NATIONAL INSTITUTE FOR MATERIALS SCIENCE

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2024 No. 4

Research Center for
Macromolecules
and Biomaterials

Flexible Progress to Revolutionize Materials

Flexible Progress to Revolutionize Materials

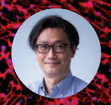
Soft materials are light-weight, flexible, and possess a range of astonishing functionalities.
Through the precise design of polymers,
soft materials can actively move under applied voltage and
adjust their shape in response to temperature or humidity, adapting their properties as needed.
Soft materials play a crucial role in diverse fields from wearable devices that seamlessly fit
the body to medical applications such as artificial cartilage and drug delivery systems.

The potential of soft materials is limitless.
The Research Center for Macromolecules and Biomaterials is advancing the development of materials
to enhance daily life while also striving to extend healthy life expectancy in an aging society.
By integrating polymer materials with metal and inorganic materials,
the center is fostering the development of advanced materials with enhanced functionality.

Aiming for a world where health and comfort ensure happiness for all,
the Research Center for Macromolecules and Biomaterials is committed to creating
the foundations for a brighter, more fulfilling future.

Data: **Research Center for Macromolecules and Biomaterials**

Director : Masayuki Takeuchi



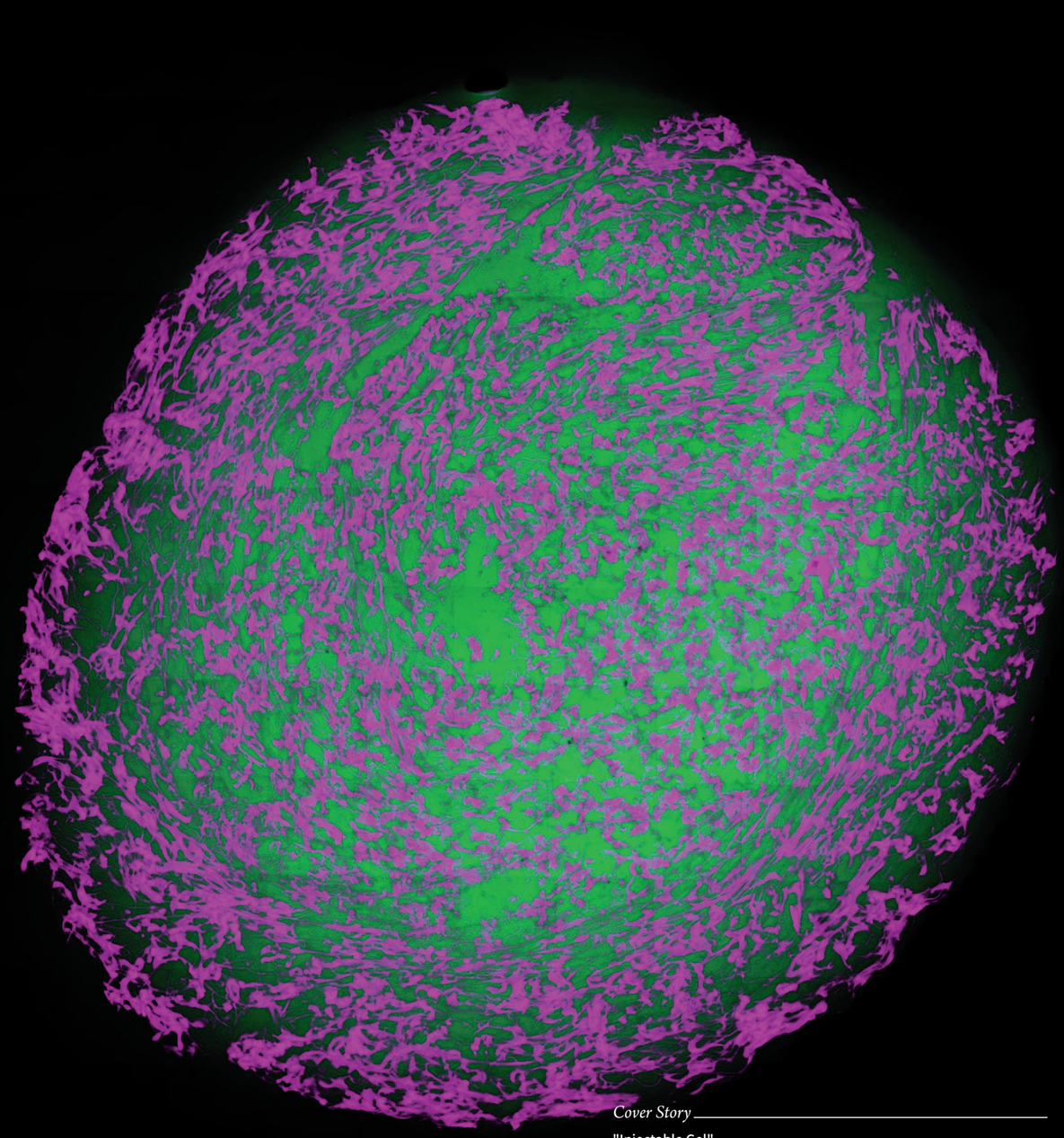
Number of permanent researchers: 54

Macromolecules Field

- Molecular Design and Function Group
- Electronic Functional Macromolecules Group
- Molecular Mechatronics Group
- Printed Electronics Group
- Electrochemical Sensors Group
- Data-driven Polymer Design Group
- Supramolecule/Polymer Materials Team
- Polymer Process Technology Team
- Polymer Surfaces and Devices Team

Biomaterials Field

- Mechanobiology Group
- Medical Soft Matter Group
- Polymeric Biomaterials Group
- Bioceramics Group
- Tissue Regeneration Materials Group
- Smart Polymer Group
- Olfactory Sensors Group
- Electrochemical Nano-Bio Group



These fluorescent microscope images show injectable gels for regenerative medicine, which are composed of three types of gelatin: "GUPy," "GTH," and "GVS." On the right, GUPy (pink) and GTH (green) are stained, while on the left, only the encapsulated "human mesenchymal cells" (red) within the gel are stained. When this gel, containing the encapsulated cells, is injected into the body, GUPy dissolves at body temperature (37°C), creating fiber-like pores that support cell growth (see p.11).

Cover Story

"Injectable Gel"

Known as "injectable gels," these medical gels are designed to remain liquid when filled into a syringe and solidify in response to body temperature or pH once injected.
Akihiro Nishiguchi has been advancing research on injectable gels using a material derived from porcine tendon gelatin modified with UPy groups.* One of the developed materials is a "medical adhesive" (as shown on the cover photo). This adhesive is designed to solidify at body temperature (37°C) by adjusting the number of UPy groups. To use it, the adhesive is first slightly heated to turn it into a liquid. It is then injected into a surgical wound, where it effectively seals the wound and prevents adhesion to the surrounding tissues.
During his research, Nishiguchi also discovered a method to create fiber-like pores within the gel. This innovation is paving the way for the development of injectable gels for regenerative medicine, capable of encapsulating transplanted cells and delivering them into the body (see p.11).

∗ UPy Groups: Ureidopyrimidinone groups

Key Projects

Overview of ongoing projects
at Research Center for Macromolecules and Biomaterials

#1 Fundamental Technologies for Developing Soft Polymer Materials Driving a Material Revolution

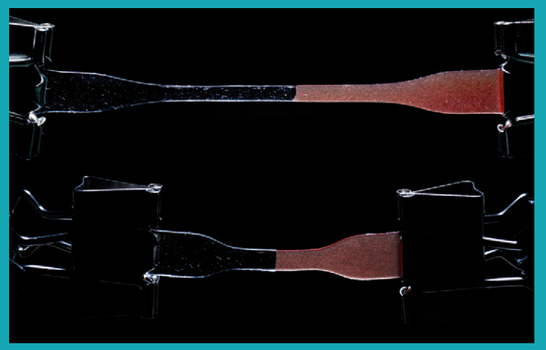
Project leader
Masanobu Naito

Aiming for Social Transformation in the Future: **Unlocking Uncharted Functions through Hierarchical Control of Soft Materials**

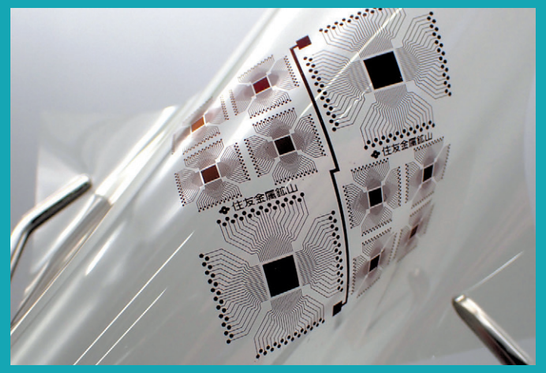
Wearable sensors for monitoring body temperature and heart rate, as well as flexible displays, rely on soft "polymer materials" that have significantly enhanced our lives. To achieve a “material revolution” enabling such advancements, this project focuses on fundamental technologies that seamlessly connect molecular synthesis techniques with device development.

Specifically, we are leveraging advanced molecular synthesis techniques to create a variety of soft materials such as conductive polymers, supramolecular substances, liquid crystal molecules, and complex molecules, exploring their unprecedented properties and functions. Through integration techniques and processes for film formation and device fabrication, we aim to develop materials with innovative functions such as high responsiveness, dynamic properties, ionic conductivity, and self-healing capabilities. This hierarchical control of materials expands the possibilities for soft materials in the future.

See Research 1 on **p. 6** See Research 2 on **p. 8**
See Research 3 on **p. 9**



"Self-Healing Ion Gel" that bonds when the cross-sections of two gels are simply pressed together (see p.8).



Using copper-based "thick-film conductive ink," jointly developed with NIMS and industry, to print electronic circuits on film substrates. Photo provided by Takeo Minari

Towards Realizing Society 5.0 and a Well-being Society: **Pursuing Diverse Aspects of Quality of Life through the Exploration of Applications**

Soft materials have the potential to transform interactions between humans and robots, making them more natural and intuitive. For example, prosthetics covered with flexible electronic skin featuring tactile sensors can provide wearers with a lifelike sensation, as if the prosthetics were part of their bodies. Additionally, self-healing and highly durable materials enhance the reliability of interfaces requiring long-term stability, such as those found in vehicle components. This project not only focuses on functionality but also emphasizes enhancing quality of life by advancing applications in medicine and healthcare. For instance, flexible sensors capable of detecting subtle changes in skin can monitor emotions and biological information, aiding daily health management.

To drive these advancements, we are also developing new analytical technologies using artificial intelligence to reveal the fundamental properties of soft materials derived from complex structures.

See Research 4 on **p. 10**

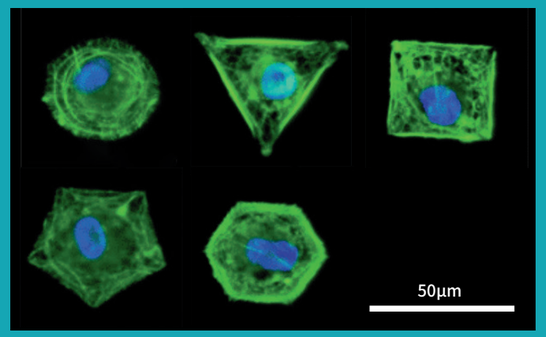
#2 Platform for the Creation of Multilayered Bioadaptive Materials

Project leader
Mitsuhiro Ebara

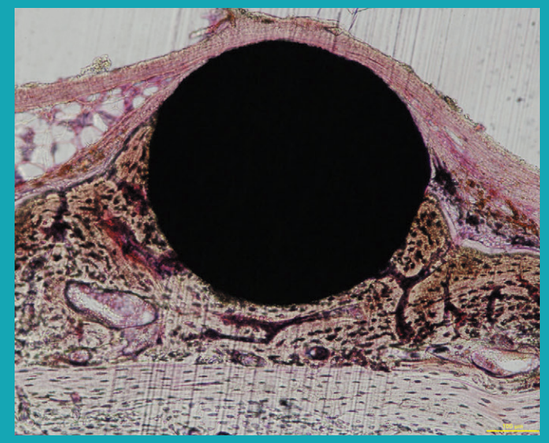
Decoding the 'Dialogue' Between Biologics and Materials: **Understanding the Science of Interactions**

Biological systems—cells, tissues, and blood—are highly complex, changing over time and space. When materials interact with these systems, phenomena occur across multiple layers, from cells to tissues and throughout the body. For example, molecular and ionic attractions lead to the adsorption of proteins onto material surfaces, followed by cell proliferation and eventual integration with biological tissues. NIMS focuses on the creation of 'bioadaptive materials' that enable advanced biological control at multiple layers. These materials actively utilize interactions between biological environments and materials to control biological functions. This project employs cutting-edge bioimaging technologies and data science to conduct complex analyses at the molecular, cellular, and tissue levels. The aim is to deepen the logical understanding of fundamental life phenomena, such as the influence of physical forces on cells and the process of bone remodeling, and to clarify the parameters that materials interacting with these phenomena should possess, paving the way for innovative bio-material designs.

See Research 5 on **p. 11** See Research 6 on **p. 12**
See Research 7 on **p. 14** See Research 8 on **p. 15**



Fluorescent microscope image of human bone marrow-derived mesenchymal stem cells cultured on micropatterned surfaces of equal area but different shapes (green: actin cytoskeleton, blue: cell nuclei). Photo provided by Guoping Chen



"Hydroxyapatite/collagen bone-like nanocomposite (HAp/Col)" coating applied to titanium artificial bone (black), accelerating bone tissue (brown) integration by three times. Photo provided by Masanori Kikuchi

Leading Biological Phenomena with Skillful Innovation: **Innovative Material Creation and Medical-Engineering Collaboration**

For materials to interact with biologics as designed, precise fabrication techniques—such as polymer porosity, particle formation, surface modification, and integration with metals—are essential to program their functions and activation timing. Furthermore, medical polymer materials must be biocompatible, imposing strict design constraints. This project is optimizing materials fabrication techniques and exploring new synthesis methods to ensure that both raw materials and production methods are biologically safe. Additionally, a significant challenge in medical materials lies in the rigorous approval process required before clinical use. The research center is strengthening collaborations with medical institutions and pharmaceutical companies to develop efficient strategies that consider safety and efficacy testing. With a focus on achieving a well-being society, we are promoting materials development with clear goals such as biological sensors, wearable devices, drug delivery, and cell cultivation.

Achieving Flexibility and Output Force with 'Liquid Crystal Polymer Actuators'

Masafumi Yoshio

Group Leader,
Molecular Mechatronics Group

Yoshio examines the response of a ring-shaped device he developed, with Chengyang Liu, a member of his team.

Actuators convert energy, such as electricity or hydraulics, into mechanical motion. With the growing demand for soft actuators, Masafumi Yoshio has been developing innovative materials that harness the unique properties of liquid crystals.

Dilemma Between Softness and High Output Force

The sensation of touching objects far away as if they were right in front of you is made possible by 'haptics,' a tactile technology that conveys touch using force, vibration, or motion. Actuators, essential components of this technology, deliver these tactile experiences. Advanced tactile reproduction could revolutionize fields such as remote medical diagnostics and virtual reality, unlocking new possibilities for enhancing daily life.

As demand grows, actuators, traditionally made of metallic materials, are expected to bend flexibly and precisely control their force. One prominent example is the 'polymer-based film actuator,' which uses a polymer membrane containing ions sandwiched between flexible electrodes. When voltage is applied, the imbalance in ion distribution causes smaller negative ions to move toward the positively charged electrode, bending the membrane in that direction (Fig. 1). This mechanism underpins its functionality.

"Our current challenge is to achieve rapid and large deformations under low voltage. Polymer membranes, however, are composed of long entangled polymer chains, which create resistance as ions move through the material. To address this challenge and enable faster, larger deformations, one potential approach is to increase the ion content in the polymer membrane. Yet, this approach makes the membrane softer, reducing its ability to generate strong force," Yoshio explains.

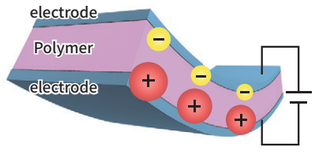


Figure 1. When voltage is applied to the electrodes, the cations in the polymer membrane move toward the cathode, and the anions move toward the anode. Since anions have a smaller ionic radius, the entire membrane bends toward the anode.

Self-Organized Ion Pathways

To maintain flexibility while enhancing the actuator's force output, the material must facilitate efficient ion transport. A critical challenge is creating pathways for ions within the material, which Yoshio tackles using "photo-crosslinked liquid crystal polymers."

Liquid crystal polymers, formed from rod- or disk-shaped molecules, self-organize into ordered yet adaptable structures. Among them, those whose structural arrangement can be fixed with light exposure are known as photo-crosslinked liquid crystal polymers.

"The advantage of liquid crystals lies in their self-organizing ability to create ordered structures. These structures, including columnar and gyroid forms, can be configured to meet specific needs based on their molecular arrangement. Additionally, incorporating photo-crosslinkable properties allows the material to be processed into films, enhancing versatility in device design. Furthermore, simpler manufacturing processes allow for the efficient production of diverse, cost-effective materials," Yoshio notes.

The process of creating ion pathways begins with an "ionic liquid crystal polymer," a type of liquid crystal polymer that promotes ion flow. Through material design, photo-crosslinkable properties can be incorporated into this polymer. Following this, "ionic liquids," which are composed exclusively of positive and negative ions, are mixed into the polymer. Although ionic liquids lack ordered structures on their own, they interact partially and integrate into the liquid crystal's ordered structure. These embedded ionic liquids create efficient pathways for ion conduction.

Yoshio has been investigating ion conductivity in various liquid crystal structures and exploring potential applications. In 2022, he developed a photo-crosslinked liquid crystal polymer actuator based on a columnar structure, which achieved greater flexibility and higher output compared to traditional polymer film actuators (see NIMS NOW Vol.20, No.1, p.12).

High-Speed Ion Transport Between Nanoscale Spheres

In 2024, Yoshio unveiled the "micelle cubic phase photo-crosslinked liquid crystal polymer actuator," capable of rapid deformation under lower voltages. The key to achieving high-speed ion conduction lies in the liquid crystal structure known as the "micellar cubic structure." This structure consists of nanoscale spheres (micelles) that are three-dimensionally interconnected to form a framework, allowing ions to move swiftly between them.

"To realize this structure, wedge-shaped ionic liquid crystal molecules with photo-curable properties were designed and synthesized. By mixing a small amount of ionic liquid with these molecules, enclosing the mixture between glass substrates, and exposing it to light, we obtained a transparent film with a micellar cubic structure made of liquid crystal polymers (Fig. 2)," Yoshio explains.

A noteworthy feature of this structure is the high elasticity of the micelles formed by the wedge-shaped molecules. This elasticity not only ensures efficient ion pathways but also enables increased output force.

"We demonstrated that a film with a thickness of only 300 micrometers could lift a 5 gram weight when a voltage of 2 volts was applied (Fig. 3). This represents a world-class level of output for a soft actuator," Yoshio adds.

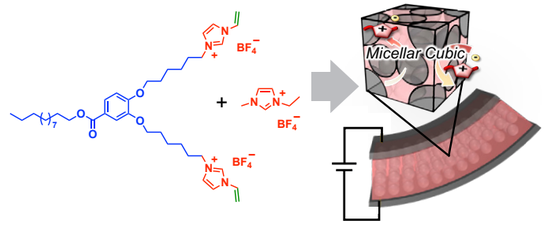


Figure 2. A schematic of the "Micellar Cubic Phase Photo-Crosslinked Liquid Crystal Polymer Actuator." Wedge-shaped ionic liquid crystal molecules (left) are mixed with a small amount of ionic liquid and enclosed between glass substrates, where nanoscale spheres (nanomicelles) align periodically. Upon exposure to light, polymerization connects the nanomicelles three-dimensionally, forming a transparent film. This film is then placed between electrodes to create a soft actuator (Figure 3).

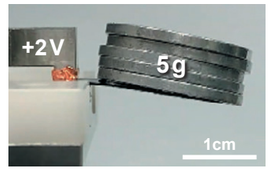


Figure 3. When 2 volts of voltage are applied to the electrodes, the actuator withstands a load equivalent to five 1-yen coins (5 g).

Towards Haptic Devices

Yoshio is further developing liquid crystal polymer membranes by combining ionic liquid crystal molecules with plastics.

"In the micellar cubic structure, ions travel through gaps within the framework. To achieve faster and larger deformations in actuators, we are now focusing on separating the framework from the ion pathways to enhance ion conduction. Additionally, plastics enable the addition of functionalities like heat resistance and self-healing properties, depending on the selected material." Yoshio explains.

By mixing ionic liquid crystal molecules and plastic materials in solution, Yoshio successfully created films that formed layered structures within a polymer network through self-organization (Fig. 4).

"Using this film, we prototyped a ring-shaped haptic device (see p.6 photo and Fig. 4 photo). Key factors in reproducing tactile sensations are the actuator's vibration frequency and the pressure applied to the skin. When humans touch objects, the 'Pacinian corpuscles' in the deeper layers of the skin detect these inputs, sending signals to the brain that generate the perception of touch. This ring-shaped device vibrates up to 110 Hz and exerts a force that presses the skin by 3 micrometers, falling within the range detectable by Pacinian corpuscles," Yoshio explains.

While many aspects of tactile realism remain unexplored, this actuator serves as a stepping stone toward greater advances. "In the future, I hope to make this haptic device a part of everyday life, allowing users to feel the texture of products during online shopping and beyond," Yoshio envisions, continuing to shape a vivid future with his innovative thinking.

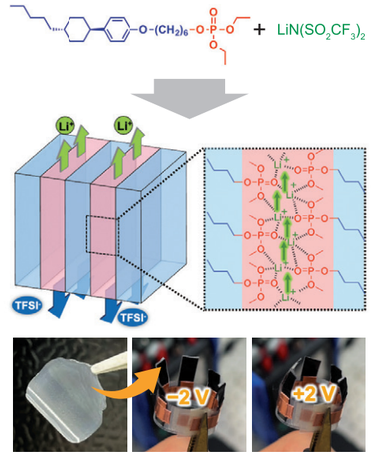


Figure 4. When ionic liquid crystal molecules (phosphate esters forming complexes with lithium salts) are mixed with plastic materials (a blend of polyvinyl chloride and vinylidene fluoride-hexafluoropropylene copolymer) in solution, the resulting liquid crystal forms a layered structure. Lithium ions rapidly move through the organized phase (pink area) formed by the phosphate esters and lithium salts. By combining this liquid crystal with other polymers, a self-supporting film is obtained (left photo). This film is applied to a ring-shaped haptic device (center and right photos).

Yoshio's expertise lies in his skillful molecular design, anticipating the final self-organized structure, and in his synthesis techniques. A variety of ionic conductive liquid crystal molecules synthesized by Yoshio and his team are lined up in the lab. The production of these "parts" involves more than ten stages of polymer chain synthesis.

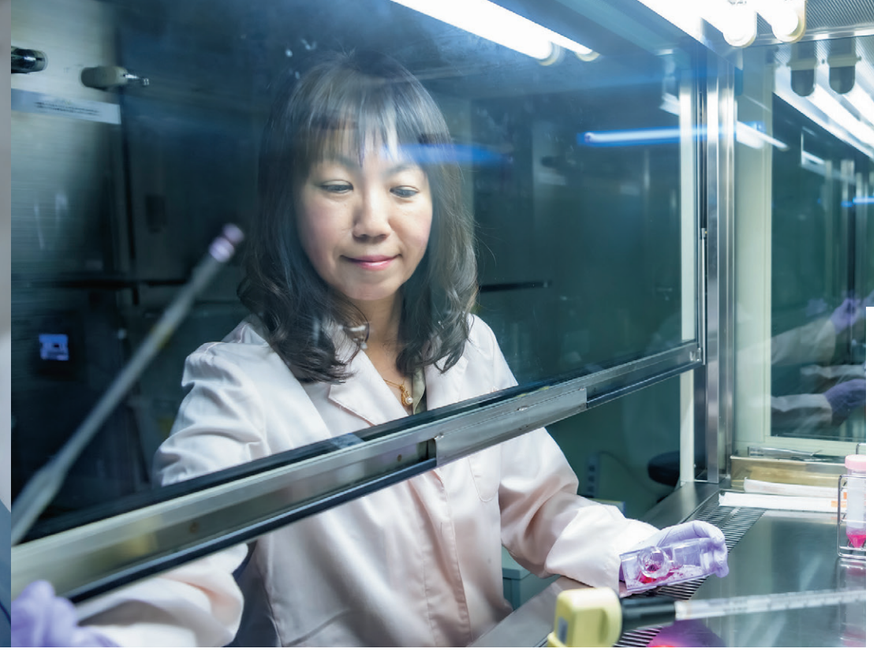
RESEARCH 2

Extending the Lifespan of Next-Generation Rechargeable Batteries with Polymer Gels

Ryota Tamate

Independent Researcher

Tamate holding a sample of polymer gel electrolyte



RESEARCH 3

Protecting Material Surfaces with Concentrated Polymer Brushes

Chiaki Yoshikawa

Team Leader,
Polymer Surfaces
and Devices Team

Lightweight and highly flexible, polymer gels are often described as having properties that bridge the gap between liquids and solids. By controlling the interactions between their two key components—polymers and solvents—Ryota Tamate has developed a range of polymer gels with unique properties. He is now actively exploring their potential applications.

Designing interactions between polymers and solvents

A polymer gel is a three-dimensional network of crosslinked polymer chains that swell by absorbing solvent. Focusing on the interactions between polymers and solvents, Tamate has developed polymer gel electrolytes with diverse functionalities by utilizing ion-conductive solvents.

One example is the self-healing ion gel he introduced in 2022, which combines high mechanical strength with self-repairing capabilities. When damaged, the gel restored itself as the surrounding polymer chains reestablish their entangled state (see Fig. 1 and p.4 photo). The gel also exhibits unique properties: it can withstand up to one ton of pressure, be reshaped, and is fully recyclable (Fig. 2).

"I have adopted various strategies to enhance the functionality of polymer gels, such as utilizing chemical bonds in addition to the physical entanglement of polymer chains," Tamate explained. "Currently, my primary focus is on extending the lifespan of lithium metal batteries."

Overcoming challenges in lithium metal batteries

Lithium metal batteries are a type of rechargeable battery that use lithium metal as their anode material. While they theoretically offer higher energy densities compared to lithium-ion batteries, repeated charge-discharge cycles lead to the formation of dendritic crystals on the lithium metal surface and the exfoliation of the metal. These phenomena degrade charge-discharge cycle performance, preventing these batteries from achieving the durability seen in lithium-ion batteries.

"To protect lithium metal from such damage, I designed a gel combining polymers and an electrolyte made from highly concentrated lithium salts and used it to coat the lithium metal," Tamate said. "The gel's elasticity allows it to adapt to the volume changes of the lithium metal as it expands and shrinks during charge-discharge cycles."

However, the primary challenge with polymer gels lies in achieving mechanical strength capable of withstanding repeated volume changes. To address this, Tamate incorporated functional groups into the gel to induce hydrogen bonding between polymer chains, enhancing the gel's mechanical strength chemically. Furthermore, by increasing the lithium salt concentration in the electrolyte, he successfully reduced the number of solvent molecules that interfere with hydrogen bond formation.

Tamate evaluated the durability of the lithium metal anode both before and after coating it with the polymer gel electrolyte. The results showed that the coating extended the anode's lifespan from approximately 200 hours to over 1,000 hours. Additionally, when paired with a high-capacity cathode, the battery's charge-discharge cycle count increased from around 50 to over 100 cycles before capacity degradation began. Tamate's efforts to explore the application of polymer gels and their unique properties continues to make steady progress.

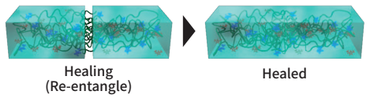


Figure 1. A self-healing ion gel composed of densely entangled polymer chains. Tamate discovered that this gel, made of ultrahigh-molecular-weight polymers (i.e., polymers with molecular weights exceeding one million), can be easily synthesized by polymerizing monomers in an ionic liquid solvent.

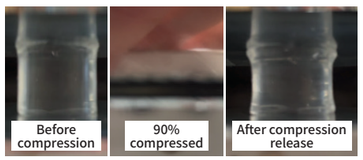


Figure 2. A self-healing ion gel undergoing mechanical strength testing. When compressed to 10% of its original volume under one ton of pressure, the gel regained its shape without collapsing. The gel is recyclable due to the absence of chemical crosslinking in its polymer components and the use of a non-volatile ionic liquid as the solvent.

Concentrated polymer brushes are a unique class of polymer coatings, where they exhibit excellent anti-biofouling properties, while minimizing protein adsorption and cellular interactions. Chiaki Yoshikawa has worked in this field over multiple decades, publishing in highly cited journals and generated multiple patents. Her research involves understanding the interactions between these coatings and biological organisms and exploring potential practical applications.

Biocompatible Surfaces Vital to Medical Applications

The surface of a material is constantly exposed to several environmental conditions, affecting its lifespan. Examples include material degradation, attachment of unwanted materials, or other physical damages—such as scratches or ultraviolet irradiation. Therefore, finding methods to protect the surface of materials can improve their use and lifespan.

When implanted within the human body, materials are exposed to biological tissue, blood, plasma, and other bodily fluids, all of which can damage the surface of the material. Proteins within the body start interacting with the surface, triggering a series of biological reactions - damaging and removing the material, leading to poor material-tissue integration. Concentrated polymer brush coatings can minimize adsorption of proteins, cells, and other biological materials, by forming a protective barrier against these threats, leading to better material integration (Fig.).

"These polymer brushes offer a promising solution, but applying and growing these brushes into a uniform and dense coating on any material surface is difficult," Yoshikawa said. "The current challenge is for more precise polymerization, which requires multiple steps, such as introducing initiating groups. Additionally, some materials are inherently difficult to grow and attach these coatings, due to the material surface properties and/or shape, which limit its applicability. To address this issue, I developed new coating materials which can be easily applied onto surfaces, forming a concentrated polymer brush coating."

Developing Easy-to-Apply, Versatile Coating Materials

Yoshikawa has developed two types of coating materials: fine particles with concentrated polymer brushes; and well-defined bottlebrush polymers (see upper and lower diagram on the right in Fig.). Both are easy to use: once dissolved in a solvent, they can be used on any surface. Films made from these materials have great adhesive properties, where they can be applied onto any surface through chemical reactions.

"I've conducted experiments to evaluate how proteins interact with these films, factoring protein size, coating thickness, and brush density," said Yoshikawa. "The insights gained from these observations have been applied to ongoing collaborative efforts between industry, academia and the public sector to develop marine paints using polymer brush technology. Our aim is to develop new paints to prevent the attachment of marine organisms, such as plankton and mollusks, onto the hull of the ship, which slows its movement. In addition, these coatings may provide a lubricating effect, deducing the drag for more efficient travel. Field testing of these coatings takes time, and challenges remain before our novel paints can be widely adopted. However, we are committed to advancing this research for everyone's benefit."

The performance of marine paints is evaluated by submerging test plates coated with the paints in seawater. The plates are inspected weekly to determine the extent to which marine organisms have attached to their surfaces.

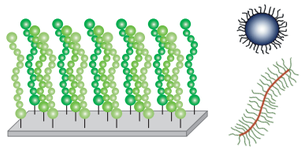


Figure. (Left) Concentrated polymer brushes grown at a high packing density on a planar substrate via surface-initiated atomic transfer radial polymerization.
(Right) Two polymer brush materials developed by Yoshikawa: fine particles grafted with concentrated polymer brushes (top); and well-defined bottlebrush polymer structure whereby an elongated polymer backbone is grafted with a multitude of polymer chains (bottom)

Hibi operating the pyrolysis gas chromatography mass spectrometer. The computer connected to the mass spectrometer outputs data processed by the newly developed machine learning algorithm, allowing instant analysis of monomer sequence distribution.

RESEARCH 4

"Polymer Sequencer" Achieved Through Mass Spectrometry and AI

Yusuke Hibi
Researcher,
Data-driven
Polymer Design Group

Polymers are chain-like molecules consisting of hundreds to tens of thousands of monomers in their basic structure. Yusuke Hibi and his colleagues developed the world's first "polymer sequencer," a tool that uses mass spectrometry and AI to quantitatively determine the frequency of monomer sequences, which impart diverse functions to polymers.

Randomly Arranged Monomers

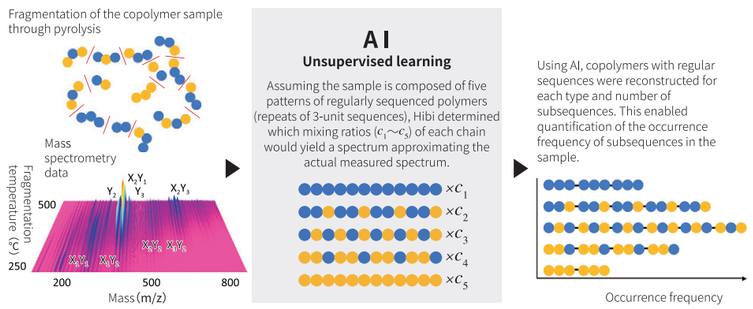
Many of the polymers abundant in our daily lives, such as plastics, are copolymers—compounds composed of two or more different kinds of monomers. Even when the same monomers are combined, differences in their sequence can drastically alter the properties and functions of the copolymer. A key factor affecting copolymer properties is the occurrence frequency of specific sequences of a few monomers (subsequences). For instance, in a copolymer consisting of monomers A and B, sequences like AAA and ABAB are examples of subsequences.

Controlling the sequence of monomers during the polymerization process is challenging; typically, random sequences are generated probabilistically. Moreover, there has been no universal analytical technique to quantitatively determine the occurrence frequency of subsequences in randomly sequenced copolymers, making it difficult to analyze the correlation between sequence and material properties or to design materials based on sequence. In response, Hibi and his colleagues proposed a strategy to analyze copolymer mass spectrometry data using AI.

World's First "Polymer Sequencer"

"We subjected copolymers made from multiple monomer types to pyrolysis mass spectrometry by heating them up to 600°C. As the temperature rises, the copolymers decompose and gasify into short fragment chains, producing spectra that reflect the mass of each fragment chain in a short time (left diagram). During this process, copolymers are cleaved probabilistically along the main chain at weaker bonds, resulting in numerous fragment chains of various lengths and sequences. However, since fragment chains of the same mass but different sequences (like ABA and AAB) can form, directly quantifying subsequences from mass spectrometry data is extremely challenging. Therefore, I utilized AI to simulate a scenario where the copolymer is composed of regularly sequenced polymers (central diagram) and analyzed which mixing ratio of regular polymers would yield spectra with closely approximating to the observed data. This approach is based on the concept that any copolymer can be considered a mixture of regularly sequenced polymers," explains Hibi.

Furthermore, Hibi succeeded in using AI to aggregate similar subsequences and reconstruct virtual polymer chains (right diagram), enabling quantitative determination of subsequence occurrence frequencies within copolymer samples. "This polymer sequencer can analyze almost all thermally decomposable polymer materials, regardless of monomer types or quantities. However, there are challenging cases, such as when polymers decompose entirely into single monomers. Our goal is to further expand our analytical capabilities," Hibi states. By addressing these challenges, Hibi seeks to pave the way for new advancements in polymer material development.



Nishiguchi synthesizing injectable gels with phase-separated, porous structures

RESEARCH 5

Developing Injectable Porous Gels to Deliver Viable Cells via Phase Separation

Akihiro Nishiguchi
Principal Researcher,
Polymeric Biomaterials Group

Cell transplantation therapy uses stem cells or other cell types to regenerate damaged organs caused by disease or injury. The cells are encapsulated in polymer gels and injected into the body. Akihiro Nishiguchi has successfully developed gels that enhance both graft survival and cell viability.

Porous structures improve the performance of cell transplantation

Spinal cord injuries and myocardial infarction currently lack established treatments. In regenerative medicine, cell transplantation therapy is emerging as a highly promising method. Among these approaches, the use of injectable gels to encapsulate cells for direct delivery into the body is gaining particular attention.

Injectable gels are designed to remain liquid in a syringe and solidify in response to specific stimuli after being administered, allowing them to stay at the target site. However, injected gels often fail to properly engraft onto target organs. Even when engraftment succeeds, the transplanted cells frequently undergo apoptosis.

Apoptosis occurs because the polymeric chains of injectable gels are densely crosslinked. This dense structure hinders the diffusion of oxygen and nutrients essential for cell growth and survival. It also prevents recipient tissue cells from infiltrating the gel, weakening intercellular communication necessary for successful integration. To address these challenges, many researchers are exploring porous gels.

"Most injectable porous gels developed so far feature pore sizes in the nanometer range," Nishiguchi explained. "While exploring the potential use of injectable gels as tissue adhesives, I discovered a method to create gels with micro-sized pores that are more suitable for cell growth."

Confirming Blood Vessel Regeneration in Mice

Nishiguchi's efforts to develop injectable gels have focused on gelatin derived from pig tendons and modified with UPy (ureidopyrimidinone), referred to as GUPy.

"When I mixed GUPy with two other types of gelatin, the latter two crosslinked, while GUPy aggregated and caused liquid-liquid phase separation, LLPS (Fig.)," Nishiguchi said. "LLPS typically occurs between different types of polymers. However, in this case, even though all components were gelatin, LLPS still occurred, likely driven by the strong hydrogen bonding promoted by the UPy groups. Additionally, GUPy is designed to dissolve at 37°C, enabling the gel to spontaneously form fibrous pores after being injected into the body."

Nishiguchi cultured human mesenchymal stem cells in this gel and observed that the cells adhered, spread, migrated, and proliferated along the fibrous pores. Furthermore, when the gel containing mesenchymal stem cells was administered into the limbs of mice with impaired blood flow, the dysfunctional blood vessels regenerated, restoring normal blood flow.

"I hope this new gel makes a significant contribution to regenerative medicine," Nishiguchi stated. "It also has potential applications in three-dimensional bioprinting and drug delivery systems, and I am actively exploring these possibilities."

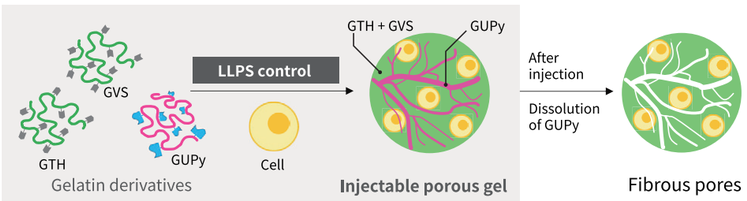


Figure. A fibrous internal network in UPy (ureidopyrimidinone)-modified gelatin (GUPy) is formed by mixing GUPy with equal portions of thiolated skin gelatin (GTH) and vinyl sulfonated gelatin (GVS). During gel formation, GTH and GVS crosslink, while liquid-liquid phase separation (LLPS) causes GUPy to create a fibrous structure. After injection into the body, the GUPy component dissolves spontaneously at body temperature (37° C), forming fibrous pores within the gel. This porous structure improves oxygen diffusion and facilitates cell infiltration within the gel.

Uto stretches a strip of smart polymer with a helical memory shape until it becomes straight. When immersed in hot water, the strip rapidly returns to its original helical form.

RESEARCH 6

Smart Polymers in Medicine: Advancing Therapies and Understanding Diseases

Koichiro Uto
Principal Researcher,
Smart Polymers Group

Smart polymers can be deformed by applying force, retaining the new shape until exposed to external stimuli, such as heat or light, which trigger a return to their original form. While medical applications for these materials are progressing, challenges remain, particularly in optimizing shape recovery at lower temperatures. Koichiro Uto has developed techniques to control smart polymers at temperatures close to human body temperature, thereby expanding their potential for medical use.

Achieving sharp polymer responses at low temperatures

Smart polymers can transition between crystalline and amorphous states in response to external stimuli such as heat, light, or magnetic fields, enabling various deformations. Due to crosslinks between their molecules, they maintain stability even in the amorphous state, preserving and retaining their permanent shape memory.

Uto focuses on temperature-responsive smart polymers for medical applications. Among these materials, he frequently utilizes poly(ε-caprolactone) (PCL), which has a relatively low melting temperature of 60°C—the temperature at which it transitions from a crystalline to an amorphous state.

"I chose PCL for its low melting temperature," Uto said. "However, 60°C is still much higher than body temperature, so it needed to be lowered. Another reason for selecting PCL is its biodegradability, which makes it environmentally friendly."

Uto precisely controlled the PCL synthesis process, creating PCLs with varying numbers of branches and arm/chain lengths. By evaluating these materials, he identified a PCL that responds to lower temperatures. He can now fine-tune the melting temperatures of PCLs with a precision of approximately 1°C, within a range of 30°C to 43°C. Smart polymers with such precise low-temperature responsiveness are exceptionally rare worldwide.

Innovative materials for enhancing quality of life

Uto and his colleagues in the Smart Polymers Group have been advancing the research and development of smart polymers for medical applications, leveraging their exceptional control over the low-temperature responsiveness of these materials.

One notable example is a fetal treatment device currently being developed in collaboration with Osaka University. The device targets tumors detected in a fetus within the mother's womb. A PCL string is inserted through the mother's abdomen and wrapped around the base of the tumor. When heat is applied, the string contracts, cutting off the tumor's blood supply and causing it to necrotize. This approach is particularly effective in confined spaces where precise tightening is required (see NIMS NOW, Vol.20, No. 1, p.14).

In addition, the Smart Polymers Group is collaborating with Aichi Cancer Center to develop a patient positioning device for radiation therapy (see photo at the top of p. 13) and a bolus designed to fit snugly against the skin, maximizing radiation delivery to the tumor. While similar shape-memory devices are commercially available, they typically have high melting temperature (around 80°C). These devices must be molded directly onto the patient's face or skin, which can cause discomfort, particularly for children. In contrast, the PCL-based products under development have significantly lower melting temperature, allowing for safer and more comfortable molding.

The group has also been developing other smart polymer-based materials to enhance the quality of life, including collaborating with Nihon L'Oréal K.K.—a subsidiary of the globally renowned cosmetics company—to research and develop hair styling agents.

Exploring disease development mechanisms using dynamic materials as cell scaffolds

In addition to these projects, Uto and his colleagues are focusing on developing cell scaffold materials for cell culture. These scaffolds act as substrates to support cell proliferation and differentiation, which are essential for tissue engineering and regenerative medicine.

"Cells in the human body are constantly exposed to dynamic environments, such as heartbeat rhythms, blood vessel contractions, and other physiological processes," Uto explained. "In contrast, cells are typically cultured in static environments, like plastic plates or gel surfaces, which differ from their natural in vivo conditions. Using smart polymers, we aim to create cell culture environments that more closely replicate real biological systems."

In recent years, researchers have discovered that the structure of cells grown on culture substrates varies significantly depending on the topography of the surface.

While studying at a university in the United States, Uto cultured cardiomyocytes (heart muscle cells) to deepen his knowledge of medical applications of his materials research. He used two types of substrates: flat surfaces and microfabricated surfaces with parallel ridges. The cardiomyocytes cultured on the microfabricated surfaces aligned along the ridges as they grew and exhibited structural characteristics closer to those of mature human cardiomyocytes compared to cells cultured on flat surfaces.

Furthermore, Uto developed a dynamic cell culture environment by using shape-memory polymers to create substrate surfaces with adjustable structures.

"I developed a smart polymer-based substrate," Uto said. "Its surface features fine, parallel ridges that rotate 90 degrees when heated. Initially, I cultured cardiomyocytes on the substrate at 32°C, with the ridges aligned longitudinally. After a set period, I raised the temperature to 37°C, causing the ridges to rotate 90 degrees. As a result, the cardiomyocytes gradually reoriented, eventually aligning with the new ridge orientation (Fig.). Surprisingly, the cells' pulsation direction also shifted by approximately 50 degrees. Using a custom observation system, I was the first to capture these dynamic cellular behaviors globally."

These findings indicate that collective cell movement can be controlled by adjusting the surface patterns of a substrate. This approach also opens up possibilities for accurately replicating heart disease development processes in vitro using smart polymer materials.

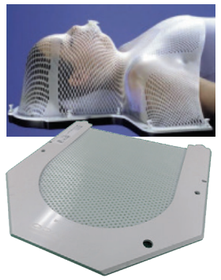
"In a healthy heart, cardiomyocytes are well-organized and aligned," Uto said. "However, in regions affected by hypertrophic cardiomyopathy or other heart diseases, these cells become noticeably misaligned. This misalignment is believed to result from structural changes in the extracellular matrix, which acts as a scaffold for the cells. Smart polymers hold significant potential for elucidating the mechanisms underlying heart disease development."

Advancements in 4D printing compatible with readily available materials

Uto and his colleagues are committed to advancing medical treatments by developing technologies that use readily available materials—those that are simple to produce and easy to apply. As part of this effort, Uto is exploring 4D printing, which adds a new feature to traditional 3D printing: the ability to change shape over time.

"The drawback of smart polymers," Uto explained, "is their lower mechanical strength compared to metals. Many medical devices, such as stents used to widen narrowed blood vessels or bronchi, require a certain level of strength. One way to overcome this limitation with smart polymers is by creating complex geometric structures, which can be efficiently produced using 3D printers. Although designing these structures involves advanced calculations, the actual manufacturing process is straightforward once the design is finalized. We are currently developing temperature-responsive shape-memory inks suitable for 3D printing. Meanwhile, another NIMS research group has prototyped a smart polymer stent based on structural calculations and 3D printing, with its performance now being evaluated by a private company. We are also considering 4D-printed medical applications, such as a foldable stent that can be inserted into a blood vessel in its compact form and expanded at the target location."

Temperature-responsive smart polymers, capable of precise activation at lower temperatures, hold immense potential for medical innovation. However, Uto notes that their high sensitivity could present challenges in certain in vivo applications. To address this, his team is investigating hybrid materials—smart polymers enhanced to respond to external stimuli such as light and magnetic fields. Through these and other strategies, they aim to expand the possibilities for the medical applications of smart polymer technologies.



Radiation therapy mask—a patient positioning device being developed jointly by the Smart Polymers Group and Aichi Cancer Center. The netted component is made of temperature-responsive smart polymers. When heated to approximately 40° C, the material softens, allowing it to be molded easily over the patient's head, creating a custom-fitted mask.

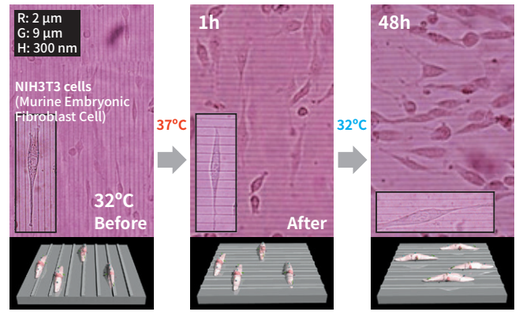
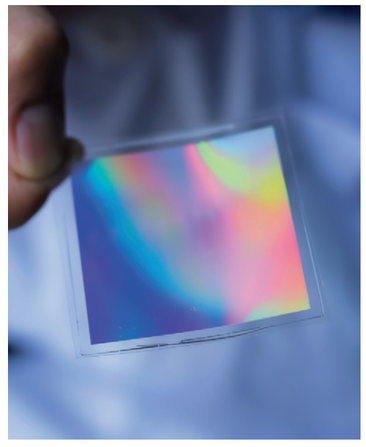


Figure. Cell behavior in response to changing substrate surface structures. Uto designed a PCL substrate that alters its surface structure in response to temperature changes. When heated to its melting temperature (37° C), the parallel ridges on the surface disappear and transverse ridges emerge. This transformed surface structure is preserved by cooling the substrate below its crystallization temperature (32° C). Using a live observation system developed by Uto, fibroblasts cultured on the substrate were observed gradually extending and reorienting themselves to align with the changing ridge orientation, demonstrating complex behavior in response to the substrate's shifting topography.



PCL substrate with a microfabricated surface featuring parallel ridges, created by Uto. "Phenomenological studies have shown that the structure of a scaffolding surface—whether flat or textured—has a significant impact on how cells develop," Uto explained. "My goal is to uncover the mechanisms by which cells sense surface structures and leverage this understanding to develop innovative techniques for controlling cell maturation and function through surface structure manipulation."

RESEARCH 7

First-Ever Successful Cell Culture at an Ionic Liquid Interface

Takeshi Ueki
Senior Researcher,
Mechanobiology Group

Ueki observing the growth and spread of cells cultured at an ionic liquid interface using a fluorescence microscope.

Figure 2. Schematic illustration of cells cultured at the interface between a cell culture medium and a hydrophobic ionic liquid.

Ionic liquids, composed solely of cations and anions, exhibit unique properties distinct from molecular liquids. Takeshi Ueki has successfully cultured cells at ionic liquid interfaces and is now working to further optimize the efficiency of this technique.

Unique liquids that barely evaporate or boil

Liquids like water and alcohol consist of molecules, while ionic liquids are composed entirely of cations and anions. Despite being in a liquid state, they have extremely low volatility and do not evaporate or boil under normal conditions. Additionally, their properties—such as hydrophilicity, hydrophobicity, and polarity—can be precisely tuned by modifying their chemical structures. By exploiting these unique properties, Ueki has been developing innovative methods for culturing cells at ionic liquid interfaces.

The standard method for cell culture today involves growing cells at the interface between a plastic dish and an aqueous protein-containing medium, a solid-liquid interface. In contrast, Ueki is exploring a novel approach to culture cells at liquid-liquid interfaces formed between an aqueous protein solution and a hydrophobic ionic liquid.

"The use of plastic plates confines cell cultures to the dish surface, however, a liquid-liquid interface allows for a significant increase in the available culture area by dispersing droplets of the medium throughout the solution. Furthermore, because ionic liquids neither evaporate nor boil, they can be reused after heating, drying, and sterilization, offering a notable advantage." Ueki explained.

The challenge, however, was the cytotoxicity of hydrophobic ionic liquids to cells. To overcome this, Ueki developed a cellular toxicity evaluation method to screen for non-cytotoxic ionic liquids and he was able to successfully culture human mesenchymal stem cells at ionic liquid interfaces for the first time globally.

Key factors that stabilize liquid interfaces and allow them to function as cell scaffolds

"Cells cultured at different ionic liquid interfaces exhibited distinct behaviors," Ueki explained (Fig. 1). "Some ionic liquids caused cells to form spherical aggregates, while others led them to spread across the interface." It is well known that cellular behavior is influenced by the mechanical properties of the scaffold. In this case, the cells appear to sense and respond to the 'stiffness' of the liquid interface.

What determines the "stiffness" of liquid interfaces as cellular scaffolds? Observations using atomic force microscopy (AFM) revealed that cells do not adhere directly to the ionic liquids but to solid nanofilms of self-assembled protein nanolayers (PNLs) formed at the interface (Fig. 2). Ueki further discovered that the stiffness and thickness of the PNLs depend on the speed of protein Brownian motion* during their formation, which is in turn governed by the chemical structure of the ionic liquids.

"These findings offer valuable insights for designing ionic liquids that promote the formation of mechanically robust PNLs," Ueki said. "We have also developed a method to convert ionic liquids into solid thin films with a gel-like structure. Interestingly, cells cultured on these gels spread over larger areas compared to those cultured at liquid interfaces. Moving forward, we will deepen our investigations to establish innovative cell culture systems using ionic liquids."

* Brownian motion refers to the random movement of particles suspended in a medium, caused by collisions with solvent molecules undergoing thermal motion.

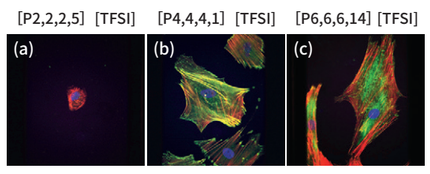


Figure 1. Fluorescence microscope images of human mesenchymal stem cells cultured at the interfaces between a cell culture medium and three different ionic liquids, corresponding to images (a)–(c). In (a), the cells formed spherical aggregates, whereas in (c), the cells spread thinly across the interface. Notably, the spreading behavior in (c) was comparable to that observed on a glass substrate. This result means that cells perceive the ionic liquid interface in (c) as having mechanical properties similar to those of glass, providing stable scaffolding for cell attachment and growth.

Toru Yoshitomi (left) and Dr. Kinji Furuya (right) monitoring the injection of a newly developed tissue marker into a pig's gastric submucosal layer using an endoscope.

RESEARCH 8

Collaborating with Surgeons to Develop a New Tissue Marker

Toru Yoshitomi
Principal Researcher,
Tissue Regeneration
Materials Group

Tissue markers enable precise surgical removal of tumors and other potentially malignant growths. In collaboration with surgeons, Toru Yoshitomi developed an advanced marker that resists diffusion, remains in tissues for extended periods, and can be injected using endoscopic needles. He is now advancing its practical application.

Unresolved Issues in Surgical Tissue Marking

Malignant stomach and colorectal tumors develop on the mucosal lining of the digestive tract, rendering them invisible from the outside during open abdominal surgery. To address this, an endoscopic needle is used to inject a tissue marker around a target tumor in advance, providing a visual guide for surgeons during the procedure.

India ink tissue markers, sterilized with high-pressure steam in hospitals, are commonly used in surgeries. However, these markers tend to diffuse and offer poor contrast, making it difficult to distinguish marked areas from surrounding tissues. Additionally, surgeries may be delayed by up to a month due to scheduling conflicts, further reducing the markings' visibility. Accurate tissue marking is essential for preserving as much healthy tissue as possible, which significantly impacts the patient's prognosis.

In January 2020, Yoshitomi attended the Young Researchers Exchange Meeting hosted by the Tsukuba Life Science Promotion Association. During the event, he met Dr. Kinji Furuya from the Department of Gastrointestinal and Hepato-Biliary-Pancreatic Surgery at the University of Tsukuba and learned about the challenges with tissue markers faced by medical practitioners. This meeting initiated a collaboration between the NIMS Tissue Regeneration Materials Group and the University of Tsukuba's digestive surgery team to develop a new tissue marker.

The Journey of Tissue Marker Development

For a tissue marker to be ideal for medical professionals, it should be injectable using an endoscopic needle, like conventional India ink markers. It must remain localized in the tissue for about one month without significant diffusion and be detectable using a near-infrared camera integrated into a laparoscopic surgery system.

"Developing a tissue marker with these characteristics involved continuous trial and error," Yoshitomi said. "Even after developing a marker with the desired fluorescent properties, we faced numerous challenges. The marker diffused and disappeared within a week after injection, clogged syringe needles, caused various other issues. Each time, our team of materials scientists collaborated closely with clinical specialists to identify and implement solutions."

After extensive efforts, Yoshitomi's group successfully developed an effective tissue marker. To test its performance, the marker was injected into a rabbit's gastric submucosal layer alongside a conventional India ink marker. One month later, the injection sites were assessed. While the India ink site had significantly spread, while the new marker site remained localized, showing no diffusion and maintaining clear fluorescence (Fig.). In another preclinical study, the new marker was endoscopically injected into the tissue of a 40-kg pig using a syringe needle. The results confirmed the marker met the expectations of medical professionals.

"We are currently preparing for non-clinical safety testing, the next step following basic research, with the ultimate goal of bringing the tissue marker into practical use," said Yoshitomi. His team is making steady progress in developing an effective tissue marker to address critical challenges and improve surgical efficiency.

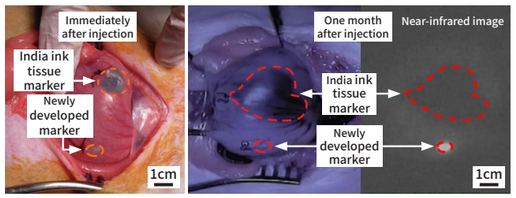


Figure. Comparison of tissue markers in diffusion and visibility
After injecting 100 μL of each marker into a rabbit's gastric submucosal layer, near-infrared imaging one month later showed the new marker remained localized with clear fluorescence, while India ink diffused significantly.

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