controlling insulin concentrations in response to fluctuations in blood glucose levels. This is important for insulin administration, allowing for the automatic management of blood glucose levels appropriately for the biological rhythms of patients with diabetes, which can be described as a biomimetic of pancreatic function. These studies are summarized in Table 1.

These studies can be classified in terms of their response function to glucose and can be broadly divided into (1) systems that use enzymes to selectively capture glucose and alter the polymer hydrogel function through the reaction products, and (2) systems that employ competitive binding to glucose. In blood glucose sensors that use electrochemical detection, glucose, a neutral molecule, is oxidized by GOD, and the redox potential is measured [39,40]. Blood glucose control devices incorporating such glucose electrodes are used to continuously measure the blood glucose levels of a patient. Molecular designs have been developed to fabricate blood glucose management systems in polymer hydrogels that are compact, lightweight, and easy to operate without requiring an electrical operating mechanism.

Several approaches have been devised to develop glucose-responsive insulin delivery systems. These systems consist of immobilized GOD in a pH-responsive polymeric hydrogel enclosing a saturated insulin solution. As glucose diffuses into the hydrogel, GOD catalyzes its conversion into electrons and gluconic acid. These products of the enzymatic reaction induce a redox reaction with the redox polymer and protonate the polyelectrolyte in the membrane microenvironment, causing swelling. Several researchers have investigated this approach for glucose-responsive

insulin delivery.

Ishihara et al. and Horbett and Ratnar et al., Hoffman's research group, conducted pioneering studies on this glucose-responsive polymer hydrogel system (Figure 2).

The first report by Ishihara et al. was based on a GOD-immobilized membrane and a redox polymer with a nicotinamide moiety (Figure 2(a)) [48]. The device consisted of two membranes. One hydrogel membrane containing immobilized GOD acted as a sensor for glucose and formed hydrogen peroxide through an enzymatic reaction; the other membrane was a redox polymer with a nicotinamide moiety that controlled the permeation of insulin through an oxidation reaction with the formed hydrogen peroxide. Oxidation of the nicotinamide group increases hydrophilicity and should thus enhance the permeability to water-soluble molecules such as insulin. The results showed an increase in insulin permeability through the membrane of 1.4 times with the addition of glucose.

It was also prepared a complex membrane composed of a GOD-immobilized membrane and a polyamine membrane by Ishihara's research group (Figure 2(b))[49]. The polyamine membrane was prepared from poly(2-hydroxyethyl methacrylate (HEMA)-*co*-N,N-diethylaminoethyl methacrylate). The polyamine membrane swells in an aqueous medium, and the degree of swelling depends on the pH of the environment. This system could achieve reversible increases and decreases in insulin permeation.

Horbett et al. immobilized GOD in a cross-linked polyamine hydrogel matrix fabricated from N,N-dimethylaminoethyl methacrylate (DMA), HEMA, and a small amount of cross-linker (Figure 2(c)) [50-52]. Membranes were prepared at -70 °C using radiation polymerization, which is a specialty of Hoffman; they have succeeded in producing stable hydrogels while maintaining enzymatic activity. To obtain sufficient insulin permeability through the gels, porous poly(HEMA-*co*-DMA) gels were prepared by polymerization under conditions that induced separation into two phases during polymerization. The rate of insulin permeation through the membranes was measured in the absence of glucose, and glucose was then added at a concentration of 400 mg/dL. The average permeability after the addition of glucose was 2.4–5.5 times higher than that before glucose was added.

Applying the same GOD-poly(amine) hydrogel system, the insulin release system of the mechanochemical pump was proposed using osmotic pressure generated by swelling the hydrogel when glucose concentration increased [53].

Glucose concentration-dependent insulin release was proposed by Langer et al. based on the fact that insulin solubility is pH-dependent [54]. Insulin was incorporated into poly(ethylene-*co*-vinyl acetate) (PEVAc) matrices in a solid form. Its dissolution and diffusion rates governed its release.

GOD was immobilized on Sepharose beads incorporated with insulin in a PEVAc matrix. To establish this mechanism at physiological pH, the insulin was modified by three additional lysine groups to obtain an isoelectric point of 7.4. When glucose enters the matrix, the produced gluconic acid causes an increase in insulin solubility and, consequently, enhances the release of insulin. In the in vivo experiments, a catheter was inserted into the left jugular vein, and two polymer matrices containing insulin and immobilized enzyme were implanted subcutaneously in the lower back of diabetic rats. A glucose solution was infused, and the rats showed a 180% increase in serum insulin concentration. Control rats that received matrices containing no insulin, insulin but no glucose oxidase, or no implants showed no change in serum insulin levels. Animal experiments involving the administration of glucose solutions at considerably higher concentrations have successfully controlled insulin release based on changes in its solubility.

Recently, GOD-immobilized hydrogels have been shown to generate hydrogen peroxide, which increases the local concentration of reactive oxygen species and enhances the proliferation activity of vascular cells, thereby promoting neovascularization in vivo [55]. This finding suggests that when a GOD-immobilized hydrogel is used as an insulin release control system, it may increase the access of glucose from blood vessels and insulin to blood vessels.

2.2 Con A-based conjugation system

Lectins, which recognize glycans in vivo, have been considered for use as a sensing element based on their functions. One lectin, concanavalin A, contains four glycan-binding sites. Therefore, glucose-responsive insulin-releasing devices have been researched based on this molecule.

The principle of competitive binding suggests the preparation of glycosylated insulins that are complementary to the major combination sites of carbohydrate binding proteins, such as concanavalin A (Con A) [56]. Con A is immobilized on Sepharose beads. The glycosylated insulin, which is biologically active, is displaced from the Con A by glucose proportionally in response to the amount of glucose present, which competes for the same binding sites. Kim et al. found that the release rate of insulin also depends on the binding affinity of insulin derivatives to Con A and is influenced by the choice of glycosylated insulin glycan groups [57]. Devices were fabricated by encapsulating Con A bound to glycosylated insulin in a suitable polymer that was permeable to both glucose and insulin. Glycosylated insulin was found to be more stable against aggregation and more biologically active than commercial insulin. The functionality of the intraperitoneally implanted devices was tested in pancreatectomized dogs using an intravenous glucose tolerance test. The effect of 500 mg/kg of administered glucose on blood glucose levels was compared between normal and pancreatectomized dogs without implants, and the two-day

blood glucose profile showed that diabetic dogs implanted with the device were able to maintain acceptable glucose levels (50–180 mg/dL) [58,59].

In a series of studies on biomolecular recognition and responsive polymer hydrogels, Miyata et al. collaborated with Hoffman to synthesize glucose-responsive hydrogels that could recognize glucose and autonomously change their volume using a sugar chain lectin complex as the cross-linking point of the gel [60-62]. First, 2-glucosyloxyethyl methacrylate (GEMA), which contains glucose in the side chain, was mixed with ConA to form a GEMA–ConA complex that was then copolymerized with a small amount of cross-linker to prepare a poly(GEMA) (PGEMA)–ConA complex gel. This gel swelled in aqueous solutions of glucose and mannose, which formed complexes with ConA depending on the concentration; however, it did not change in aqueous solutions of galactose, which did not form complexes. The change in cross-linking density was investigated by measuring the elastic modulus, and the results showed that the cross-linking density of the PGEMA–ConA complex gel gradually decreased in aqueous glucose solution. Therefore, the glucose-responsive behavior of the PGEMA–ConA complex gel was due to the dissociation of the PGEMA–ConA complex with free glucose as a dynamic cross-linking point, resulting in a decrease in cross-linking density. Next, to increase the response and reversibility of the swelling and contraction of the gel in the presence of glucose, polymer functional groups were introduced into the ConA molecule, and ConA was immobilized in the PGEMA network via polymerization. The gel swelled and contracted in response to changes in the glucose concentration, demonstrating reversible glucose-responsive swelling–shrinking behavior. Based on these results, they concluded that it was possible to construct an autonomous insulin release system by utilizing the swelling and contraction behaviors in response to changes in glucose concentration.

2.3 Artificial receptors containing hydrogels

The concept of selective, concentration-dependent, and reversible binding–dissociation between lectins and sugars, a biological reaction, has been investigated in artificial systems. Phenylboronic acid (PBA) compounds, which are used as protecting groups in glycosylation reactions in organic chemistry, can bind to various sugar compounds [63]. The use of insoluble carriers with bound PBA groups allow for the chromatographic separation of various sugar compounds or biomolecules with sugar chains [64-66]. In other words, using the PBA group may induce selective binding and dissociation in conjunction with the binding constant for compounds with diol groups, such as sugars. This reaction is of interest for the fabrication of glucose-responsive polymer hydrogels.

When PBA groups are incorporated into water-soluble polymers and mixed with polymers

containing polyhydric hydroxyl groups in an aqueous solution, gelation is observed. Kataoka's research group used this working principle to suggest the possibility of glucose-responsive control of insulin release in a fully synthetic polymer system [67-69]. As shown in Figure 3, the first polymer with a PBA unit was a copolymer of 3-acrylamide phenylboronic acid (AAPBA) and N-vinylpyrrolidone (VPy). When poly(VPy-*co*-AAPBA) was mixed with an aqueous solution of poly(vinyl alcohol) (PVA) containing polyvalent hydroxyl groups, the viscosity of the solution increased significantly. Increasing the molecular weight of PVA can make it more hydrogel-like. When glucose is added to the system in this state, the viscosity of the solution gradually decreases, and it is believed that the bond between PVA and the PBA group is replaced by glucose. In an aqueous medium, an important property of phenylboronate compounds is the equilibrium between the uncharged and charged forms. Because only charged PBA group can form stable complexes with glucose in aqueous solutions, the equilibrium shifts in the direction of increasing charged phenylboronates due to complexation with glucose. The direct complexation of uncharged PBA with glucose is unstable in water because it is susceptible to hydrolysis. As a result, as the concentration of glucose in water increases, the proportion of charged PBA group also increases, and the entire system becomes hydrophilic. Therefore, a glucose concentration-dependent change in the ratio of uncharged to charged PBA groups in the polymer chain should have a decisive influence on the solubility of the polymer if the polymer chain shows amphiphilic nature. Indeed, in a hydrogel system composed of poly(NIPAAm-*co*-AAPBA), the water content changed rapidly with the glucose concentration [70]. Thus, introduction of PBA groups to PNIPAAm-based hydrogels with a phase change in the swelling ratio with respect to the temperature resulted in a marked change in the water content of the hydrogel. The release of insulin from inside the gel was also shown to respond to the addition of glucose within a short time and became pulsatile. Kataoka et al. attributed this dramatic change in water content to the formation and disruption of the surface barrier layer of the gel, which resulted in a dramatic change in the solute transport properties and allowed for on-off control of insulin release from the gel [71].

Kim et al. prepared a hydrogel by cross-linking a water-soluble polymer based on polyacrylamide (PAAm)-based polymer with PBA groups using a bifunctional sugar compound; the insulin encapsulated in the hydrogel was released by changing the external glucose concentration. When glucose enters the gel, the cross-links are displaced by an exchange reaction at the cross-linking points, and the water content of the gel increases or the gel dissolves, thus releasing insulin [72]. Another research group used PBA group-modified insulin as a cross-linking agent for PVA to form hydrogels and achieved glucose-responsive insulin

release from the hydrogel [73].

Many studies have explored glucose-responsive hydrogel systems that secrete insulin in response to changes in blood glucose levels(See Table 1) [74-76]. To develop a polymer hydrogel system that responds to glucose, it is necessary for the hydrogel to exchange and react with glucose in a biological environment. Therefore, the dissociation constant of the boronic acid group can be controlled by introducing a substituent into the PBA group [77]. In addition, the introduction of a tertiary amino group in the polymer has been attempted under the hypothesis that the dissociation of the boronic acid group can be promoted by creating the periphery of the PBA group in a basic environment [78,79]. Although clear material designs have been developed regarding the mechanism of action, they have yet to contribute to the actual treatment of patients with diabetes. Langer et al. synthesized a new injectable glucose-responsive hydrogel composed of a single polymer. They optimized the structure based on the importance of the ratio of PBA group to glucose groups on the polymer chain during hydrogel formation and found that polymers composed of 10%–60% PBA groups, with the remainder modified with glucose groups, were suitable for hydrogel formation. These hydrogels were shown to have shear-thinning and self-healing properties and the ability to recover to a gel state within seconds after shear-induced flow. Systems that utilize single-molecule polymers in this way are expected to exhibit improved responsiveness [80].

Recently, Matsumoto et al. introduced functional groups into PBA group to control its affinity with various sugars, including glucose [81]. They named this artificial lectin “boronolectin” and are working to develop applications for this compound. In particular, by combining the boronolectin system with microneedle or catheter technology, they have explored developing a safer and portable insulin administration system that responds to changes in glucose concentration [82,83]. They have also considered the application of sialic acid recognition in drug delivery carriers [84]. We look forward to seeing the future of this research, which will lead to improved quality of life for patients with diabetes. Sugar compounds are generally distributed throughout the biological environment and support various biological reactions. Research has explored different applications of polymers and polymer hydrogel systems with PBA chemistry, which change their function in response to changes in sugar concentration. These systems are also relevant to tissue regenerative medicine and the establishment of fundamental materials science. This is discussed in the following sections.

3. Bio-inspired extracellular hydrogel matrix systems

3.1 Formation of hydrogel systems containing living cells

3.1.1. Complexation between phenylboronic acid and poly(vinyl alcohol)

As described previously, the PBA group in the polymer can bind to polymers with hydroxyl groups in the PVA main chain. In general, PVA hydrogels are stable and elastic and can be prepared by repeatedly freezing and thawing an aqueous solution to strengthen the hydrogen bonds between the PVA chains. By utilizing the reversible covalent bonds between PBA group and polyol compounds in an aqueous system, spontaneously formed reversible hydrogels can be prepared. Furthermore, the presence of low-molecular-weight sugars is expected to cause substitution reactions at the cross-linking points and change the state of the hydrogel. This phenomenon is interesting, and its use as an ECM to encase cells has been investigated. In recent years, the properties of ECMs in biological tissues have attracted attention in tissue regenerative medicine [85-87]. If these properties can be arbitrarily controlled, it may be possible to understand the factors involved in the cellular environment during tissue growth.

Konno and Ishihara reported the molecular design and synthesis of a water-soluble 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer with PBA groups: poly(MPC-*co*-*n*-butyl methacrylate (BMA)-*co*-*p*-vinylphenylboronic acid (VPBA)) (PMBV)(See Figure 4(b)) [88]. The MPC was designed using a bioinspired concept and industrially produced based on the procedure developed by Ishihara et al [89,90]. MPC is hydrophilic, electrically neutral, and exhibits good polymerization ability [91]. It is well-known that MPC polymers show excellent biocompatibility and cytocompatibility [92-94]. In particular, poly(MPC) does not interact significantly with cells. PMBV easily forms a hydrogel with PVA at room temperature, even in a cell culture medium [88]. The chemical structure of PMBV and the mechanisms of hydrogel formation and dissociation with PVA are shown in Figure 4(a). When solutions of PMBV and PVA are mixed together, a hydrogel is formed in a short period. Moreover, when low-molecular-weight sugar compounds, such as D-fructose and D-sorbitol, are added, the PMBV/PVA hydrogel reversibly dissociates owing to an exchange reaction with PVA (See Figure 4(c)) [88]. It should be noted that cell culture medium generally contains D-glucose, and the exchange reaction with glucose needs to be reduced or sufficiently slowed when cell culture is performed for long durations with this hydrogel. As the concentrations of PMBV and PVA are less than 5.0 wt%, the remaining part of the hydrogel is composed of more than 95 wt% cell culture medium. Therefore, sufficient oxygen and other nutrients for cell growth, as well as cell function-regulating molecules such as cytokines, can diffuse into the hydrogel. The dissociation of the PMBV/PVA hydrogels depends entirely on the chemical structure of the sugar compounds. In fact, in solutions containing D-fructose and D-sorbitol, the weight of the hydrogel decreases rapidly and eventually becomes a solution. In contrast,

D-glucose does not initiate substantial dissociation of the PMBV/PVA hydrogels. The complexation constants of PBA and sugar molecules were measured and were found to decrease in the following order: D-fructose (4370 M^{-1}) > D-galactose (276 M^{-1}) > D-glucose (110 M^{-1}) [95]. The complexation constants of PVA and PMBV are intermediate, between those of D-fructose and D-glucose. Therefore, PMBV/PVA hydrogels can form even when the cell culture medium contains D-glucose. The storage modulus of a hydrogel is related to its cross-linking density. As the cross-links of PMBV/PVA hydrogels are formed by chemical bonds between PBA groups and hydroxyl groups, the storage modulus of the hydrogel is related to the concentration of sugar compounds in the cell culture medium and the complexation constant. When cell culture medium containing D-glucose is added to the PMBV/PVA hydrogel, the volume of the hydrogel increases. In other words, as the volume of the hydrogel increases, the cross-linking density decreases. Therefore, the storage modulus of the PMBV/PVA hydrogel can be controlled by the addition of cell culture medium.

3.1.2 Reversible cell immobilization with biomimetic polymer hydrogels

In the field of cell engineering, many strategies have been proposed for the design of polymer matrices. These polymer matrices have the potential to provide artificial ECMs for organizing cells into 3D structures [85,87]. Synthetic ECMs can also guide cell growth and the formation of desired tissues. In cell engineering for tissue regeneration, the requirements for artificial ECMs vary depending on the target tissue. Various polymer hydrogels, which are typically composed of hydrophilic polymer chains of macromolecules or synthetic polymers, have been used for this purpose. Among these, hydrogels made from biomolecules such as collagen and hyaluronic acid, which are found in the ECM that constructs natural tissues, have been widely used in the fields of biochemistry and cell engineering. These hydrogels function equivalently to the ECM of living tissues and actively interact with cells to induce cellular responses. Most natural hydrogels degrade spontaneously under biological conditions via simple hydrolysis of the backbone and enzymatic reactions. In contrast, synthetic polymer hydrogels are fabricated by cross-linking water-soluble polymers. In addition,, the entanglement of these polymer chains is useful for obtaining physically cross-linked hydrogels. Representative examples of such polymers include poly(ethylene glycol), PVA, PNIPAAm, and zwitterionic polymers. The physical properties of these synthetic polymer hydrogels can be controlled using appropriate cross-linkers and cross-linking densities, which can significantly affect the function of cells encapsulated in the hydrogel matrix. This includes the hydrogel’s oxygen and solute permeability, elastic modulus, and direct interaction with cells. Synthetic polymer hydrogels with MPC units are employed as raw materials for soft contact lenses and have excellent

properties such as low biological reactivity and low interaction with the resulting cells [96]. Therefore, MPC polymer hydrogels can be used as cell-encapsulation matrices because of their excellent cytocompatibility.

In cell engineering, establishing technologies that can provide cells with high activity and functionality and preserve cultured cells is important for effective tissue regeneration. Cells are typically preserved by freezing, but the physical stress caused by temperature changes and chemical damage caused by anti-freeze methods can significantly reduce cell function [97-99]. Furthermore, this common cell cryopreservation procedure should not be applied to susceptible cells such as embryonic stem (ES) cells and induced pluripotent stem cells. Therefore, stem cell-based cell engineering requires cell preservation, improved viability, and control of differentiation [100]. Applying ECM-like hydrogels that do not affect cell organization to stem cell encapsulation may solve these problems. Thus, hydrogel systems that spontaneously form and reversibly dissociate under mild cell culture conditions are promising carriers for cell preservation. Table 2 summarizes the cell types encapsulated in PMBV/PVA hydrogels [101]. After dissociating the PMBV/PVA hydrogel by adding D-sorbitol, the cells can be collected as a suspension in the cell culture medium. For example, in the approach used for the encapsulation and collection of cells in PMBV/PVA hydrogels, a cell suspension was prepared using mouse fibroblasts (L-929 cells) cultured under normal conditions in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and D-glucose. After normal cell culture and preparation of a cell suspension by trypsinization, the L-929 cells were suspended in a PMBV solution containing DMEM. Next, the cell suspension in the PMBV solution was mixed with PVA solution and slowly permeated to form a PMBV/PVA hydrogel containing encapsulated cells. After cell culture for several days under standard conditions, D-sorbitol solution was added to the PMBV/PVA hydrogel to separate the hydrogel matrix, and the encapsulated cells were collected by centrifugation. Thus, the cells could be preserved and recovered in the PMBV/PVA hydrogel without the need for freezing.

Konno et al. focused on the dissociation rate of PMBV/PVA hydrogels during cell recovery, and newly prepared poly[MPC-*co*-3-methacrylamidophenylboronic acid (MAPBA)]/PVA hydrogel system. They successfully achieved the improvement of the dissociation rate of the poly(MPC-co-MAPBA)/PVA hydrogel by the addition of D-sorbitol compared with that of PMBV/PVA hydrogel [102].

The PMBV/PVA hydrogel system can also be used for new developments in cell culture environments. Recently, cell culture in a microenvironment has attracted considerable attention in the fields of medicine and pharmaceuticals owing to the development of organ-on-chip

technology aimed at analyzing biological reactions and discovering new bioactive molecules. This technology has the advantage that cells can be organized with dimensions equivalent to those in vivo using microchips and microfluidic channel systems that precisely manage fluids and soluble bioactive molecules. Analysis on a microchip provides many advantages, such as small sample volumes and reduced analysis times [103,104]. If cell lines can be added to this system, cell-based microfluidic systems can be realized. However, a method to preserve live cells on the chip under normal assay conditions is essential for the miniaturization, integration, and commercialization of cell-based microfluidic systems. As cell engineering technology has progressed, hydrogels have been used to encapsulate cells. Although hydrogels used for cell encapsulation must allow for a sufficient distribution of cells and have suitable properties as tissue-engineering scaffolds, even in microfluidic systems, these hydrogels have potential limitations for cell preservation. These limitations can be addressed by using the PMBV/PVA hydrogel system. Xu et al. used PMBV/PVA hydrogels incorporated in microchips to investigate spontaneous packaging and cryopreservation of mammalian cells under refrigeration (4 °C) and room temperature (25 °C) conditions [105,106]. The cryopreservation of the cells in this system was evaluated over 16 d. The results showed that the PMBV/PVA hydrogels in the microchips allowed the cryopreservation of cells at 4 °C for more than a week with very high viability, high intracellular esterase activity, sufficient cell membrane integrity, and slight morphological changes. Furthermore, after the stored cells were retrieved by dissociating the hydrogel through the addition of fructose solution, the collected cells showed the ability to adhere to the surface of cell culture plates and grow under standard cell culture conditions. Thus, cells can be stored under flexible ambient temperature conditions that can be easily established, even in locations outside the laboratory. This extended storage period allows for transportation using worldwide express delivery services. Therefore, the packaging and storage of cells using PMBV/PVA hydrogels that reversibly form and dissociate depending on the sugar concentration and are incorporated with microchips may be a potential alternative approach for the flexible supply and delivery of small amounts of cells locally and globally. This approach would also be very useful for the development of various cell-based applications, from bench to bedside, after conducting many case studies with different cell types.

3.2 Regulation of cell functions in the hydrogel

The required properties of cell-encapsulation hydrogels include maintaining the solute permeability of the hydrogel and controlling the elastic modulus of the hydrogel matrix surrounding the encapsulated cells. In biological systems, cells function within ECMs with various elastic moduli to form unique tissues. Therefore, it is necessary to control the elastic

modulus of the hydrogel matrix to obtain a suitable cell encapsulation environment [107]. Several reports have shown that the storage modulus of hydrogel matrices for cell encapsulation significantly affects the cell function. Mooney et al. reported the regulation of stem cell activity in hydrogel matrices with tunable stress relaxation [108,109]. They observed that cell functions such as spreading, proliferation, and osteogenic differentiation of mesenchymal stem cells were enhanced during cell culture in hydrogels with faster relaxation. Thus, the cell–hydrogel interaction is a key parameter controlling cell functions. If the elastic modulus of the hydrogel matrix can be continuously changed during cell encapsulation, the effect of the matrix on the cell function could be discussed more clearly. This would allow for the molecular design of polymer hydrogels for use in tissue engineering.

In MPC polymer hydrogels, the interaction between the encapsulated cells and the polymer matrix is negligible owing to the unique hydrophilicity of the MPC polymer chains and the very low protein adsorption [94,110]. This suggests that only the physical properties of the hydrogel matrix affect the properties of the encapsulated cells. To elucidate the influence of the physical properties of the artificial ECM on cell function, the relationship between the storage modulus of the hydrogel and cell proliferation has been evaluated. Oda et al. reported that mouse mesenchymal stem cells (C3H10T1/2 cells) were efficiently encapsulated in PMBV/PVA hydrogels without functional degradation and cultured under normal cell culture conditions [111,112]. Figure 5(a) shows the relationship between the relative proliferation rate of the C3H10T1/2 cells encapsulated in PMBV/PVA hydrogels and the storage modulus of the hydrogel. The storage modulus of the hydrogel is shown to affect the cell proliferation rate. In PMBV/PVA hydrogels with a storage modulus of 1.2 kPa, cells can be immobilized in the hydrogel, maintained in a single-cell state, and uniformly dispersed within the hydrogel matrix. After one day of culturing, taking advantage of the properties of the PMBV/PVA hydrogel, it is possible to decrease the elastic modulus to approximately 0.7 kPa by adding DMEM during cell culture (Figure 5(b)). In this case, the environment around the encapsulated cells changes, and the cells proliferate (Figure 5(c)). This on-off control of cell proliferation in response to continuous changes in the elastic modulus is interesting for providing cell sources. In particular, when the elastic modulus is high and cell proliferation is halted, the percentage of cells in a specific cell proliferation cycle increases (Figure 5(d)). This phenomenon is associated with the inhibition of cell proliferation in the hydrogel. During cell proliferation, cells cycle from the early G1 phase to the S phase, where DNA synthesis occurs in the nucleus [113]. The cells then progress to the G2 phase, where cell proliferation occurs, and the M phase, where cell division occurs, repeating this cycle. When C3H10T1/2 cells are encapsulated in PMBV/PVA hydrogels,

the proportion of cells in the G1 phase is increased compared with C3H10T1/2 cells cultured in cell culture dishes under normal conditions. In particular, cell proliferation is inhibited in the PMBV/PVA hydrogels when the storage modulus is 1.1 kPa; as a result, after one day of culture, more than 95% of the cells are in the G1 phase. The doubling time of C3H10T1/2 cells is approximately 20 h, indicating that all cells are arrested in the G1 phase during a single cell proliferation cycle. When the diffusion of low-molecular-weight solutes and proteins in PMBV/PVA hydrogels with this modulus was examined, the permeability coefficient was found to change depending on the molecular weight; however, these molecules diffused relatively easily when the molecular weight was approximately 10–100 kDa [114,115]. In other words, convergence of the cell cycle to the G1 phase was not related to nutrient deficiency within the cells. This suggests that the observed results are strongly related to the influence of the physical properties of the surrounding PMBV/PVA hydrogel matrix. With advancements in molecular and cellular biology, the differentiation of stem cells into target cells using external signaling molecules has become an important technique [116,117]. In this case, an environment that efficiently induces cell differentiation using signaling molecules is required. The encapsulation of cells in a matrix is effective because it allows the concentration of external signaling molecules to be adjusted during the diffusion process and suppresses nonspecific reactions between cells. When differentiating ES cells into target cells for use in cell engineering, it is necessary that the matrix in which the cells are immobilized can be reversibly dissociated after differentiation induction and that the cells can be extracted under gentle conditions.

The effect of the PMBV/PVA hydrogel properties on the differentiation of cells encapsulated in the hydrogel has been reported. To differentiate C3H10T1/2 cells into chondrocytes, they were stimulated with bone morphogenetic protein 2 (BMP-2) (Figure 6) [112]. C3H10T1/2 cells were pre-cultured in a PMBV/PVA hydrogel with a storage modulus of 1.2 kPa for 1 d. The cells were then maintained in DMEM supplemented with BMP-2 for 3 d. This process increased the medium content in the hydrogel, decreasing the storage modulus from 1.2 kPa to approximately 0.70 kPa, and thus allowing cell proliferation. D-sorbitol solution was then added to dissociate the PMBV/PVA hydrogel matrix, and the cells were harvested. A polymerase chain reaction was then performed to analyze the mRNA produced by the cells. The addition of BMP-2 induced the differentiation of C3H10T1/2 cells into osteoblasts, even under normal cell culture conditions in cell culture plates. Cells encapsulated in the PMBA/PVA hydrogels differentiated into early osteoblasts at a four-fold higher rate and into late osteoblasts at a five-fold higher rate than those grown in cell culture plates under normal conditions. The reason for this difference was that the progression of the proliferation cycle was inhibited in cells encapsulated in hydrogels with a

storage modulus of 1.2 kPa. Upon induction of BMP-2 signaling, cells converged to the G1 phase, and cell proliferation resumed when the storage modulus of the hydrogel dropped to approximately 0.70 kPa. Thus, regulating proliferation by induction with BMP-2 increases the differentiation efficiency. This result is consistent with the differentiation process occurring during cell turnover, as well as sensitivity to signaling molecules associated with the G1 phase of the cell cycle.

Matrices that change their properties in response to the concentration of sugar compounds, such as PMBV/PVA hydrogels, are expected to play a role as new artificial ECMs that preserve cells and regulate differentiation induction.

3.3 Integration of hydrogels containing living cells

When cells form tissues, the cells may come into direct contact, but they more commonly interact indirectly through the ECM. Using a synthetic ECM to define specific distances between encapsulated cells is a suitable method for studying intercellular responses that mimic biological tissues. In other words, the multicellularity and three-dimensionality of biological tissues affect the behavior of individual cells. Cells cooperatively cultured with different cell types in a 3D matrix will exhibit properties similar to those of cells in vivo [118,119]. By alternatingly layering the polymers that make up the self-forming hydrogel, a stable cell layer containing cells can be created. For this system, the use of a water-soluble polymer having PBA groups, including a bio-derived polymer, and a polymer system having polyvalent hydroxyl groups is an attractive combination [120-122]. Gao et al. used a spin-coated layer-by-layer procedure to prepare a precise 3D spatial multilayer PMBV/PVA hydrogel [123-125]. The PMBV/PVA hydrogel multilayer film enabled the fabrication of a hydrogel matrix that encapsulated heterogeneous cell-containing layers with adjustable spacing. In addition, the residual unreacted PBA and hydroxyl groups on the surface stabilized the interface of the overlapping layers to form a continuous hydrogel layer. A cell-laden multilayer hydrogel was used to investigate the intercellular communication between HeLa cells and L929 fibroblasts in co-culture. As PMBV/PVA hydrogels are non-adhesive to biomolecules and cells, biomolecules can diffuse freely through the hydrogel matrix. Therefore, empty polymer layers without cells can be prepared to separate cell and soluble biomolecule interactions, i.e., cell–cell contact and cell–ECM interactions, from other parameters. The results showed that the dynamics of the cell proliferation cycle progression of HeLa cells were distinctly different depending on the distance from the co-cultured L929 fibroblasts. The diffusion of fibroblast-derived soluble biomolecules in multi-layered hydrogels varied with distance. These results provide initial insights that may guide the design of future co-culture systems.

Specifically, the anticancer drug paclitaxel was permeated through a hydrogel layer in which human vascular endothelial cells (HUVEC) and HeLa cells were immobilized at a distance from each other (Figure 7(a)) [125]. This was thought to mimic the basic diffusion process of anticancer drugs from the blood to the affected area. Thus, this provides an important method for evaluating drug efficacy through an artificial ECM rather than simply examining the pharmacological effects in cultured cell systems.

Dynamic covalent bonds between PBA units and multi-hydroxy compounds have great potential for the preparation of self-healing and adhesive hydrogels. Narain et al. synthesized two MPC polymers with a benzoxaborole group and 2-gluconamidoethyl methacrylamide (GAEMA) unit in the side chains (Figure 7(b)) [126]. As the pKa value of benzoxaborole is 7.2, when these two copolymers are mixed, complexes between the benzoxaborole groups and GAEMA units are efficiently formed in phosphate buffered saline, pH 7.4, and gelation occurs easily. The resulting hydrogel has self-adhesive properties and can adhere to other hydrogels because of the two functional groups present on its surface. This indicates that hydrogel matrices containing different cell types can be stacked in close proximity to each other. It has been shown that the cells are encapsulated and remain sufficiently active. In addition, the benzoxaborole groups exhibit excellent sugar responsiveness. For example, when D-fructose solution was added to the hydrogel, the hydrogel dissociated. These polymers and hydrogels showed no adverse effects on the cells. Based on these results, it was concluded that the hydrogel matrix was useful for 3D cell culture.

When tissues are damaged, immature cells invade, and the healing process begins immediately. At this time, various cytokines control the biological reaction. However, there are many unknowns regarding this reaction, such as the conditions required for the concentration and secretion of cytokines and their effects on molecular reactions between cells. Ishihara et al. encapsulated mature and undifferentiated cells in a PMBV/PVA hydrogel layer at a certain distance and examined the effect on differentiation induction. When mouse mesenchymal stem cells (MSCs), C3H10T1/2 cells, were encapsulated in the PMBV/PVA hydrogel and cultured in the presence of bone morphogenetic protein 2 (BMP-2), the alkaline phosphatase (ALP) activity in the cells increased (Figure 7 (c)) [127]. This indicates that MSCs can differentiate into mature osteoblasts. Next, when MSCs encapsulated in the PMBV/PVA hydrogel were cultured in close contact with mature osteoblasts in the hydrogel layer, higher ALP activity was observed compared with cells cultured separately. This indicates that mature cells induce the differentiation of MSCs into osteocytes. However,, when the cells were cultured in contact with a hydrogel layer that did not contain mature cells, cell differentiation did not occur. Furthermore,

when the two cell-containing hydrogels were not in contact, the immature cells did not differentiate. Therefore, it can be concluded that differentiation of MSCs in the hydrogel layer is induced by cytokines diffused from mature osteoblasts encapsulated in another hydrogel layer. These results indicate that mature cells secrete cytokines that induce the differentiation of immature cells, and cell differentiation occurs when the local concentration reaches a differentiation-inducing threshold. This model system was shown to be useful for representing the process by which rapid healing occurs and precise tissue is formed around the injury site.

3.4 Acceleration of tissue response in spontaneously forming hydrogels

Zhang et al. proposed combining the controllable properties of synthetic polymer hydrogels and the ECM to promote tissue formation by cells [128,129]. The cells are immobilized in the hydrogel in advance and treated as part of the material. When cell proliferation is activated within the hydrogel, the cells secrete their own ECM to fill the voids in the hydrogel network. When the hybrid hydrogel is implanted into living tissue, the host cells can invade the hydrogel and form a good connection between the hybrid ECM and living tissue. This procedure is referred to as “active cell immobilization.” Utilizing bioactive molecules such as growth factors can activate the immobilized cells and accelerate the induced wound healing process. In practice, basic fibroblast growth factor (bFGF) was selected and immobilized in the hydrogel matrix (Figure 8(a)). The bFGF plays an important role in cell function, promoting cell proliferation, differentiation, and secretion of ECM during the wound healing process. The immobilized bFGF hybridized the ECM produced by the cells with the hydrogel, forming a bond between the host cells and the material scaffold (Figure 8(b)). When L929 cells were grown in the hydrogel, they were well-dispersed and secreted a large amount of ECM in response to bFGF stimulation. The cross-section of the hybrid ECM hydrogel also showed that the inside of the hydrogel was filled with ECM (Figure 8(c)) [129]. Furthermore, when bFGF and laminin (LAM) were co-immobilized on the PMBV chain, collagen production within the hydrogel became more effective, confirming the generation of hybrid ECM. These results suggest that the binding of such a cell-encapsulated hydrogel layer to the surface of medical device material can effectively immobilize the medical device based on the healing response when the medical device comes into contact with biological tissue, thus reducing the inflammatory response and preventing infection. In addition, when removing a medical device, a minimally invasive procedure can be applied by treating it with a sugar-containing solution to peel off the hydrogel layer at the interface. Although further research is required, this suggests a new aspect of the functionality of stimuli-responsive polymer hydrogels.

When PBA groups were introduced into gelatin (APBA-gelatin), which has cell adhesive

properties, and the gel was mixed with PVA to form a hydrogel, encapsulating MSCs led to the formation of cell aggregates (Figure 9(a)) [130]. This not only served as a scaffold for forming cell aggregates in 3D culture, but also allowed the preparation of cell scaffold hydrogel microspheres that could well control and release growth factors at any time by non-cytotoxic stimuli. When MSCs were cultured, in the presence of APBA-gelatin/PVA hydrogel microsphere containing growth factors, cells formed cell aggregates that uniformly incorporated the microspheres (Figure 9(b)) [130]. When D-sorbitol was added to the culture medium, the APBA-gelatin-PVA hydrogel microspheres disappeared from the cell aggregates over time. Microspheres containing bFGF or BMP-2 released the respective growth factors as the microspheres disappeared. From this, it was concluded that the sugar-responsive water-solubilized microspheres are promising as scaffolds for cell aggregates and have the ability to release growth factors in the cell aggregates when needed. Research has been reported on 3D-cell encausulation in a system in which the establishment of dynamic covalent crosslinks via boronate esters formation between synthetic polymers, PBA group bearing poly(N,N-dimethyl acrylamide) and PVA. Two model of human cell-line, human breast cancer cells and pulmonary fibroblasts are encapsulated and co-cultured. (Figure 9(c)) [131]. And on artificial ECMs to repair tissue defects (Figure 9(d)) [132]. These are interesting bioinspired material systems for the regeneration of biological tissues and are considered to be essential technologies for tissue regenerative medicine, and further developments in this field are expected. Additionally, hydrogel formation between PBA group-modified LAM and alginate (ALG) produces a shear-thinning bioink upon mixing of the two polymers and pre-osteoblasts, fibroblasts, or cancer cells at physiological condition [133].

Using biomimetic science, Asha et al. applied the properties of catechol groups to form a hydrogel layer by reacting it with an MPC polymer containing benzoxaborole groups on the surface. They found that the surface inhibited bacterial adhesion [134]. Furthermore, washing the surface with a solution containing fructose caused the captured bacteria to detach, resulting in a clean surface again. This was shown to be an effective surface for preventing biofilm formation caused by bacterial debris left on sterilized surfaces. The body has defense mechanisms to prevent bacterial adhesion and biofilm formation, and this is an example of taking advantage of the self-cleaning properties of sugar-responsive polymer hydrogels.

4. Future perspectives

The molecular design of bioinspired or biomimetic polymers is a pioneering area in conventional materials science, and further developments are expected in the future, particularly

considering the unique nature of biological environments. Figure 10 shows the significant characteristics of bioinspired and biomimetic hydrogels and their expected fields of application. The interior of a cell is a molecularly crowded environment in which biomolecules are highly concentrated, and it is difficult to reproduce this in an artificial system [135,136]. Therefore, systems that exhibit characteristics similar to those of cells have been devised by simplifying the chemical reaction process that expresses a particular function. Furthermore, detailed research on the ECM has been conducted in recent years based on the knowledge that the ECM controls cellular functions [137-139]. Further simplifying this and studying the characteristics of cells supported in an artificial ECM would provide significant contributions to engineering and medicine fields that utilize cells.

One of the potential in vivo applications of bioinspired polymer hydrogels is the prevention of adhesion between repaired musculoskeletal and surrounding tissues during the healing process [132,140-143]. This application is enabled by the inhibition of cell adhesion on the surfaces of these polymers. Tendon tissue healing and adhesion are intricately driven by the chemotaxis of exogenous fibroblast precursor cells. To prevent the formation of postoperative adhesions, several strategies have been developed using polymeric materials as physical barriers. These polymeric materials have several requirements, including excellent mechanical and biological tissue compatibility in vivo, prevention of severe inflammatory responses, and allowing essential elements such as cytokines, nutrients, and oxygen to penetrate the healing area while preventing cells from penetrating the polymeric material. As discussed in the previous section, spontaneously forming and reversibly dissociating polymeric hydrogel systems are ideal candidates for the release of bioactive molecules and the encapsulation of cells. In addition, for minimally invasive endoscopic surgery, it is desirable to generate hydrogels in the affected area through a catheter. Polymeric systems that can form hydrogels in situ from a solution state are promising for this purpose. Furthermore, the therapeutic effect can be improved by complexing drugs within the hydrogel as a reservoir [144,145].

Spontaneous forming and reversibly dissociating polymer hydrogels have shown promise for in vitro and in vivo applications. They have potential applications in the design of future 3D cell culture systems. Such systems can serve as powerful tools in tissue engineering and cell biology to address basic questions related to the spatiotemporal signaling of bioactive molecules [43, 146-148]. For example, the recapitulation of bioactive molecule gradients within 3D structures and investigation of their effects on the fate of developing pluripotent stem cells will be possible. Furthermore, these bioinspired polymer hydrogels are useful as cell immobilization matrices for the efficient production of useful biomolecules, such as bioactive molecules and antibodies.

Advanced 3D printing systems and nano-/microfiber-integrated systems can be used to fabricate hydrogel-based devices [45,46,133]. It is anticipated that this system could also be used as a bioreactor using cells or bacteria. For example, it has been reported that when *Shewanella oneidensis*, an electric current-producing bacterium, is encapsulated in the hydrogel composed of PMBV with an electronically conductive ferrocene group and PVA, an electric current is generated because the bacteria produce electrons [149]. This has the potential for use in biological batteries. When the activity of the bacteria decreases, they can be replaced by flushing with a sugar solution, allowing the battery device to be reused. These applications are expected to develop further as material technologies that are useful to society.

5. Afterword

Professor Allan Hoffman has made significant contributions to the establishment of biomaterials science and the expansion of the research field based on polymer science and surface science. These achievements have had a great impact on biomaterials researchers and fields related to the analytical chemistry of biomolecules, cell engineering, and pharmaceutical science. In honor of these research achievements, the author dedicates this review to Professor Hoffman.

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Figure captions

Figure 1. Basic concept of bioinspired material design.

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Figure 6. Differentiation of cells after incubation of PMBV/PVA hydrogels. (a) mRNA expression of C3H10T1/2 cells three days after the addition of BMP-2; the expression of *Collagen type1 alpha1* as the early stage of osteoblasts (open column), *Bone gamma carboxygultamate protein 1* as the late stage of osteoblasts (closed column), *Collagen type2 alpha1* as the early stage chondrocytes (striped column), and *Peroxisome proliferator-activated receptor gamma* as the initial adipocytes (dotted column) are normalized to a house-keeping

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Figure 7. (a) Confocal microscopic image of the cell-encapsulated layer in PMBV/PVA multilayered hydrogels. The cells are human vascular endothelial cell (HUVECs in green fluorescence), and HeLa cells (in red fluorescence) located in individual layers in the multilayered hydrogel. Reprinted with permission from [123]. Copyright (2013) Elsevier. (b) Schematic illustration of dynamic hydrogels based on MPC polymers with pendent benzoxaborole (PMBG) and sugar residues. (c) Demonstration of the self-healing property of the PMBG hydrogel. (b) – (c): Reprinted with permission from [126]. Copyright (2018) American Chemical Society. (d) Total activity of alkaline phosphatase based on the differentiation of mesenchymal stem cells (MSCs; C3H10T1/2 cells) stimulated by mature osteoblast cell aggregates in the double-layered hydrogel system cultured for 10 d. (A) double-layered hydrogels containing MSC aggregates and MSCs; (B) two hydrogels containing osteoblast cell aggregates and MSCs, respectively; and (C) double-layered hydrogels containing osteoblast aggregates and MSCs. Reprinted with permission from [127]. Copyright (2022) Royal Society of Chemistry.

Figure 8. Active cell immobilization using a cytokine-binding PMBV/PVA hydrogel (PMBV-bFGF/PVA) system. (a) Structure of the bFGF binding polymer and hydrogel formation mechanism. (b) PMBV-bFGF/PVA hydrogel with immobilized cells after 5 d; scale bar: 100 μm. (c) 3D laser scanning confocal microscopy images of the distribution of fluoresceine-conjugated type I collagen in the PMBV-bFGF/PVA hydrogel with immobilized L929 cells after five days of culturing. Reprinted with permission from [128]. Copyright (2019) Royal Society of Chemistry.

Figure 9. (a) Structure of PBA group modified gelatin and its hybridization mechanism with PVA. (b) Phase-contrast microscopic images of mesenchymal stem cells (MSC) after 0 day (a, e, i), 1 day (b, f, j), 3 days (c, g, k), and 7 days (d, h, l) incubation with mixed PBA group modified gelatin/PVA hydrogel microspheres in wells of agarose-coated flat-bottom plate. The number of cells initially seeded was 1×10^4 cells per well. The hydrogel microspheres were seeded 0, 1×10^3, or 5×10^3 per well. Scale bar is 500 μm. (a) – (b): Reprinted with permission from [130]. Copyright (2020) Wiley. (c) Human pulmonary fibroblasts (CCL151, green) and human breast

cancer cells (MDA MB 231, pink) were co-cultured for 7 days in self-healing boronic acid-based hydrogels. Reprinted with permission from [131]. Copyright (2018) American Chemical Society. (d) Hyaluronic acid-based glucose-responsive hydrogel platform with responsive antioxidant activity for rapid repair of diabetic wounds. Reprinted with permission from [Ref 132]. Copyright (2022) Elsevier.

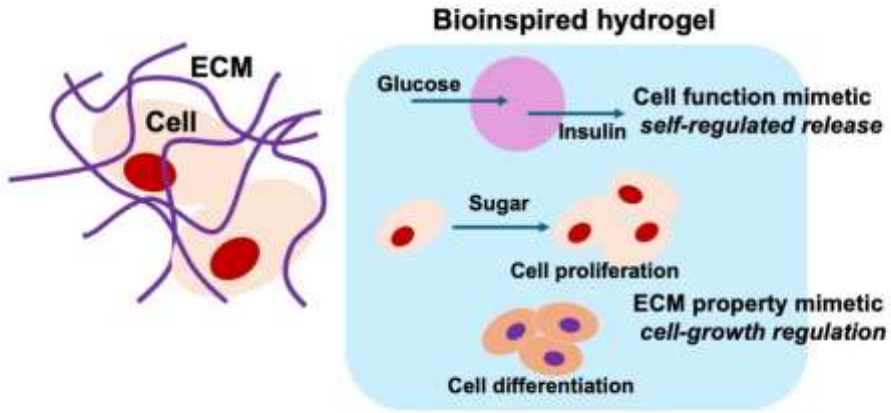
Figure 10. Characteristics of bioinspired or biomimetic hydrogels and their expected fields of application.

Table 1. Glucose responsive hydrogel system

Sensing moiety	Mechanism	Insulin	Hydrogel matrix	Function	References
GOD	Oxidation	Unmodified	GOD-immobilized PAAm / redox polymer complex membrane	Permeation through membrane	[48]
GOD	Protonation	Unmodified	GOD-immobilized PAAm / poly(amine) complex membrane	Permeation through membrane	[49]
GOD	Protonation	Unmodified	GOD-immobilized poly(amine) complex matrix	Releasing from polymer matrix	[50-52]
GOD	Protonation	Unmodified	GOD-immobilized poly(amine) complex matrix	Increase in osmatic pressure	[53]
GOD	Protonation	Lysine-modified insulin	GOD-immobilized poly(amine) complex matrix	Increase in solubility increase	[54]
Con A	Binding ability	Glycosyl insulin	Con A encapsulated polymer capsule	Exchange binding	[58]
Con A	Binding ability	Unmodified	Con A-immobilized polymer/PGEMA	Exchange binding at crosslinkings	[60,61]
Phenyl boronic acid moiety	Binding ability	None	PVPy hydrogel with phenylboronic acid group	Decrease in the viscosity of complex solution	[64]
Phenyl boronic acid moiety	Binding ability	None	PAAm hydrogel with phenylboronic acid group crosslinked by glycosyl compound	Exchange binding for dissociation	[69]

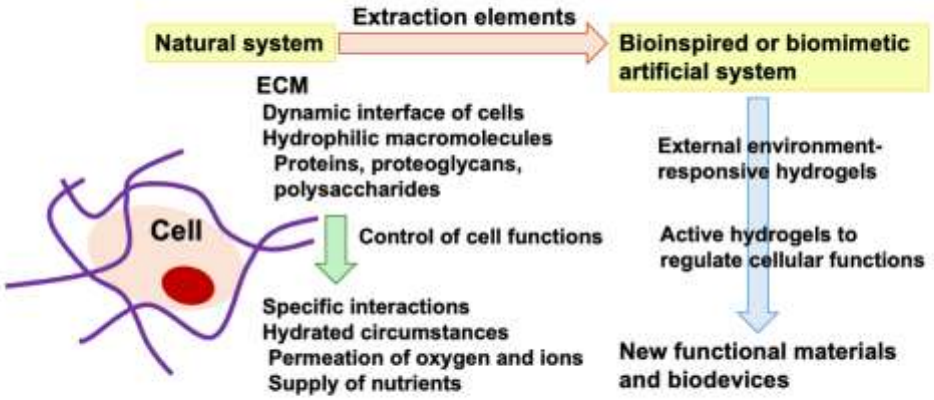
Table 2. Cell encapsulated in the PMBV/PVA hydrogels

Cells	**Cell type**	**Culturing period**	**Condition**	**Differentiation ability**
L929	Mouse fibroblast	>2 weeks	Bulk hydrogel (>10^5 cells/mL)	-
			Microcapsules (single cell)	-
			Layer by layer (Co-culture)	-
NIH3T3	Mouse fibroblast	2 weeks	Bulk hydrogel	-
Hela	Human cervical cancer cells	>1 week	Bulk hydrogel	-
			Microcapsules	-
			Layer by layer	-
A431	Human epidermoid carcinoma	>1 week	Layer by layer	-
HUVEC	Human umbilical vein endothelial cell	24 h	Layer by layer	-
HEAC	Human aortic endothelial cells	>1 week	Microcapsule	-
HepG2	Human hepatocellular carcinoma	2 weeks	Bulk hydrogel	-
Caco-2	Human epithelial colonic carcinoma	4 weeks	Bulk hydrogel	-
J774.1	Mouse macrophage	>1 week	Bulk hydrogel	-
HL60	Human promyelocytic leukemia cell	>1 week	Microcapsule	Preserved
MC3T3-E1	Mouse osteoblast	>1 week	Bulk hydrogel	Preserved
C3H10T1/2	Mouse mesenchymal stem cell	3 days	Bulk hydrogel	Preserved
ES(129/SvEv)	Embryonic stem cell	3 days	Bulk hydrogel	Preserved
ES(C57BL/6)	Embryonic stem cell	3 days	Bulk hydrogel	Preserved



Graphical abstract

Graphical Abstract



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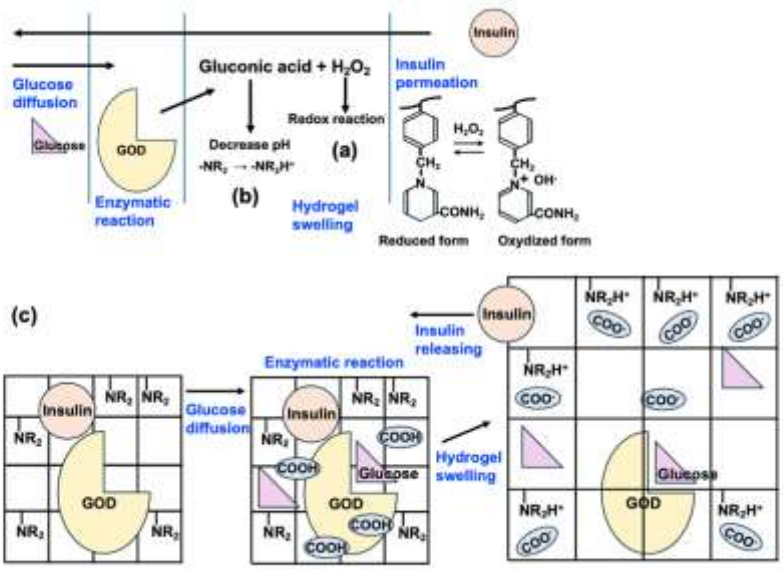

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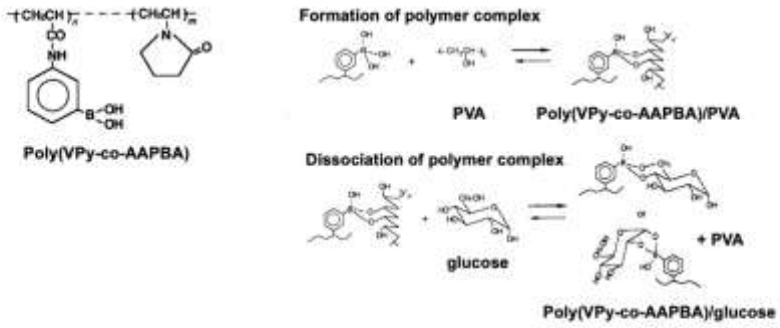

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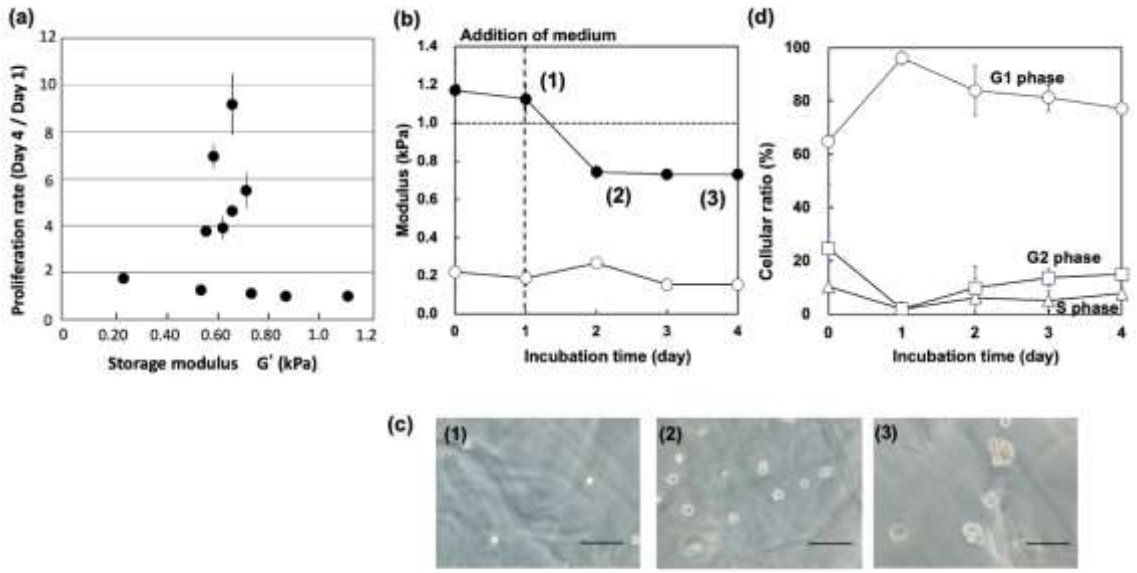

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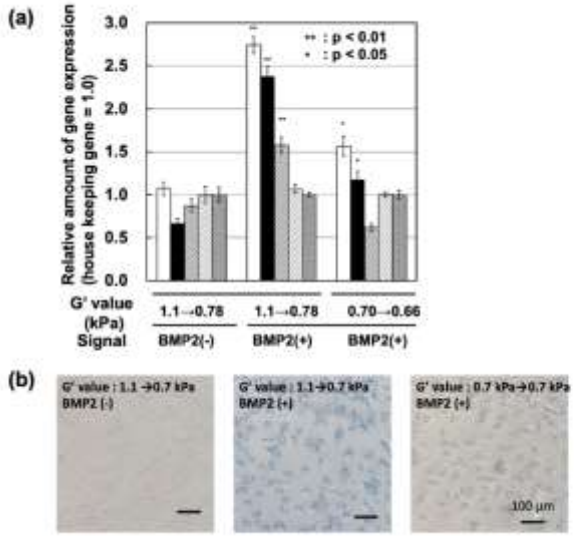

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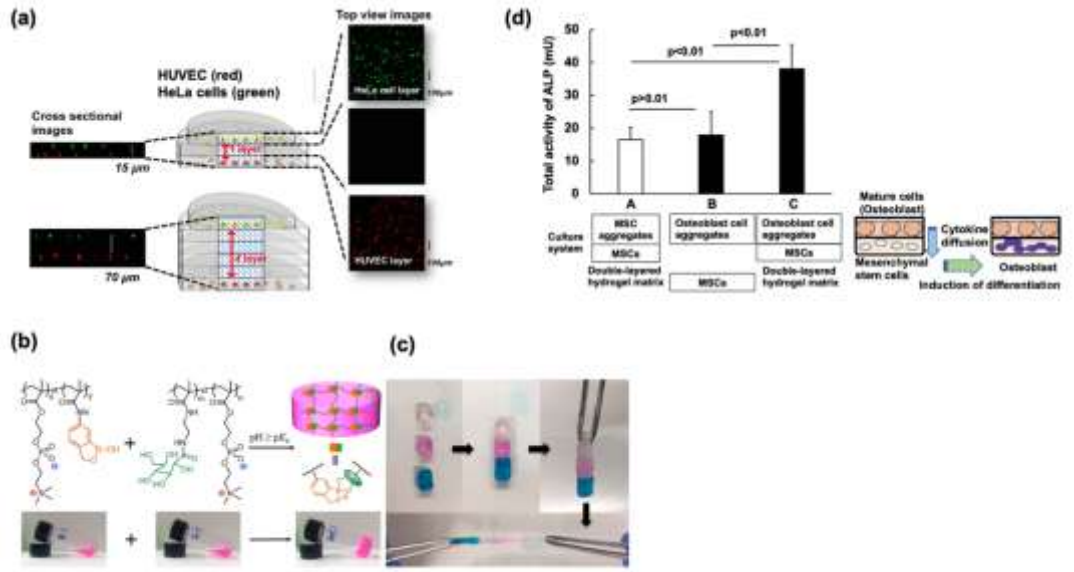

Figure 7

Figure 7. (a) Confocal microscopic image of the cell-encapsulated layer in PMBV/PVA multilayered hydrogels. The cells are human vascular endothelial cell (HUVECs in green fluorescence), and HeLa cells (in red fluorescence) located in individual layers in the multilayered hydrogel. Reprinted with permission from [123]. Copyright (2013) Elsevier. (b) Schematic illustration of dynamic hydrogels based on MPC polymers with pendent benzoxaborole (PMBG) and sugar residues. (c) Demonstration of the self-healing property of the PMBG hydrogel. (b) – (c): Reprinted with permission from [126]. Copyright (2018) American Chemical Society. (d) Total activity of alkaline phosphatase based on the differentiation of mesenchymal stem cells (MSCs; C3H10T1/2 cells) stimulated by mature osteoblast cell aggregates in the double-layered hydrogel system cultured for 10 d. (A) double-layered hydrogels containing MSC aggregates and MSCs; (B) two hydrogels containing osteoblast cell aggregates and MSCs, respectively; and (C) double-layered hydrogels containing osteoblast aggregates and MSCs. Reprinted with permission from [127]. Copyright (2022) Royal Society of Chemistry.

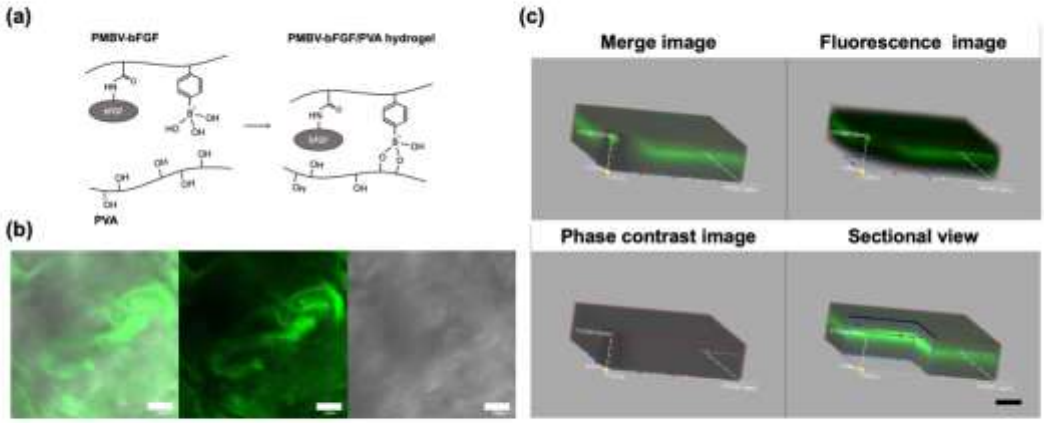

Figure 8

Figure 8. Active cell immobilization using a cytokine-binding PMBV/PVA hydrogel (PMBV-bFGF/PVA) system. (a) Structure of the bFGF binding polymer and hydrogel formation mechanism. (b) PMBV-bFGF/PVA hydrogel with immobilized cells after 5 d; scale bar: 100 μm. (c) 3D laser scanning confocal microscopy images of the distribution of fluoresceine-conjugated type I collagen in the PMBV-bFGF/PVA hydrogel with immobilized L929 cells after five days of culturing. Reprinted with permission from [128]. Copyright (2019) Royal Society of Chemistry.

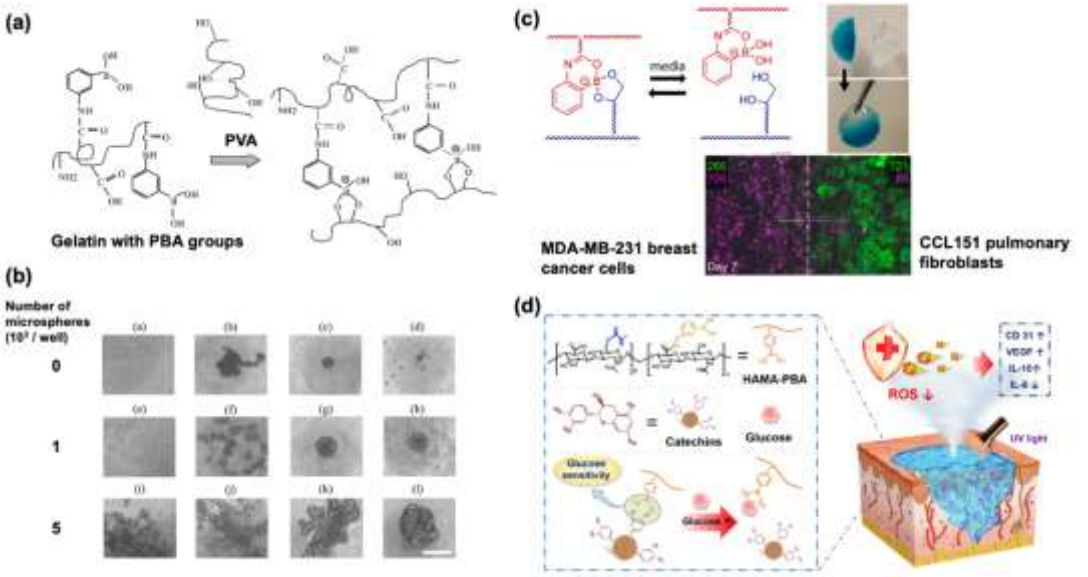

Figure 9

Figure 9. (a) Structure of PBA group modified gelatin and its hybridization mechanism with PVA. (b) Phase-contrast microscopic images of mesenchymal stem cells (MSC) after 0 day (a, e, i), 1 day (b, f, j), 3 days (c, g, k), and 7 days (d, h, l) incubation with mixed PBA group modified gelatin/PVA hydrogel microspheres in wells of agarose-coated flat-bottom plate. The number of cells initially seeded was 1×10^4 cells per well. The hydrogel microspheres were seeded 0, 1×10^3, or 5×10^3 per well. Scale bar is 500 μm. (a) – (b): Reprinted with permission from [130]. Copyright (2020) Wiley. (c) Human pulmonary fibroblasts (CCL151, green) and human breast cancer cells (MDA MB 231, pink) were co-cultured for 7 days in self-healing boronic acid-based hydrogels. Reprinted with permission from [131]. Copyright (2018) American Chemical Society. (d) Hyaluronic acid-based glucose-responsive hydrogel platform with responsive antioxidant activity for rapid repair of diabetic wounds. Reprinted with permission from [132]. Copyright (2022) Elsevier.

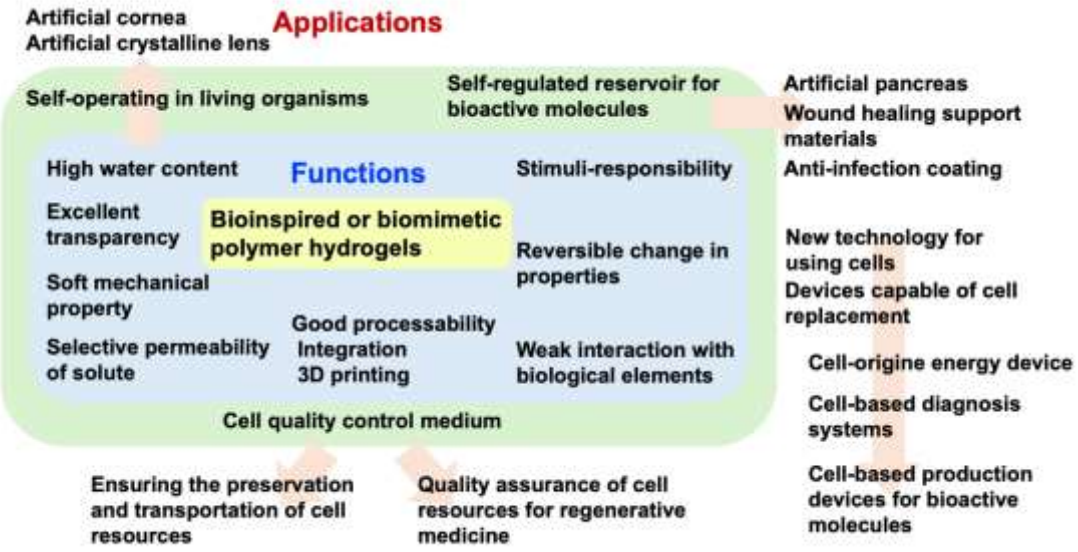

Figure 10

Figure 10. Characteristics of bioinspired or biomimetic hydrogels and their expected fields of application.

This review manuscript provides a bird's-eye view of the bioinspired polymer hydrogel systems that make up new polymer biomaterials with stimuli-responsiveness. Research into hydrogels has a long history, but research into bioinspired hydrogel systems that change their properties in response to external stimuli only began in the 1980s. Early on, there was research by Dr. Hoffman and others, followed by research into temperature- and pH-responsive hydrogels, and further research regarding spontaneously and reversibly forming hydrogels into their use as controlled drug release carriers and artificial ECMs that encapsulate cells. Currently, these studies contribute to the development of pharmacology and basic medicine. It emphasizes the perspective of a bioinspired polymer hydrogel system for the controlled release of bioactive molecules and cell-based tissue engineering in this review.

Biographic note:

Dr. Kazuhiko Ishihara has been researching functional polymers, especially biocompatible polymers that can be applied to the medical and biotechnology fields, based on the molecular design and synthesis of polymers. In 2021, he retired as a professor in the Department of Materials Engineering at the University of Tokyo and is now an emeritus professor at the University of Tokyo, a specially appointed professor at Osaka University, and a visiting professor at Kansai University. He is the inventor of 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers, their biomaterials, and several medical devices using the MPC polymers. He has opened the science of biomimetic and bioinspired polymers based on this research. He has been awarded by many academic societies and government organizations including the Honor Medal with purple ribbon.