

Stimuli-Responsive Hydrogels and Their Application to Functional Materials

Ryo Yoshida and Teruo Okano

Abstract Many kinds of stimuli-responsive polymer gels that respond to the change in their surroundings such as solvent composition, temperature, pH, and supply of electric field have been developed. They are of interest as intelligent (or smart, biomimetic) materials which have sensor, processor, and actuator functions. This article is related to stimuli-responsive gels and their application for bio- or biomimetic materials designed as self-oscillating gels.

Introduction

With the discovery of “volume phase transition” phenomena by Tanaka in 1978 [1], research using gels as functional materials was activated. Many stimuli-responsive polymer gels, that change volume abruptly in response to a change in their surroundings, such as solvent composition, temperature, pH, and supply of electric field, light, have been developed. Their ability to swell and deswell according to conditions makes them interesting for use as new intelligent materials. Applications for biomedical fields have three functions; (a) sensing an external signal (sensor function), (b) evaluation (processor function), and (c) action (actuator function) which were developed as “intelligent gels” or “smart gels.”

Stimuli-responsive gels and their application to functional materials, mainly to biomaterials and biomimetic materials are very important. Particularly, intelligent material systems that use temperature-responsive poly(*N*-isopropylacrylamide) (poly(NIPAAm) or PNIPAAm). Several intelligent systems have been created using the bulk properties and the surface properties of PNIPAAm gels, such as intelligent drug delivery systems (DDS), temperature-responsive chromatography and cell-sheet engineering. In addition, biomimetic materials exhibiting self-oscillating behavior have been developed based on the PNIPAAm gels.

Stimuli-Responsive Gels as Functional Materials

The functions of stimuli-responsive gels can be roughly classified into three categories; (a) mechanical motion, (b) mass transport, and (c) conversion and transmission of information.

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Function of Mechanical Motion

In the 1980s and 1990s, several mechanical devices using gels were devised and developed. These devices were controlled by changing temperature or an electric field (artificial muscles, robot hands to lift or grasp objects, and as artificial fish that swim by a repeating flexing motion) [2]. In addition, several chemo-mechanical gels (biochemo-mechanical gels) that can connect when glucose was added to an external solution were demonstrated [3]. Recently, more interest is shown in electrically stimulated systems using polyelectrolyte gels, organogels [4], gels consisting of carbon nanotube, and ionic liquid [5]. For example, an ion conductive polymer actuator obtained by plating gold to both sides of a perfluoro carboxylic acid film causes a bending motion and biomimetic motion in an electric field [6]. Another actuating system, driven by a magnetic field using a PVA gels-containing film magnetite (Fe_3O_4) particles (ferrogel) provides dynamic motion in response to magnetic field [7].

Function of Information Transmission and Transformation

The function to memorize or convert information is key for stimuli devices. The molecular designs to memorize structure at the molecular level in a polymer network have the following important characteristics:

Shape Memory

Osada et al. [8] pioneered the “shape memory gels” that consisted of acrylic acid and styryl acrylate with long hydrophobic side chains. The gels return to their original shape by heating. Several potential applications including artificial valves, artificial anus and medical implements, sporting goods, are being pursued.

Optical Function

Materials that change color reversibly are important for displays, recorders and sensors that lead to gels with optical conversion functions have been prepared [9–11]. New sensor materials that determine analyte concentrations by wavelength changes of diffracted light and “gels opals” that exhibit color changes with temperature by covalently bonding self-assembled PNIPAAm gels particles using divinyl sulfone [12] and porous PNIPAAm gels with “structural color” with reversible opal structure [13, 14]. The periodically ordered interconnecting porous structures were created in these gels by using a closest-packing silica colloidal crystal as a template. The porous gels changes “structural color” corresponding to temperature, which can be tuned by changing the amount of the crosslinker. Porous glucose-responsive gels that change the structural color in response to glucose concentration were prepared using glucose-responsive gels with phenyl boronic acid groups to be applied as a sensor for blood sugar levels [15].

Dimmer materials using PNIPAAm gels with high pigment concentrations were made by arranging the gels particulates containing carbon black on a glass substrate. The glass transmitted light changes reversibly with temperature [16]. To diffuse and condense pigment by volume change is similar to the function of pigments in cells of living organisms.

Molecular Recognition

Gels that recognize specific chemical substances, like a catalyst or an enzyme, have been prepared by molecular imprinting [17]. Target molecules are mixed into the solution containing the monomer with a recognition site, then polymerized so that a complex is formed by the interaction between the target molecule and the monomer. After polymerization, the target molecules are removed since the information for the target molecules is memorized in the polymer network. For example, to target theophylline, it was crosslinked in a methacrylic acid polymer network using a high concentration of crosslinker (>70%) to produce a rigid network [18].

Several hydrogels were developed to recognize environmental stimuli, such as gels that captured metal ions at different temperatures [19]. Biomolecule-responsive gels were developed that are glucose-responsive and antigen-responsive by utilizing biomolecular interactions to establish reversible crosslinking [20, 21].

Function of Mass Transport

Pulsatile Drug Release Control Using Hydrogels

Several gels that function to capture or release a chemical or biological substance and to separate or purify substances are used for biomedical applications [22, 23]. A major use is drug delivery (DDS) by stimuli-responsive gels, intelligent DDS with auto-feedback mechanism that release the drug only when it is needed and stops the release at normal state. An example is the release of antipyretics only when the body temperature rises; another senses an increase in glucose in the blood and releases insulin automatically. The on–off control for drug release by small temperature changes in the body can be realized using temperature-responsive NIPAAm copolymer gels (Fig. 1) [24].

Several regulating systems for insulin release have been developed [25–29]. An on–off regulation for insulin release is effective in response to external glucose concentration by utilizing the reversible complex formation between glucose and the phenylboronic acid group (Fig. 2).

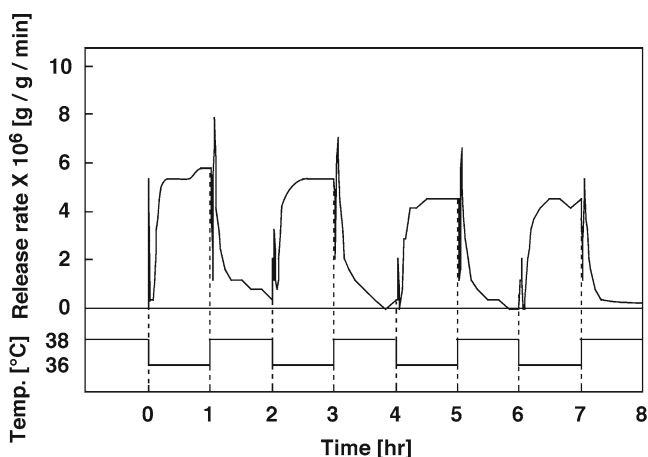


Fig. 1. Indomethacin release from poly(NIPAAm-co-dimethylacrylamide-co-butyl methacrylate) gels in response to stepwise temperature changes between 36 and 38°C in PBS (pH 7.4).

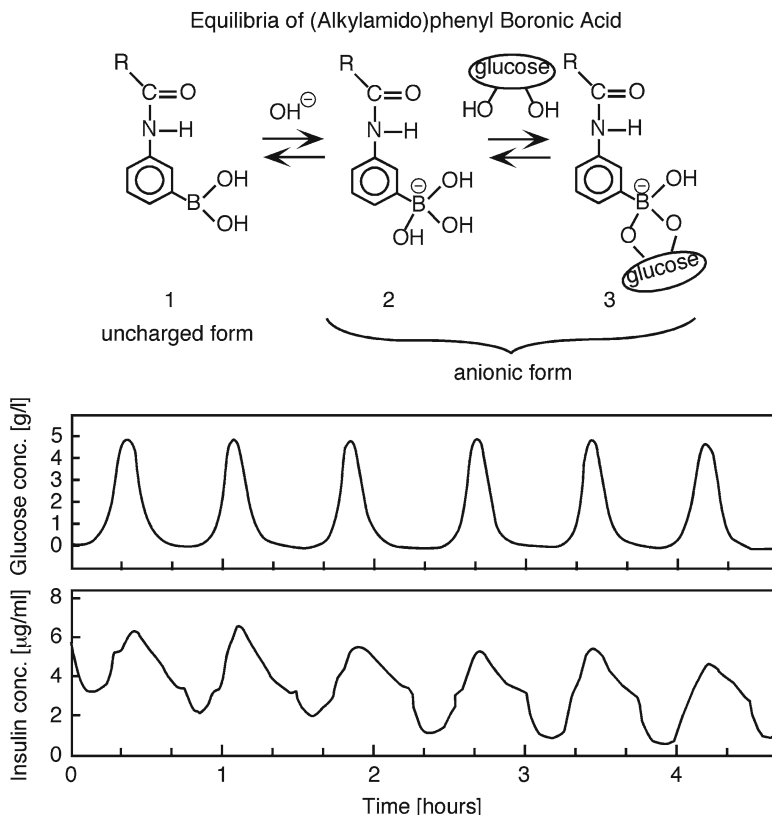


Fig. 2. Equilibria of (alkylamio) phenyl bronic acid in an aqueous solution in the presence of glucose (*top*) and repeated on-off release of FITC-insulin from the hydrogels at 28°C, pH 9.0, in response to external glucose concentration (*bottom*).

The gels operate at physiological pH bearing phenylborate derivatives as a glucose-sensing moiety [30]. There are many other self-regulated DDS that response to pH, electrical, magnetic changes [31–33].

Intelligent Surfaces for Bioseparation

These self-regulated DDS utilize changes in permeability or diffusivity of the gels by structural changes that accompany swelling and deswelling changes. New modulation systems, to control the surface properties or solubility of materials in response to an external signal, are designed with the stimuli-responsive polymers on a material surface, or by modifying the surface with bioactive substances, such as enzyme. This technology is used in biomedical field for separation, purification, diagnosis, and analysis.

PIPAAm (PNIPAAm) exhibits temperature-responsive and soluble/insoluble changes in aqueous solution. Temperature-responsive surfaces that demonstrate controlled hydrophilic/hydrophobic alterations, such as PIPAAm-grafted surfaces, as “intelligent surfaces” controlled by external modulation of temperature have been developed [34, 35]. Typically, water contact angles on surfaces change reversibly with temperature as shown in Fig. 3. At temperatures below 32°C, PIPAAm molecules are highly hydrated, and the PIPAAm-grafted surfaces are hydrophilic. Above 32°C, extensive PIPAAm dehydration occurs, with an abrupt transition to hydrophobic surfaces. This change is completely reversible with temperature.

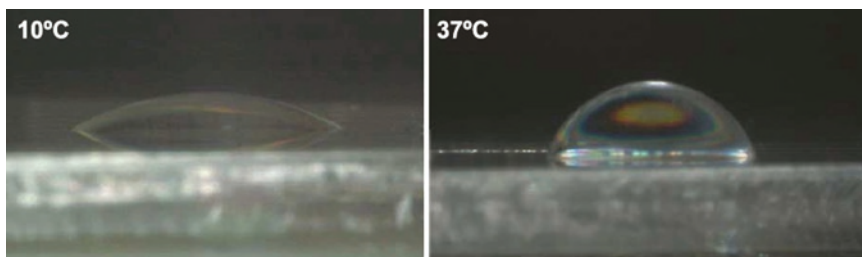


Fig. 3. Temperature-dependent wettability changes for PIPAAm-grafted surfaces at 10 and 37°C.

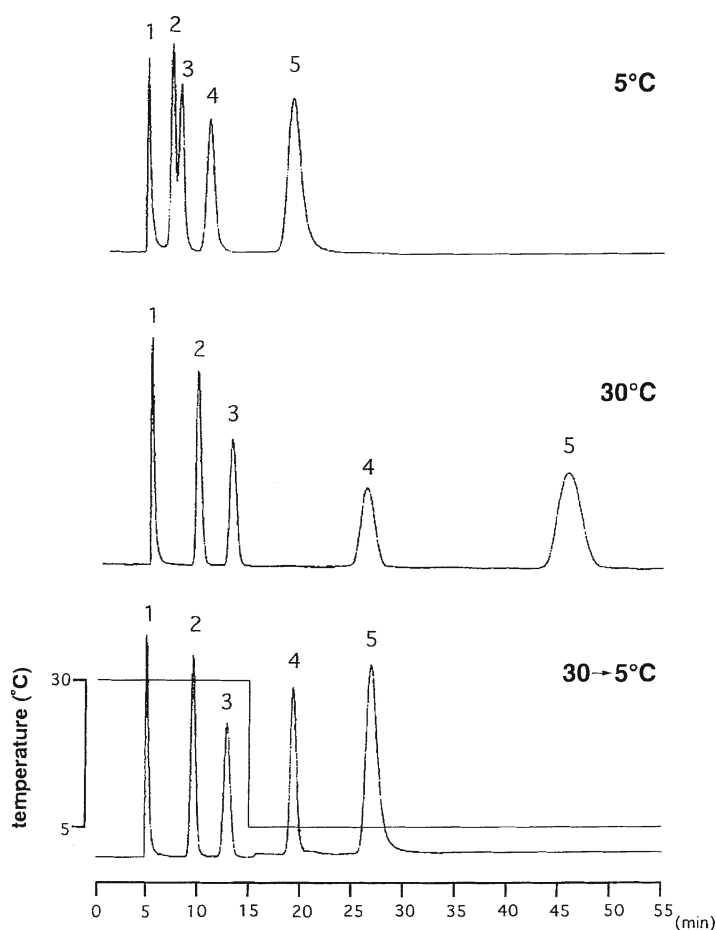


Fig. 4. Chromatograms of a mixture of four steroids and benzene with step gradient by changing column temperature. Peaks: 1, benzene; 2, cortisone; 3, prednisolone; 4, hydrocortisone acetate; and 5, testosterone. Column, NIPAAm-BMA copolymer-modified silica; eluent, water; flow rate, 1.0 ml/min; detection, UV 254 nm.

These surface characteristic changes are used for aqueous separations of bioactive compounds, including; steroid hormones [36, 37], polypeptides and proteins [38–41], and nucleic acids [42].

NIPAAm copolymer on the surface of silica gels can act as a temperature-responsive chromatograph enabling high-speed separation of mixtures by controlling the interaction between the carrier surface and substance by the temperature (Fig. 4) [36, 37]. By applying stepwise temperature changes from higher temperature to lower temperature, the retention time

for hydrophobic component is controllable. Since the mobile phase is water, an organic solvent is not used for the separation and analysis of physiologically active substances or cells.

Cell-Sheet Engineering Using an Intelligent Surface

The development of tissue engineering has progressed significantly from the original concepts [43, 44]. The current paradigm involves constructing scaffolds from biodegradable polymers with seeded cells that proliferate and deposit extracellular matrix (ECM) molecules, such as collagen and fibronectin, eventually regaining their native structure and tissue morphology as the scaffold degrades [45].

Some success was achieved for cell-sparse tissues (using large amounts of ECM and relatively few cells) such as heart valves [46], bone [47], and cartilage [48], as well as the reconstruct of human ears on the backs of mice [49]. However, these polymer scaffolds have undesirable consequences; inflammatory reactions due to the implantation of nonnatural materials with scaffold degradation, the space formerly occupied by the polymer is usually filled by cells and deposits of ECM, that can lead to pathological fibrosis which poorly resembles the native tissue structure. It is also commonly seen that cells on the periphery of the scaffolds are maintained and closely resemble native tissues, whereas there is significant cell necrosis at the interior due to restricted passive diffusion to delivery of nutrients and the removal of metabolic wastes. Therefore, current technologies are still severely lacking in terms of achieving successful tissue reconstruction.

Cell-Sheet Engineering

The architecture of many tissues, such as the heart or liver, mainly consist of associated and with comparatively little associated ECM. To recreate these tissues, cell-dense structures that mimic normal structure and function need to be engineered. To meet this purpose, a new approach using cell-sheet engineering with temperature-responsive culture dishes (Fig. 5)

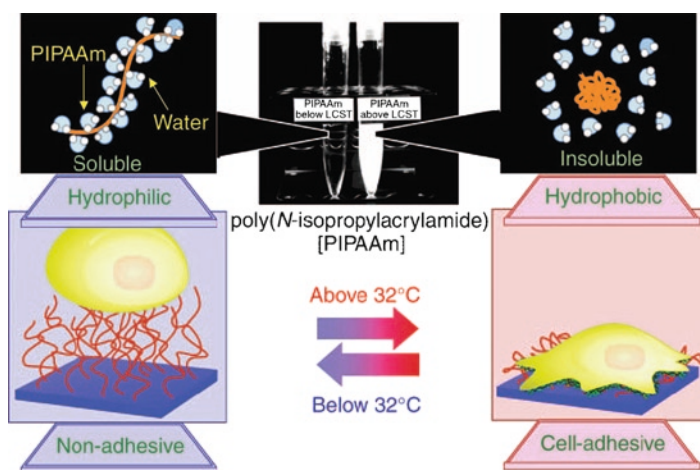


Fig. 5. Temperature-responsive culture dishes. The temperature-responsive polymer poly(*N*-isopropylacrylamide) (PIPAAm) exhibits a transition from hydrophobic to hydrophilic across its lower critical solution temperature (LCST) of 32°C. After electron-beam polymerization and grafting to normal tissue-culture polystyrene (TCPS) dishes, temperature-responsive culture surfaces can be produced. The noninvasive harvest of various cell types as intact sheets, along with deposited extracellular matrix, can be achieved by reducing the culture temperature.

[50, 51] must be followed. To create these surfaces, the temperature-responsive polymer, poly(*N*-isopropylacrylamide) (PIPAAm), is covalently grafted onto normal tissue-culture polystyrene (TCPS) dishes with radical polymerization. The PIPAAm-grafted culture surfaces provide controlled cell adhesion with simple temperature changes by exploiting the property changes of the polymer at the lower critical solution temperature (LCST) of 32°C. In culture conditions at 37°C, the surface is slightly hydrophobic, allowing cells to adhere and grow similarly to normal TCPS dishes. After the necessary culture period, the temperature is lowered to 20°C, causing the polymer to become hydrophilic. Under these conditions, the polymer swells and a hydration layer is created at the surface-cell interface and the cells are detached spontaneously, enabling them to be harvested as intact sheets [52] (Fig. 6). During cell harvesting, PIPAAm remains on the culture dish surface because the temperature-responsive polymer is covalently bonded to the dish. Normally in cell-based therapies, including tissue engineering, cells are grown in TCPS dishes and harvested using proteolytic enzymes, such as trypsin or dispase to degrade the adhesive molecules and ECM that the cells are attached to. However, treatment with these enzymes also degrades the surface-cell molecules, including growth factor receptors, ion channels, and cell-to-cell junction proteins that are vital for the differentiated functions of the cells. Using temperature-responsive surfaces, the need for proteolytic enzymes is avoided and crucial cell-to-cell and cell-to-ECM interactions are preserved [52].

Using this noninvasive cell-harvesting method, intact cell sheets can be recovered along with their deposited ECM on the basal surface. Cells harvested in this fashion can also be transferred to other surfaces, such as new cultural dishes [53, 54], other cell sheets [55] and even directly to host tissue [56–59]. The deposited ECM acts as a “molecular glue,” providing direct contact and adhesion to these surfaces without the need for additional mediators, such as carrier substrates or sutures.

Tissue reconstruction in the body can be accomplished in many ways using cell-sheet engineering. It can be transplanted as single cell sheets, for use as corneal surfaces [57, 58] (Fig. 7) and periodontal ligament tissue [60], as well as in the reconstruction of the bladder [59] and skin [61], using two-dimensional manipulation. It can be used to recreate three-dimensional structures by homotypic layering of cell sheets, as in the case of cardiac

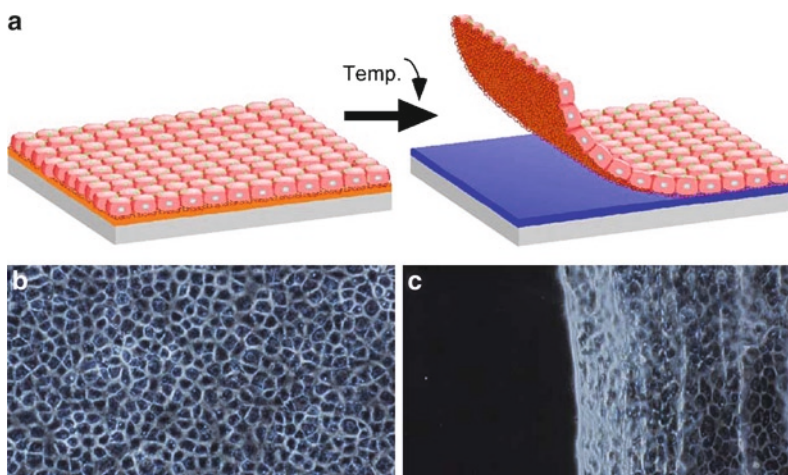


Fig. 6. Cell-sheet engineering using PIPAAm-grafted surfaces. (a) Schematic illustration for temperature-induced recovery of intact monolayer cultures. (b) Confluent culture of endothelial cells on PIPAAm-grafted dishes at 37°C. (c) Detaching endothelial cell sheet by lowering culture temperature to 20°C.

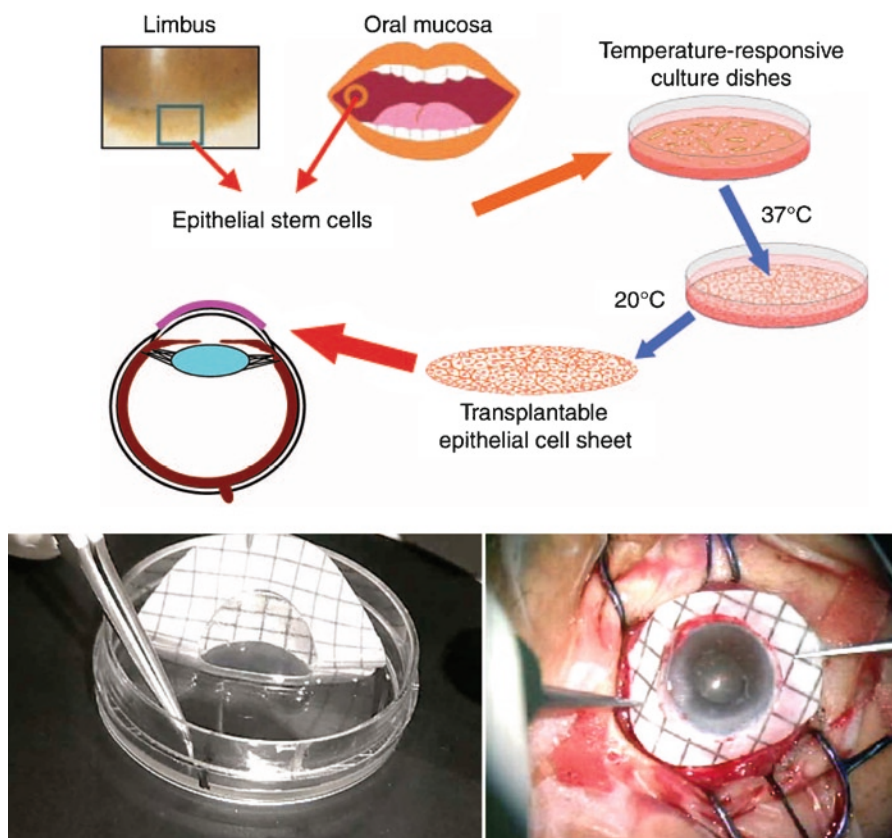


Fig. 7. Corneal surface reconstruction. Small biopsies from the limbus (the border between the cornea and neighboring conjunctiva) or from oral mucosa provide for the isolation of epithelial stem cells. Cell sheets fabricated on temperature-responsive culture dishes are harvested and transplanted directly to the ocular surface without the need for carrier substrates or sutures.

muscle [56] (Fig. 8), and it can be formed into laminar structures to recreate liver lobules [55] or kidney glomeruli by using heterotypic stratification of different cell sheets.

Using cell sheets, various cell-dense tissues that demonstrate differentiated functions while avoiding the need for biodegradable scaffolds, whose applicability is strictly limited can be engineered.

Intelligent Surfaces

Based on the intelligent surface properties of temperature-responsive culture dishes, cell-sheet engineering has been used to reconstruct various tissues without the need for biodegradable scaffolds. These temperature-responsive surfaces can be expanded for other applications.

Immobilization of Cell-Adhesive Peptides

Currently, cell culturing methods generally use animal-derived products, such as fetal bovine serum or mouse 3T3 feeder cells, to enhance cell attachment and growth. However, due to safety issues attributed to the risk of pathogen transmission, the use of these products is best avoided.

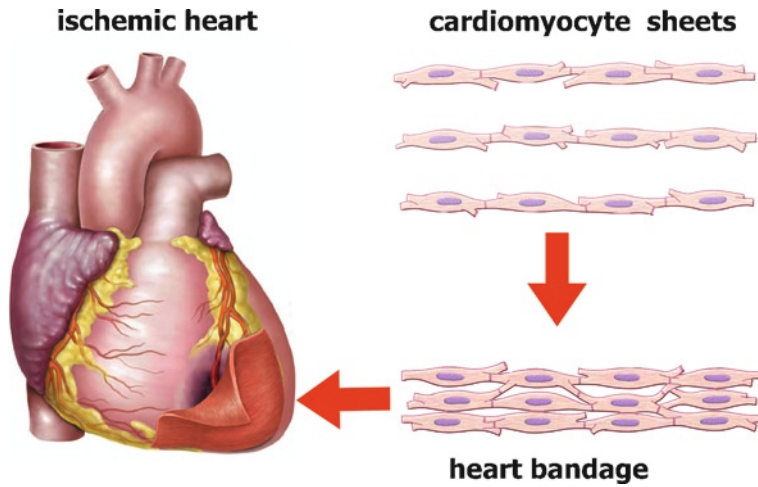


Fig. 8. Myocardial cell-sheet engineering. Cardiomyocyte sheets harvested from temperature-responsive culture surfaces and layered to form three-dimensional tissues that beat synchronously and simultaneously. The layered cardiomyocyte sheets can act as a “heart bandage” for the recovery of ischemic cardiac tissue.

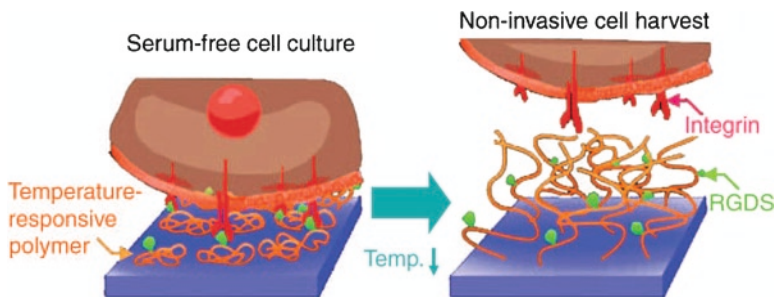


Fig. 9. Immobilization of Arg-Gly-Asp-Ser (RGDS) peptides to temperature-responsive surfaces. Cells can be cultured in serum-free conditions by immobilizing the synthetic cell-adhesive RGDS peptide to temperature-responsive culture dishes. By decreasing the culture temperature, the cells can still be noninvasively harvested, while the RGDS peptides remain attached to the temperature-responsive polymer surface.

Additionally, the US Food and Drug Administration classifies tissue-engineered constructs cocultured with animal cells, such as mouse 3T3 cells as xenografts, thus delaying their clinical application.

To overcome the need for serum, we immobilized the synthetic adhesive peptide Arg-Gly-Asp-Ser (RGDS) onto temperature-responsive dishes. The temperature-responsive PIPAAm surfaces were functionalized by copolymerization with a reactive comonomer containing a free carboxyl group, after which the synthetic RGDS peptide is immobilized on the temperature-responsive surface [62, 63]. Cells attach, spread, and grow to confluency at 37°C, even in serum-free conditions supplemented with recombinant growth factors. After reaching confluency, cells can be harvested as intact sheets by simple temperature changes, in the same manner as normal PIPAAm dishes. Cells cultured on these dishes in serum-free conditions resemble cells cultured in a medium containing 10% fetal bovine serum. In addition, cell harvest from these surfaces is achieved by controlling the binding affinity between the RGDS ligand and cell-surface integrin proteins, with the RGDS ligand remaining on the culture surface after cell sheet removal (Fig. 9).

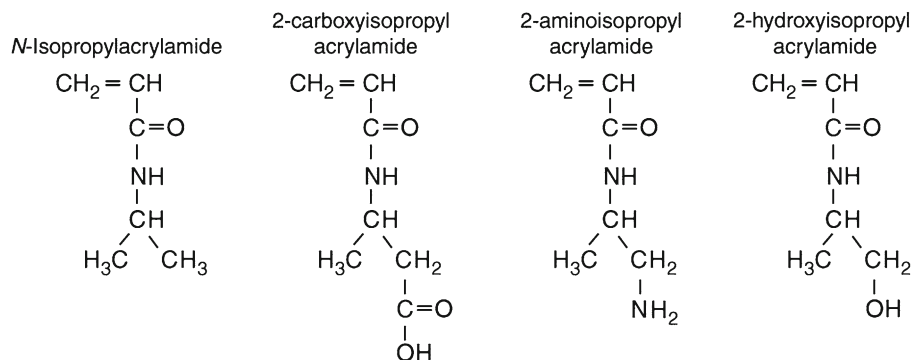


Fig. 10. Synthesis of functionalized temperature-responsive copolymers with various functionalized comonomers, such as 2-carboxyisopropyl acrylamide, 2-aminoisopropyl acrylamide, and 2-hydroxyisopropyl acrylamide. These comonomers contain the isopropylacrylamide backbone chain of poly(*N*-isopropylacrylamide) (PIPAAm) and reactive pendant groups. These functionalized PIPAAm derivatives show sensitive phase transitions in response to temperature change similar to PIPAAm, enabling the immobilization of various ligands to the temperature-responsive surfaces.

Currently, work is under way on immobilizing other growth factors onto temperature-responsive surfaces. The copolymerization with other functionalized comonomers containing amino or hydroxyl groups enable the attachment of various ligands (Fig. 10). This eliminates the need for other animal-derived products, such as feeder cells to increase cell growth, such that, the length of culture can be dramatically decreased.

Micropatterned Surfaces

To mimic normal function, it is necessary to recreate a 3D tissue architecture with the integration of multiple cell types. As shown with liver reconstruction, interactions between various cell types are needed to preserve differentiated cell functions. Layering of various patterned cell sheets is thought to be able to create structures that resemble normal tissues. However, the creation of micropatterned surfaces is generally very complex and must exploit differences in cell adhesion for coculture, thus significantly limiting applicability. Therefore, a novel method was created for micropatterned cell seeding [64, 65].

Using metal micropatterned masks, PIPAAm was selectively grafted onto TCPS dishes to create surfaces controlled by localized temperature-responsive regions. Hepatocytes seeded at 20°C, a temperature at which PIPAAm-grafted portions are hydrophilic, attached only to ungrafted TCPS portions and conform to the pattern. After 5 h, the culture temperature is raised to 37°C, and fibroblasts seeded at this time are only attached to the PIPAAm-grafted regions, creating and maintaining a micropatterned coculture system. This technique conceivably enables the facile design and creation of micropatterned cell sheets that can be manipulated to create 3D structures that mimic normal tissues, if the surfaces are grafted with different temperature-responsive polymers (Fig. 11).

It was shown that by incorporating a hydrophobic monomer, *n*-butyl methacrylate, into the PIPAAm feed, the LCST of the copolymers can be systematically lowered [66]. Using this technique, it is possible to create micropatterned surfaces with different temperature-responsive domains across the entire culture surface to provide the intact harvest of cell sheets with various cell types. This approach can be used to create cell sheets consisting of cardiomyocytes or hepatocytes cocultured with endothelial cells that mimic microvascular networks within the tissues.

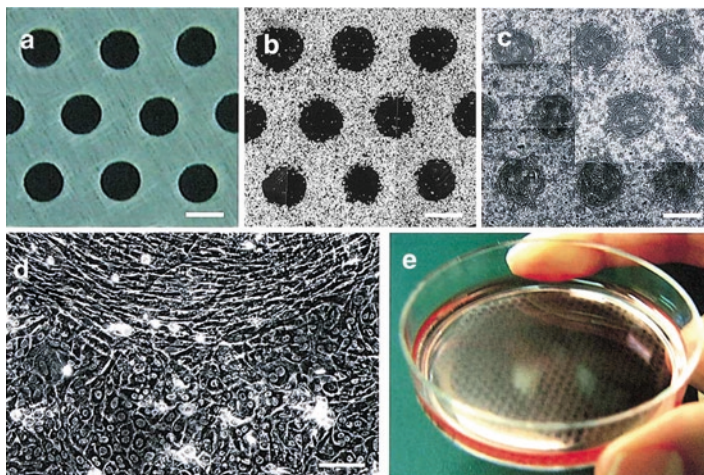


Fig. 11. Patterned co-culture of hepatocytes and fibroblasts. (a) Metal patterned mask having circular holes (1 mm in diameter). (b) Hepatocytes are seeded and cultured on PIPAAm-pattern-grafted dishes for 5 h at 20°C, then spread at 37°C only on nongrafted TCPS domains. (c) The second cell type, fibroblasts, are seeded at 37°C, and cocultured in an organized pattern. (d) Magnified image of the border of two domains after a 7-day culture at 37°C. Note the fibroblast orientation around the circumferences of nongrafted domains. Bar 1 mm in (a)–(c), 100 μ m in (d). (e) Macroscopic view of pattern-seeded hepatocytes before the second cell seeding. Circular domains spread all over the 60-mm dish surfaces.

Although the field of tissue engineering has made significant advances during the past 20 years, there still remains considerable difficulty in recreating tissues and organs because of the limitations of traditional scaffold-based methods. Cell-sheet engineering, which utilizes temperature-responsive intelligent surfaces, should overcome many of the problems that have limited conventional approaches in the past and establish a new basis for regenerative medicine.

Design of Network Structure for Functional Gels

To design gels with unique property and function, several attempts to create unique network structure in nanoorder scale by molecular and supramolecular design have been done.

Topological Gels, Double Network Structure Gels, Nanocomposite Gels

Polyrotaxane has a supramolecular structure in which many cyclic molecules, α -cyclodextrin (α -CD), are threaded on a single polymer chain, poly(ethylene glycol) (PEG), and are trapped by capping the chain with bulky end group. Ito et al. [67] chemically crosslinked α -CDs contained in a polyrotaxane and developed a gels with the polymer topologically interlocked by figure-of-eight crosslinks. The figure-of-eight crosslinks can pass the polymer chains freely to equalize the tension of the threading polymer chains just like pulleys. Therefore, stress may be automatically relaxed in the gels. As a result, transparent gels with good tensility, low viscosity, and large swelling ability in water are obtained. Considering these properties, application to biomaterials, such as a soft contact lens and an artificial joint, should be possible.

Since gels are generally weak and fragile, the application area is restricted. To solve this disadvantage, Osada et al. [68] developed gels with extremely high mechanical strength

by preparing interpenetrating polymer network (IPN, or double network (DN)) structures. The DN gels consisting of PAMPS and PAAm showed very high mechanical strength to compression or cutting. Haraguchi et al. [69] have found out that physical properties are improvable by constructing an organic-inorganic network at nanolevels. Nanocomposite hydrogels (NC gels) composed of PNIPAAm in which inorganic clay (Na-hectrite) forms physical crosslinking points was synthesized. In the NC gels, the distance between crosslinking points are long and uniform, as a result that the polymer behaves like a linear polymer and the gels has extraordinary mechanical, optical, and swelling/deswelling properties.

Graft Gels

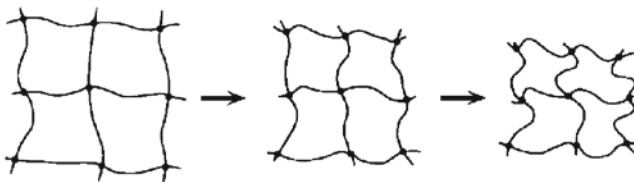
Several potential applications of stimuli-responsive gels, such as smart actuators, when a fast response is needed. The kinetics of swelling and deswelling in these gels are typically governed by diffusion-limited transport of the polymeric components of the network in water, the rate of which is inversely proportional to the square of the smallest dimension of the gels. Several strategies have been explored to increase the response dynamics, such as controlling the size and shape of gels or introducing porosity to control macroscopic structure.

On the other hand, molecular designs for rapid response by tailoring the gels architecture at the molecular level attract is attracting attention. We prepared a thermosensitive gels with a comb structure by grafting PNIPAAm chains onto the crosslinked networks of the same PNIPAAm (Fig. 12) [70, 71]. Within the gels, terminally grafted chains have freely mobile ends, distinct from the typical network structure in which both ends of the PNIPAAm chains are crosslinked and relatively immobile. With increasing temperature, grafted PNIPAAm chains begin to collapse from their expanded (hydrated) form to compact (dehydrated) forms. This collapse occurs before the PNIPAAm network begins to shrink, because of the mobility of the grafted chains. The grafted polymer chains dehydrate to create hydrophobic nuclei which enhance aggregation of the crosslinked chains. Whereas similar gels lacking the grafted side chains take more than a month to undergo full deswelling; the graft gels collapse in about 20 min (Fig. 12). As the grafted chains are lengthened the deswelling rate becomes faster. To control the response, the introduction of other grafted polymer chains, such as PEG or the copolymer of NIPAAm and dimethylacrylamide, to change the hydrophilicity and the phase transition temperature of grafted chains [72].

Microfabrication of Gels

Application to micromachines, μ -TAS: Microfabrication technology, such as photolithography or X-ray lithography, can be employed in the preparation of microgels. Since any shape of gels can be created by these methods, applications to micromachines and μ -TAS such as soft microactuator, microgel valve, and gels displays [73], can be made. Ito et al. synthesized thermosensitive polymer with photo-reactive groups by introducing azidoaniline into the -COOH site in the copolymer of NIPAAm and acrylic acid (AAc) [74]. After coating the polymer on a glass plate, it was irradiated with UV Light through a photo-mask. By removing the unreacted polymer, a micropatterned microgel was obtained. By this method, the lattice-shaped PNIPAAm gels with a quick response was prepared with a specific surface area. Beebe et al. [75, 76] coated the pH-sensitive gels (AAc/HEMA copolymer gels) around a post in microchannel by photolithography, and designed the microgel valve to change the direction of flow in the microchannel in response to the pH of the fluid. At higher pH, the gels swells and stops the flow to the right direction, but allows the flow by deswelling at lower pH.

The structures made by photolithography are two-dimensional, using microfabrications of gels with three-dimensional structure are being attempted. Two-photon initiated

Homopolymer gel**Comb-type graft polymer gel**

○ : Hydrophobic cluster

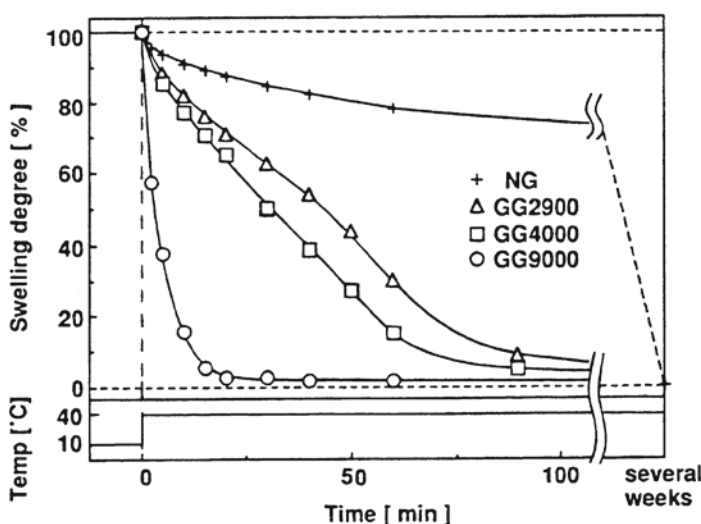


Fig. 12. (Top) Structure and shrinking mechanisms for conventional homopolymer and comb-type grafted PNI-PAAm gels undergoing temperature-induced collapse in aqueous media (bottom). The time course of deswelling of the hydrogels undergoing shrinking, at 40°C, in response to stepwise temperature changes from 10°C.

polymerization was applied to prepare micromachines and integrated circuits for chemical operation with 3D-structure. This method was used to fabrication of microgels and microcantilevers made of photo-responsive gels to deflect under illumination [77].

Self-Oscillating Gels as Novel Biomimetic Materials

Autonomous oscillation is one of the characteristic behaviors in living systems that spontaneously changes with temporal periodicity (called “temporal structure”) such as heartbeat, brain waves, pulsatile secretion of hormone, cell cycle, biorhythm, and so forth, are examples. From the standpoint of biomimetics, several stimuli-responsive polymer systems have been studied, but the polymer systems undergoing self-oscillation under constant condition without any on–off switching of external stimuli are not available. If such autonomous

polymer systems like a living organism could be realized, by using completely synthetic polymers, then unprecedented biomimetic materials will be created.

In order to realize the autonomous polymer system by tailor made molecular design, we focused on the Belousov–Zhabotinsky (BZ) reaction [78–80], which is well-known for exhibiting temporal and spatiotemporal oscillating phenomena. The BZ reaction is often analogically compared with the TCA cycle (Krebs cycle), which is a key metabolic process taking place in the living body. The overall process of the BZ reaction is the oxidation of an organic substrate, such as malonic acid (MA) or citric acid, by an oxidizing agent (bromate ion) in the presence of a strong acid and a metal catalyst. In the course of the reaction, the catalyst undergoes spontaneous redox oscillation. When the solution is homogeneously stirred, the color of the solution periodically changes, like a neon sign, based on the redox changes of the metal catalyst. When the solution is cast as a thin film in stationary conditions, concentric or spiral wave patterns develop. The oxidation wave propagates in the medium at a constant speed and is called a “chemical wave.”

Design of Self-Oscillating Gels

We attempted to convert the chemical oscillation of the BZ reaction to the mechanical changes of gels and generate an autonomic swelling–deswelling oscillation under nonoscillatory outer conditions. A copolymer gels which consists of NIPAAm and ruthenium tris(2,2'-bipyridine) ($\text{Ru}(\text{bpy})_3^{2+}$) was prepared. $\text{Ru}(\text{bpy})_3^{2+}$, acting as a catalyst for the BZ reaction, was appended to the polymer chains of NIPAAm (Fig. 13). The poly(NIPAAm-co- $\text{Ru}(\text{bpy})_3^{2+}$)

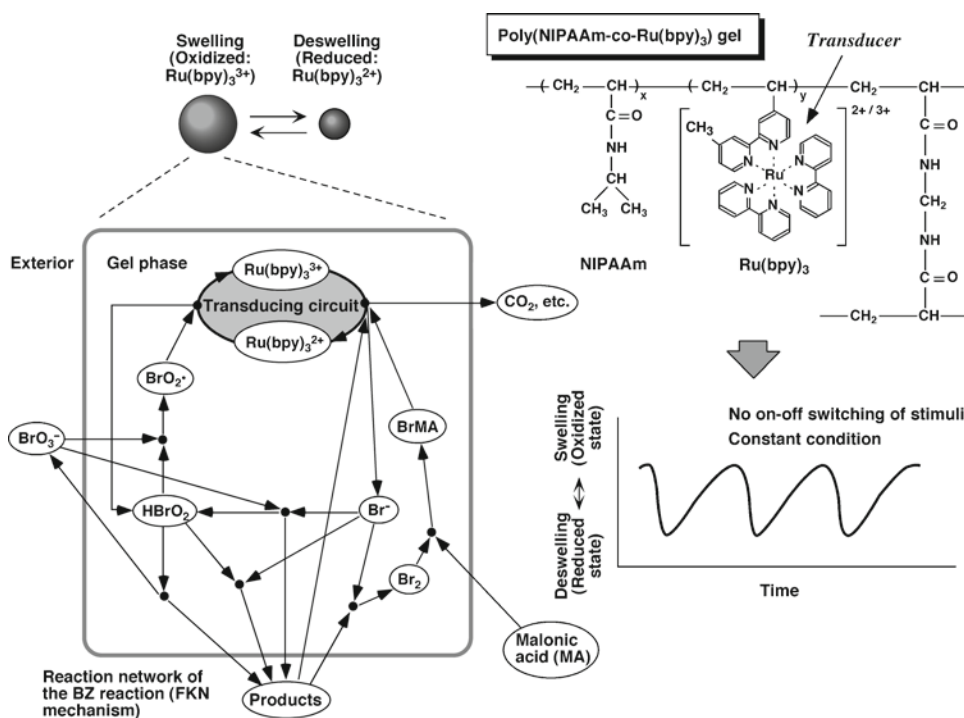


Fig. 13. Mechanism of self-oscillation for poly(NIPAAm-co- $\text{Ru}(\text{bpy})_3^{2+}$) gels coupled with the Belousov–Zhabotinsky reaction.

gels has a phase transition temperature due to the thermosensitive constituent NIPAAm. The oxidation of the $\text{Ru}(\text{bpy})_3^{2+}$ moiety caused not only an increase in the swelling degree of the gels, but also a rise in the transition temperature. These characteristics may be interpreted by considering an increase in hydrophilicity of the polymer chains due to the oxidation of $\text{Ru}(\text{II})$ to $\text{Ru}(\text{III})$ in the $\text{Ru}(\text{bpy})_3$ moiety. As a result, it is expected that the gels undergoes a cyclic swelling–deswelling alteration when the $\text{Ru}(\text{bpy})_3$ moiety is periodically oxidized and reduced under constant temperature. When the gels is immersed in an aqueous solution containing the substrates of the BZ reaction (MA, acid, and oxidant) except for the catalyst, the substrates penetrates into the polymer network and the BZ reaction occurs in the gels. Consequently, periodical redox changes induced by the BZ reaction produce periodical swelling–deswelling changes of the gels (Fig. 13). Since first being reported in 1996 as a “self-oscillating gels” [81, 82], we have been systematically studying the self-oscillating polymer and gels as well as their applications to novel biomimetic materials [83].

Self-Oscillating Behavior of the Gels

Self-Oscillation of the Miniature Bulk Gels

Shown in Fig. 14 is the observed oscillating behavior under a microscope for the miniature cubic poly(NIPAAm-co- $\text{Ru}(\text{bpy})_3^{2+}$) gels (each length of about 0.5 mm). In miniature gels sufficiently smaller than the wavelength of the chemical wave (typically several millimeter), the redox change of ruthenium catalyst can be regarded to occur homogeneously without pattern formation [84]. Due to the redox oscillation of the immobilized $\text{Ru}(\text{bpy})_3^{2+}$, mechanical swelling–deswelling oscillation of the gels autonomously occurs with the same period as for the redox oscillation. The volume change is isotropic and the gels beats as a whole, like a heart muscle cell. The chemical and mechanical oscillations are synchronized without a phase difference (the gels exhibits swelling during the oxidized state and deswelling during the reduced state).

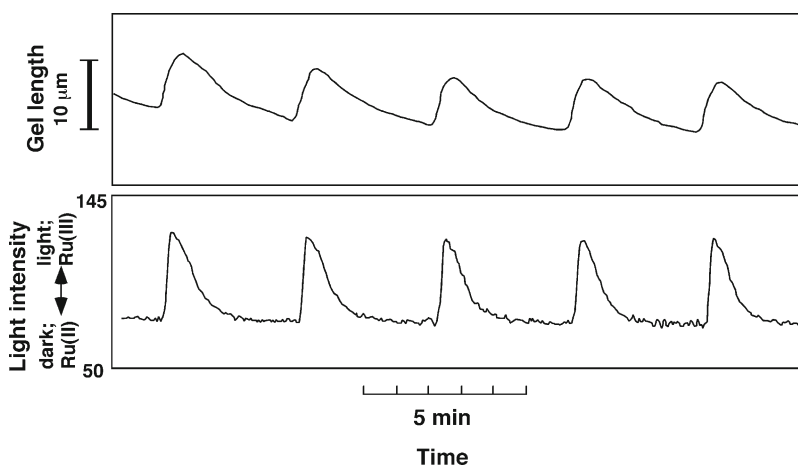


Fig. 14. Periodic redox changes of the miniature cubic poly(NIPAAm-co- $\text{Ru}(\text{bpy})_3^{2+}$) gels (*lower*) and the swelling–deswelling oscillation (*upper*) at 20°C. Color changes of the gels accompanied by redox oscillations (orange: reduced state, light green: the oxidized state) were converted to 8-bit grayscale changes (dark: reduced, light: oxidized) by image processing. Transmitted light intensity is expressed as an 8-bit grayscale value. Outer solution: $[\text{MA}] = 62.5 \text{ mM}$; $[\text{NaBrO}_3] = 84 \text{ mM}$; $[\text{HNO}_3] = 0.6 \text{ M}$.

Control of Oscillation Period and Amplitude

Typically, the oscillation period increases with a decrease in the initial concentration of substrates. The swelling–deswelling amplitude of the gels increases with an increase in the period and amplitude of the redox changes. Therefore, the swelling–deswelling amplitude of the gels is controllable by changing the initial concentration of substrates.

On–Off Regulation of Self-Beating Motion

Inherently, in a BZ reaction there is an abrupt transition from steady state (nonoscillating state) to oscillating state with a change in any controlling parameter, such as chemical composition or light. Utilizing this characteristic, reversible on–off regulation of self-beating triggered by the addition and removal of MA was successfully achieved [85]. Also, since the NIPAAm gels is thermosensitive and so the beating rhythm can be also controlled by temperature [86].

Peristaltic Motion of Gels with Propagation of Chemical Wave

When the gels size is larger than chemical wavelength, the chemical wave propagates in the gels by coupling with diffusion of intermediates. Then peristaltic motion of the gels is created. Shown in Fig. 15 is a cylindrical gels immersed in an aqueous solution containing the BZ reactants. The chemical waves propagate in the gels at a constant speed in the direction of the gels length [87]. Considering the orange (Ru(II)) and green (Ru(III)) zones represent the shrunken and swollen parts, respectively, the locally swollen and shrunken parts move with the chemical wave, like the peristaltic motion of living worms. The tensile force of the cylindrical gels with oscillation was also measured [88].

It is well known that the period of oscillation is affected by light illumination for the Ru(bpy)₃²⁺-catalyzed BZ reaction [89]. Therefore, we make a pacemaker with a desired period (or wavelength) by local illumination of laser beam to the gels. The period (or wavelength) can be changed by local illumination to a pacemaker that already exists in the gels. Chemical and optical control of the self-sustaining peristaltic motion of the porous structural gels are possible [90–93].

Design of Biomimetic Micro-/Nanoactuator Using Self-Oscillating Polymers and Gels

Self-Walking Gels

Further, we successfully developed a novel biomimetic walking-gels actuator made of self-oscillating gels [94]. To produce directional movement gels, asymmetrical swelling–deswelling is desired. For these purposes, as a third component, hydrophilic 2-acrylamido-2-methyl-propanesulfonic acid (AMPS) was copolymerized into the polymer to lubricate the gels and to cause anisotropic contraction. During polymerization, the monomer solution faces two different hydrophilic glass surface and a hydrophobic Teflon surface; since Ru(bpy)₃²⁺ monomer is hydrophobic, it easily migrates to the Teflon surface. As a result, a nonuniform distribution along the height is formed by the components, and the resulting gels gradient distribution component in the polymer network.

To convert the bending and stretching changes in one-directional, we fabricated a ratchet with an asymmetrical surface structure. The gels was repeatedly bent and stretch autonomously on the ratchet base, resulting in the forward motion of the gels, while backward

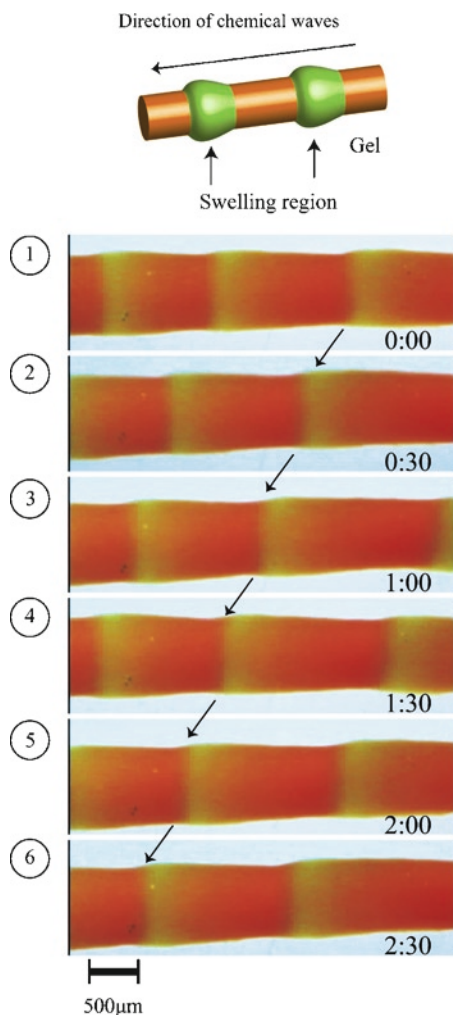


Fig. 15. Time course of peristaltic motion of poly(NIPAAm-co-Ru(bpy)₃²⁺-co-AMPS) gels in a solution of the BZ substrates (MA, sodium bromate, and nitric acid, 18°C). The green and orange colors correspond to the oxidized and reduced states of the Ru moiety in the gels, respectively.

movement was prevented by the teeth of the ratchet. Successive profiles of the “self-walking” motion of the gels is shown in Fig. 16. The walking velocity of the gels actuator was approximately 170 μm/min. Since the oscillating period and the propagating velocity of the chemical wave changes with substrate concentration in the outer solution, the walking velocity of the gels can be controlled. By using the gels with a gradient structure, another type of actuator that generates a pendulum motion was also developed [95].

Design of mass transport surface utilizing peristaltic motion of gels: An attempt was made to transport an object by utilizing the peristaltic motion of poly(NIPAAm-co-Ru(bpy)₃-co-AMPS) gels. As a model object, a cylindrical poly(acrylamide) (PAAm) gels was put on the gels surface. It was observed that the PAAm gels was transported on the gels surface with the propagation of the chemical wave as it rolled (Fig. 17) [96]. A model was proposed to describe the mass transport phenomena based on the Hertz contact theory, based on the relation between the transportability and the peristaltic motion. The functional gels surface

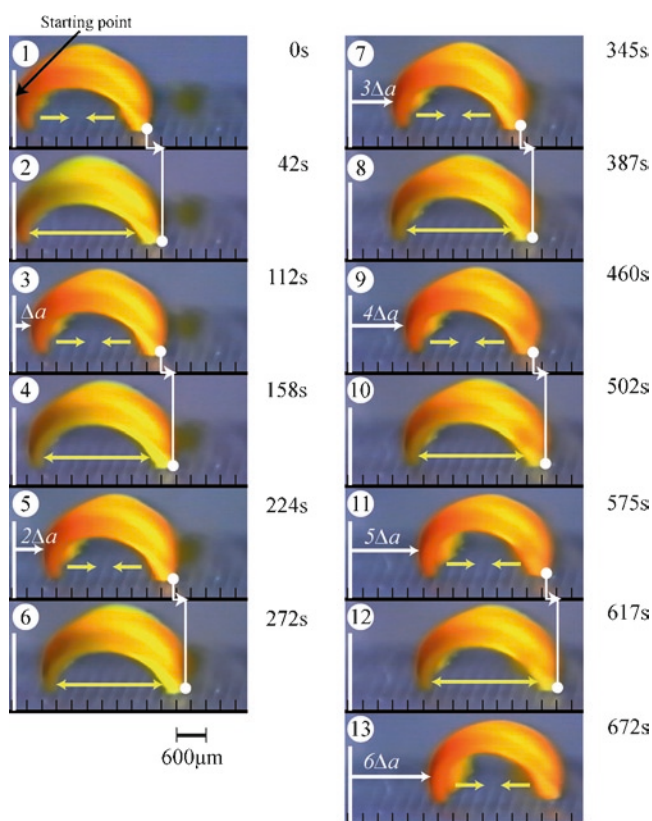


Fig. 16. Time course of self-walking motion of the gels actuator. During stretching, the front edge can slide forward on the base, but the rear edge is prevented from sliding backwards. Oppositely, during bending, the front edge is prevented from sliding backwards while the rear edge can slide forward. This action is repeated, and as a result, the gels walks forward. Outer solution: $[MA]=62.5$ mM, $[NaBrO_3]=84$ mM, $[HNO_3]=0.894$ M, 18°C .

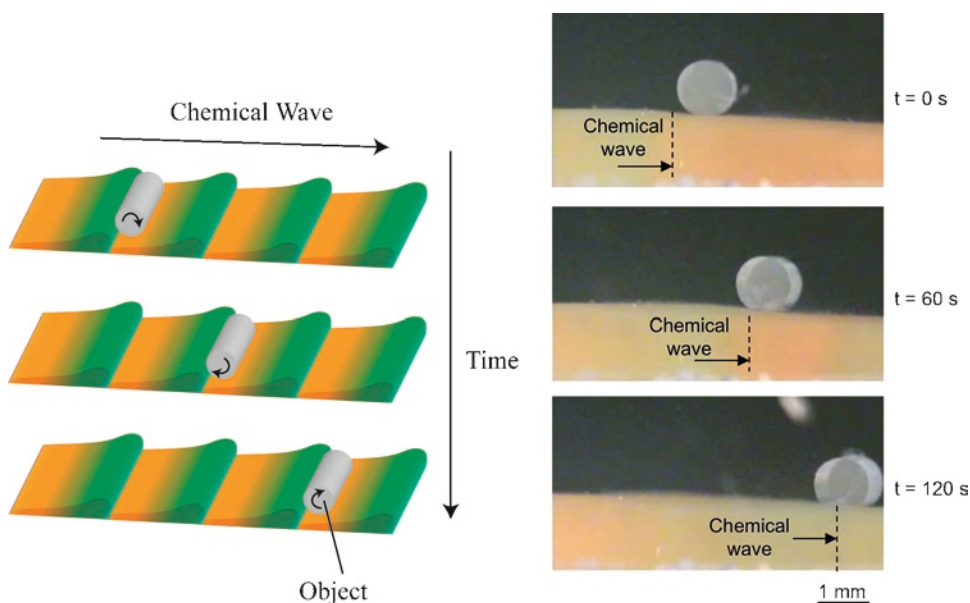


Fig. 17. Schematic illustration of mass transport on the peristaltic surface (*left*) and observed transport of cylindrical PAAM gels on the poly(NIPAAm-co-Ru(bpy)₃²⁺-co-AMPS) gels sheet.

generating autonomous and periodic peristaltic motion has potential for several applications such as to transport soft materials, formation of ordered structures of micro- and/or nanomaterials and a self-cleaning surface.

Microfabrication of the Gels by Lithography

Microfabrication of self-oscillating gels by lithography for application to ciliary motion actuator (artificial cilia) [97]. The gels membrane with micro projection array on the surface was fabricated by utilizing X-ray lithography (LIGA) method. With the propagation of chemical wave, the micro projection array exhibits dynamic rhythmic motion like cilia. The actuator may also serve as a microconveyer.

Control of Chemical Wave Propagation in Self-Oscillating Gels Array

A chemo-mechanical actuator utilizing a reaction-diffusion wave across the gap junction was constructed to make a new microconveyer by micropatterned self-oscillating gels array [98]. Unidirectional propagation of the chemical wave the BZ reaction was induced on gels arrays. Using a triangle-shaped gels as an element of the array, the chemical wave is propagated from the corner side of the triangle gels to the plane side of the other gels (C-to-P) across the gap junction, whereas, it propagated from the plane side to the corner side (P-to-C) in the case of the pentagonal gels array (Fig. 18). By fabricating different shapes of gels arrays, control of the direction is possible. The swelling and deswelling changes of the gels follow a unidirectional propagation of the chemical wave.

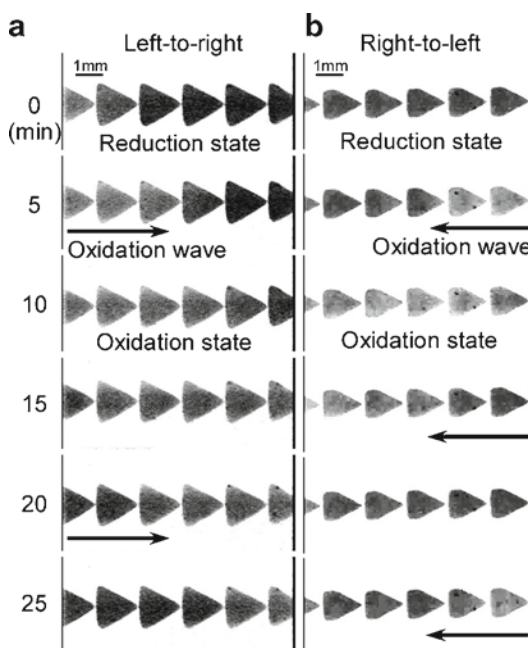


Fig. 18. Propagating behavior of the chemical wave on the (a) triangle gels array and (b) pentagonal gels array.

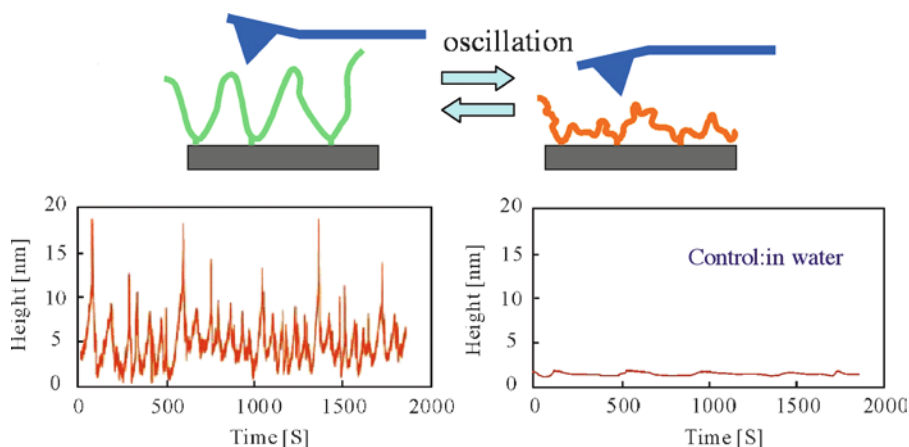


Fig. 19. Self-oscillating behavior of immobilized polymer in the BZ substrate solution ($[MA]=0.1$ M, $[NaBrO_3]=0.3$ M, $[HNO_3]=0.3$ M) measured by AFM.

Self-Oscillating Polymer Chains as a “Nano-oscillator”

The periodic changes of linear and uncrosslinked polymer chains can be easily observed as cyclic transparent and opaque changes for the polymer solution with color changes due to the redox oscillation of the catalyst [99]. Synchronized with the periodical changes between Ru(II) and Ru(III) states of the $Ru(bpy)_3^{2+}$ site, the polymer becomes hydrophobic and hydrophilic, and exhibits cyclic soluble–insoluble changes. Further, by grafting the polymers or arraying the gels beads on the surface of substrates, self-oscillating surfaces as nanoconveyers were designed. The self-oscillating polymer was covalently immobilized on a glass surface and self-oscillation was directly observed at a molecular level by AFM [100]. The self-oscillating polymer with *N*-succinimidyl group was immobilized on an aminosilane-coupled glass plate. While no oscillation was observed in pure water, nanoscale oscillation was observed in an aqueous solution containing the BZ substrates (Fig. 19). The amplitude was about 10–15 nm and the period was about 70 s, although some irregular behavior was observed due to no stirring. The amplitude was less than that in solution, as observed by DLS (23.9 and 59.6 nm). This smaller amplitude may be due to the structure of the immobilized polymer that was a loop-train-tail: the moving regions were shorter than the soluble polymer, as illustrated in Fig. 19. The amplitude and frequency were controlled by the concentration of reactant in the solution. The oscillation polymer chain may be used as a component for nanoclocks or nanomachines.

Self-Flocculating/Dispersing Oscillation of Microgels

A submicron-sized poly(NIPAAm-co- $Ru(bpy)_3^{2+}$) gels beads were prepared by surfactant-free aqueous precipitation polymerization, and the oscillating behavior analyzed [100–104]. Shown in Fig. 20, the oscillation profiles of transmittance for the microgel dispersions. At low temperatures (20–26.5°C), on raising the temperature, the amplitude of the oscillation became larger. The increase in amplitude is due to increased deviation of the hydrodynamic diameter between the Ru(II) and Ru(III) states. Furthermore, a remarkable change in waveform was observed between 26.5 and 27°C. The amplitude of the oscillations dramatically decreased at 27.5°C, and the periodic transmittance changes

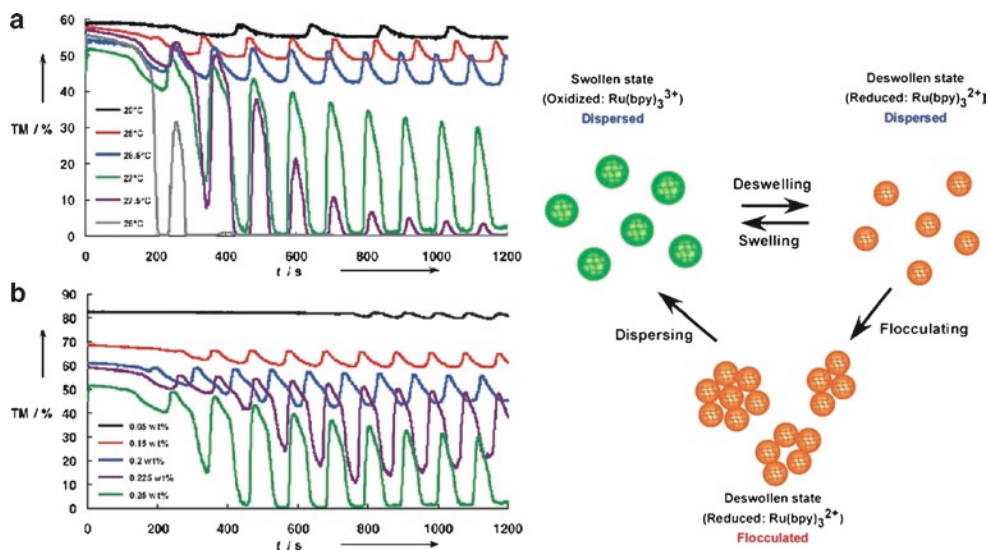


Fig. 20. Self-oscillating profiles of optical transmittance for microgel dispersions. The microgels were dispersed in aqueous solutions containing MA (62.5 mM), NaBrO₃ (84 mM), and HNO₃ (0.3 M). Microgel concentration was 0.25 wt%. (a) Profiles measured at different temperatures. (b) Profiles measured at different microgel dispersion concentrations at 27°C.

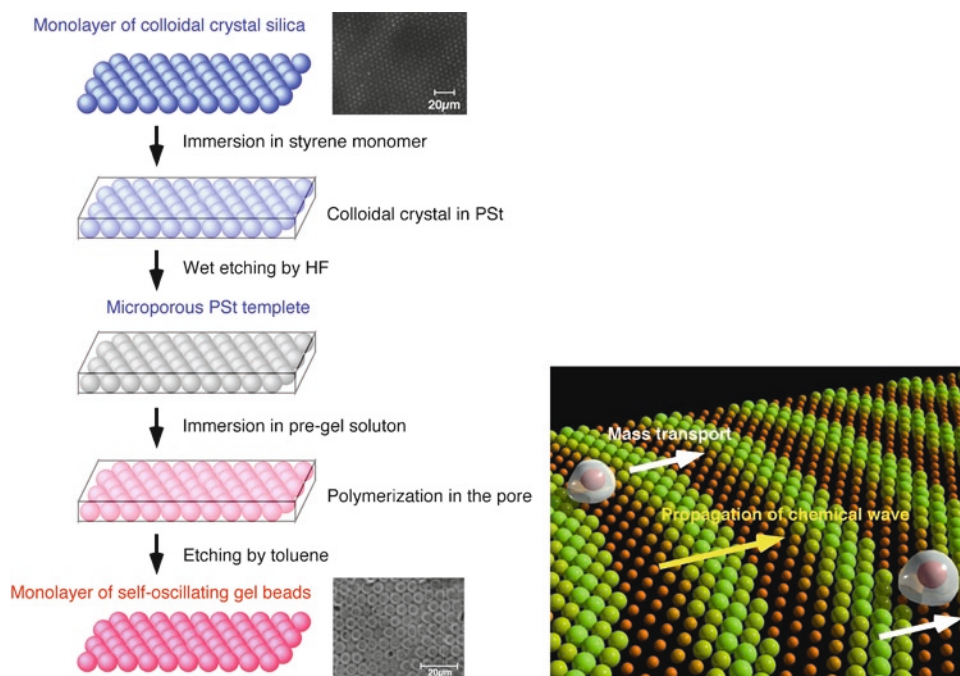


Fig. 21. Preparation of self-oscillating gels beads monolayer by two-step template polymerization and schematic illustration of functional surface (nanoconveyor) using self-oscillating gel beads array. The operating conditions of self-oscillating polymer systems are limited to the nonphysiological environment where the strong acid and the oxidant coexist.

could no longer be observed at 28°C. The sudden change in oscillation waveform should be related to the difference in colloidal stability between the Ru(II) and Ru(III) states. The microgels should fluctuate due to the lack of electrostatic repulsion when the microgels were deswollen. The remarkable change in waveform was only observed at higher dispersion concentrations (greater than 0.225 wt%). The self-oscillating property makes microgels more attractive for future developments such as microgel assembly, optical and rheological applications.

Fabrication of Microgel Beads Monolayer

The construction of micro/nanoconveyers by grafting or arraying self-oscillating polymer or gels beads by fabrication method for organized monolayers of microgel beads was investigated (Fig. 21) [105]. A 2D close-packed array of thermosensitive microgel beads was prepared by double template polymerization. A 2D colloidal crystal of silica beads with 10 μm diameter was obtained by solvent evaporation. This monolayer of colloidal crystal serves as the first template for preparation of macroporous polystyrene. The macroporous polystyrene trapping the crystalline order can be used as a negative template for fabricating a gels bead array. By this double template polymerization method, functional surfaces using thermosensitive PNIPAAm gels beads were fabricated. It was observed that topography

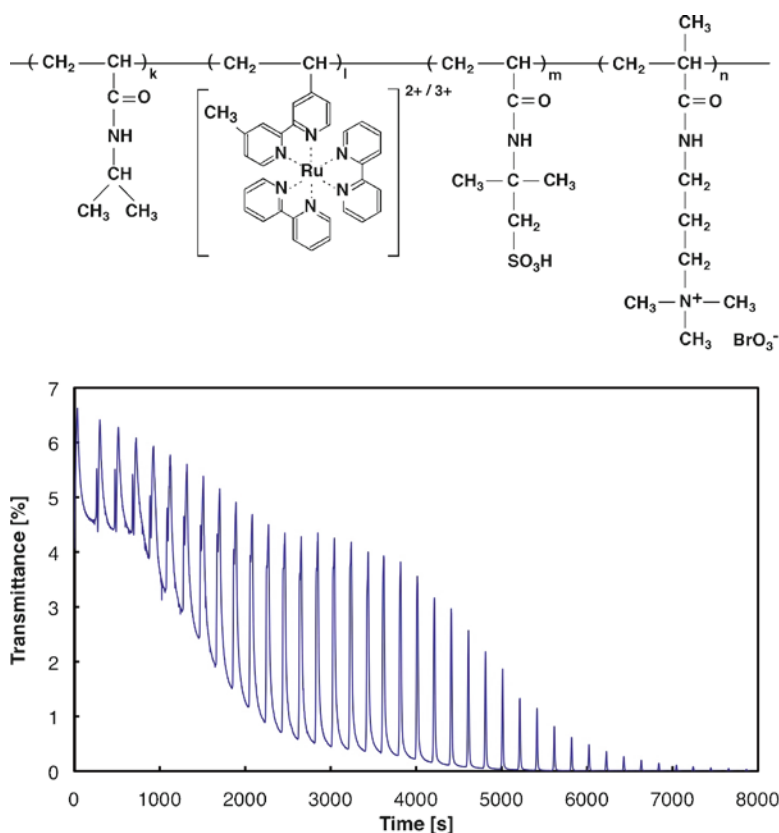


Fig. 22. Chemical structure of poly(NIPAAm-co-Ru(bpy)₃²⁺-co-AMPS-co-MAPTAC) (*upper*) and the oscillating profiles of the optical transmittance for the polymer solution at 12°C when only MA (0.7 M) is added to the solution (*lower*).

of the surface changed with temperature. The fabrication method demonstrated here was so versatile that many kinds of gels beads could be obtained. This method may be a key technology to create new functional surface.

Self-Oscillation Under Physiological Conditions

To extend the application field to biomaterials, more sophisticated molecular designs for self-oscillation under physiological condition are needed. The integrated polymer system was constructed so that all of the BZ substrates, other than biorelated organic substrate, were incorporated into the polymer chain [106–109]. The quarternary copolymer was synthesized that includes both of the pH-control and oxidant-supplying sites in the poly(NIPAAm-co-Ru(bpy)₃) chain at the same time. As a pH-control site, 2-acrylamido-2-methyl-propanesulfonic acid (AMPS) was incorporated for pH-control; methacrylamidopropyltrimethylammonium chloride (MAPTAC) with a positively charged group was incorporated as a capture site for the anionic oxidizing agent (bromate ion). By using this polymer, self-oscillation under biological conditions where only the organic acid (malonic acid) exists was achieved (Fig. 22).

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