1. Research Content

Polyimide (PI) films for coating glass substrate are commonly used to align the liquid crystal (LC) molecules in liquid crystal display (LCD) devices. Among them, PI containing steroidal side chains, as shown in Fig. 1, is expected to be useful because it provides a wide range of pretilt angles according to its diamine ratio. Unfortunately, the orientation and conformation of molecules at the PI surface after rubbing have not been fully known yet so far, especially so far as a steroidal structure and alkyl chain parts are concerned.

![Chemical structure of PI containing steroidal side chains](image)

**Figure 1.** Chemical structure of PI containing steroidal side chains used in this study.

Ullah *et al.* reported that the second harmonic generation (SHG) intensity of a rubbed PI film with units possessing a 30% fraction of steroidal side chains (PI-30) shows anisotropic SHG intensity patterns as a function of the sample rotation angle. To analyze the experimental result, they assumed that only the steroidal side chains of the PI-30 have microscopic optical nonlinearity. However, Ullah *et al.* have not checked the validity of the assumption yet. In this study, I first
observed SHG response from unrubbed PI film surfaces at several different molar fractions of side-chain diamine in PI containing steroidal side chains, PI-0, PI-30, PI-60, PI-90, and PI-100, to examine whether or not their assumption was correct. I found that both the PI main chains and PI side chains contribute similarly to the SHG intensity. The SHG intensity in S\text{in}/P\text{out} polarization configuration normalized by P\text{in}/P\text{out} was the smallest for the unrubbed PI-0 film, or the PI without steroidal side chains. This fact indicates that the microscopic nonlinear dipoles are standing nearly upright at the surface of the unrubbed PI-0 film. In contrast, their orientational spread is wider when the side chains are involved.

The molecular orientation of steroidal side chains at the rubbed PI surfaces was investigated by sum frequency generation (SFG) vibrational spectroscopy to establish a correlation between the molecular structure and LC alignment. Several analytical techniques are used to study polymer films, such as infrared spectroscopy, atomic force microscopy, and near-edge X-ray absorption fine structure spectroscopy. However, they are not helpful for analyzing the steroidal structure and the alkyl chain parts. On the other hand, SFG vibrational spectroscopy has been demonstrated as a useful surface analytical technique. Because this technique is sensitive to non-centrosymmetric parts of materials, it is commonly used to determine vibrational resonances of molecules adsorbed at surfaces and interfaces.

In SFG measurements, I have investigated three types of PI-30 films, including unrubbed PI-30 film, rubbed PI-30 film, and rubbed PI-30 film with a poly (methyl methacrylate) (PMMA) overlayer. Before analyzing the details of the SFG spectra of the PI-30 film, I examine whether or not the SFG response originates from the PI-30 film surface. For this purpose, I measured the SFG spectra of the rubbed PI-30 films with and without a PMMA layer for PPP and SSP polarization combinations. The thickness of the PMMA layer was $\sim 5$ nm. The SFG intensity decreased dramatically after the sample was covered with the very thin PMMA layer for the PPP polarization combination. Furthermore, the peaks in the spectra changed drastically after PMMA deposition for the SSP polarization combination. These results indicate that the SFG of the rubbed PI-30 film mainly originates from the PI film surface, and the SFG contribution from the PI/glass substrate interface and the bulk PI can be ignored.

In the SFG spectrum of the rubbed PI-30 film in the CH stretching region from 2800 to 3000 cm$^{-1}$ at an azimuthal angle of $\gamma = 0^\circ$ and for a PPP (P-polarized SFG, P-polarized visible, P-polarized IR) polarization combination, I found that the SFG intensity of the CH$_2$ symmetric stretching mode at 2842 cm$^{-1}$ is weaker than those of the CH$_3$ groups at 2859 and 2874 cm$^{-1}$, although the number of CH$_2$ groups in one side chain is larger than the number of CH$_3$ groups. The result means that the CH$_2$ groups in the rubbed PI-30 film have a small net polar orientation.
To analyze the effect of the rubbing process on the orientation of the CH₃ groups at the PI side chain, I focus on the CH₃ symmetric stretching modes in 2850-2890 cm⁻¹ region. The SFG spectra of the rubbed PI-30 film at azimuthal-angle intervals of 45° for a PPP polarization combination were observed. The spectra showed that the isopropyl group is pointing toward the air side from the PI surface, but their average orientation is not affected by rubbing because the length of the alkyl chain in this PI is short. However, a slight anisotropy of the symmetric stretching mode of the CH₃ group next to the steroidal structure was observed in the SFG spectra after rubbing. Its average tilt angle was estimated as $\theta_0 = 40° \pm 10°$ in the rubbing direction. These results indicate that the rubbing appears to cause conformational changes of the CH₃ group near the steroidal structure, but the rubbing does not significantly affect the orientation of the isopropyl group at the end of the PI side chain.

2. Research Purpose

The correlation between the molecular orientation at the surface of the PI containing steroidal side chains and the alignment of LC molecules on this rubbed PI film is important from a scientific perspective. It is also desirable from an industrial perspective because it helps to understand the mechanism of LC alignment on the rubbed polymer surface and provides a reference for the design of new PI containing steroidal side chains for future commercial research in the display industry. Nevertheless, to the best of my knowledge, there is no study investigating the molecular orientation and conformation of the steroidal structure and the alkyl side chain at the rubbed PI film surface like the one in this study. Hence, it is unknown yet which part of the rubbed PI film containing steroidal side chains is most strongly correlated with LC alignment. Therefore, the ultimate purpose of my study was to establish which structural factor of rubbed PI films with steroidal side chains is most strongly correlated with LC alignment.

Four possible structural factors were checked; (1) the phenyl rings in the PI side chain, (2) the phenyl rings in the PI main chain, (3) the steroidal structure and the adjacent CH₃ group, and (4) the isopropyl group and the neighboring methylene group in the PI side chain. Among them, factors (2) and (4) can be excluded because they do not show any anisotropy after rubbing. Consequently, only two factors (1) and (3) are possibly correlated with LC alignment. Unfortunately, I cannot determine so far which of them is mainly related to LC alignment, and further study is necessary for resolving this issue. Generally, chemical structures located near the main chain within the side chain of a PI tend to have weaker interactions with LC molecules. I, therefore, suggest that factor (1) may not be the main factor in LC alignment. In this case, factor (3) is the main factor causing LC alignment. However, further study is necessary for precise judgment of the correlation between factor (3) and LC alignment.
3. Research Accomplishment

Publications


International Symposiums


Domestic Meetings


Awards

1. Student Award at *12th International Symposium on Atomic Level Characterizations for New Materials and Devices (ALC’19)*, October 2019.
2. JAIST Foundation Research Grant for Student, March 2020.
3. MEXT (Monbukagakusho) scholarship, October 2018-September 2021.
**Title of the Dissertation:** Stabilizing High-Performance Li-Ion Battery Anodes by Strategic BIAN-Based Functional Polymer Binders Design for Controlled Solid-Electrolyte Interphase (SEI) Formation

**Intended Degree:** Doctor of Materials Science

**Laboratory:** Matsumi Laboratory

**Student Number:** 1820407

**Name:** Agman Gupta

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**Research Content**

The development of high energy density lithium-ion batteries (LIBs) for future applications in electric vehicles (EVs), hybrid electric vehicles (HEVs), portable electronic devices, etc. is one of the prime areas of research across the globe. In this regard, various suitable high-capacity alternatives like Sn, Ge, and Si have been explored to replace commercial graphite anode in a LIB. Among various alternatives, Si has been recognized as the next generation anode active material because of its high theoretical capacity (~4200 mAhg\(^{-1}\)) and natural abundance. However, commercial graphite anode and its potential next generation alternative Si have their own sets of drawbacks. But the quality of solid-electrolyte interphase (SEI) is one common drawback associated with both that destabilizes their long-term cycling performance with poor reversible capacity. As a remedy to mitigate all the drawbacks, especially those associated with the interphase formation on these anodes, the utilization of polymers as binders has been recognized as a good strategy. Compounds of the family bis(aryl)acenaphthene (Ar-BIAN) are famous for their ability to act as ligand for transition metal complexes in catalysis as they can act as electron reservoir and offer rich redox chemistry (Figure 1). Also, they have exhibited impressive synthetic versatility as they are potent with structural modification, functionalization, and have tunable electronic properties because of the availability of suitable precursors. Most importantly, their low-lying lowest unoccupied molecular orbital (LUMO) empowers them with the possibility of undergoing n-doping in the reducing cathodic environment to restrict the electrolyte decomposition and control the SEI formation if utilized as binders for anodes in LIBs. Therefore, this dissertation work was aimed to focus on design, synthesis, and application of novel polymers belonging to the family of compounds bis(aryl)acenaphthenequinone (Ar-BIAN) as binders (Figure 2) to stabilize Li-ion battery (LIB) anodes by the controlled SEI formation, administering mechanical robustness, and maintaining electrical conductivity within the electrode laminate.

Experimental results obtained during the research work have been mentioned and discussed as follows:
BIAN-based functional polymer binder to stabilize graphite anode for ultra-long cycling performance.

PVDF has been the conventional polymer binder to stabilize graphite anodes in a LIB. However, because of its non-conducting nature, inability to undergo doping in the cathodic environment to influence the SEI formation, and poor mechanical robustness disqualify it from being a go-to binder choice for ultra-long cycling of graphite anode at a high current rate for future applications. Therefore, this chapter details the design, synthesis, and application of a novel BIAN-p-phenylene (P-BIAN) copolymer as binder for graphite in a LIB. The salient features of P-BIAN that prove it to be superior to PVDF are (Figure 3): (i) better mechanical robustness (higher Young’s modulus), (ii) latching of the electrode laminate on to the current collector through electron rich diimine nitrogen atoms of P-BIAN, (iii) conjugated framework provides inherent electrical conductivity within the electrode laminate, (iv) its ability to establish tentative π-π interaction with graphite framework to administer mechanical robustness, and (v) most importantly, its low-lying LUMO enables it to undergo n-doping in reductive environment to restrict the excessive electrolyte decomposition on the anode surface to control the optimal SEI formation. The synergy between all the above-mentioned salient features stabilized the graphite anode in a LIB for over 1700 cycles at a high current rate of 1C with reversible capacity of 260 mAhg\(^{-1}\) and 95% capacity retention. The anodic half-cells were electrochemically characterized by cyclic voltammetry (CV) studies and dynamic electrochemical impedance spectroscopy (DEIS) studies to understand the interphase formation and its effect on the performance of graphite anode. Further, the postmortem characterization of anodes after electrochemical evaluation was carried out by XPS and FESEM techniques to understand the SEI thickness and surface morphology of the anode.

BIAN-based functional n-type conducting self-healing composite binder to stabilize silicon anode in a LIB.

Silicon is potent with 10 times higher gravimetric capacity (4200 mAhg\(^{-1}\)) than the commercial graphite (372 mAhg\(^{-1}\)). Therefore, it is regarded as the next generation anode active materials that can lead to development of LIBs with higher energy density. Also, it can be more economical than graphite because of its natural abundance. However, it has several drawbacks that have hindered its full-scale commercialization in the past decade. The drawbacks are as follows (Figure 4): (i) rapid volume expansion (~300%) on repeated charge-discharge that pulverizes Si particles into smaller fragments, (ii) the pulverization causes loss of interparticle electrical contact and electrical contact of particle to current collector, and (iii) continuous decomposition of electrolyte components on the newly pulverized Si surfaces causes thick SEI formation on the anode surface. These drawbacks together destabilize the Si anodes and cause rapid capacity fade with poor
cyclability. Therefore, various functional polymer binders that can mitigate these drawbacks have been explored. However, the synthesis of a versatile binder that can collectively mitigate all the drawbacks of silicon anodes is an important area of research. In this regard, this chapter reports the strategic design, synthesis, and application of a novel electrostatically hydrogen bonded P-BIAN/PAA composite binder (Figure 5) that is potent with excellent mechanical robustness, self-healing property, can maintain electrical conductivity within the electrode laminate, and can undergo n-doping by virtue of its low-lying LUMO to restrict the excessive electrolyte decomposition to form a thin SEI. Thus, the silicon anode was stabilized for over 600 cycles of charge-discharge with a high reversible capacity of 2100 mAhg\(^{-1}\) and 95\% capacity retention. The fabricated anodic half-cells were electrochemically characterized by CV and DEIS studies, respectively to understand the interfacial impedance and properties. Also, the electrochemically characterized anodes were subjected to XPS and FESEM techniques to understand the SEI thickness and anode’s surface morphology after cycling for over 600 cycles.

**BIAN-based functional n-type conducting covalently crosslinked composite binder to stabilize silicon anode for ultra-long cycling performance.**

As reported and discussed earlier, the electrostatic hydrogen bonding between the P-BIAN and PAA polymeric chains could empower the P-BIAN/PAA composite binder with an ability to stabilize Si anodes. However, for future applications, the demand of LIB with robust and durable cyclability is inminent. Therefore, in Chapter 4, a novel covalently crosslinked P-BIAN polymer matrices were designed, synthesized, and utilized as binder to improve the cyclic stability of Si anodes with higher reversible capacity. The covalently crosslinked P-BIAN binder matrices can restrict drastic volume expansion of Si particles for longtime because of the improved mechanical robustness in comparison to its hydrogen bonded counterpart in chapter 3 as shown in Figure 6. Also, the covalent crosslinking via diimine nitrogen backbone in the P-BIAN would tentatively lower its band energy of LUMO furthermore in comparison to the hydrogen bonded counter in chapter 3. Therefore, by the synergistic effect of its ability to render improved mechanical robustness and n-doping driven SEI formation, Si anodes were stabilized for over 1000 cycles with higher reversible capacity of 2500 mAhg\(^{-1}\) and 99.1\% capacity retention. The fabricated anodic half-cells were electrochemically characterized by CV and DEIS studies, respectively to understand the interfacial impedance and properties. Also, the electrochemically characterized anodes were subjected to XPS and FESEM techniques to understand the SEI thickness and anode’s surface morphology after cycling for over 1000 cycles.

**Research Purpose**

The pursuit of alternative anode active material that can potentially replace the conventional graphite anode and increase the energy density of next generation Li-ion batteries has been the prime focus of academia and industry. The emergence of Si as potential competitor to replace graphite by virtue of its two-fold significance (i) natural abundance for making LIBs cost-efficient and (ii) 10 times higher theoretical gravimetric capacity than graphite to increase the energy density
of LIBs has led to extensive research towards making Si a commercial choice. However, inherent failure mechanism of Si has kept its commercialization at bay. Therefore, to have comprehensive understanding of the chemical and electrochemical response of Si anodes during charge and discharge and stabilize its performance, researchers belonging to academia have been conducting extensive research activity get a breakthrough. In this dissertation work, to stabilize Si anodes to deliver high reversible capacity with long cyclability, the author has explored impact of novel BIAN-based functional polymers as binders by exploring their rich redox chemistry, electrochemical significance, and structure-property relationship to stabilize high-performance LIBs. The author has hierarchically tried to stabilize graphite and Si anodes in a LIB by designing BIAN-based polymers that can mitigate major drawbacks associated with these electrodes and most importantly tailor the formation of SEI on the electrode surface. The highlighting features of BIAN family of compounds are electron reservoir property, n-doping ability in reducing anodic environment, rich redox chemistry, intrinsic electronic conductivity, tunable electronic properties, and coordinating ability because of electron rich nitrogen atoms. Also, the compounds of this family have been utilized as ligands for transition metals for their catalytic utility. Therefore, their exceptional coordinating capability via the imine moiety has potential to form variety of composite polymers and monomers with an external dopant to diimine framework. This creates a wider scope of opportunity to explore a plethora of conducting/crosslinked, conducting/self-healing, and self-healing type BIAN-based polymers with varying electronic property to tune the interface as needed for stabilizing not only Si anodes but also other high-capacity materials like phosphorous, boron, MoS2, etc. in next-generation LIBs that suffer drastic volume expansion/contraction on charge-discharge. This class of compounds can find its versatile application in future energy storage technologies like Na, K, and Mg-ion batteries.

Research Accomplishment

Research Article


Conference Presentation Awards

1. Best Presentation Award of JAIST World Conference (JWC) 2020 - International Symposium for Innovative Sustainable Materials & The 7th International Symposium for Green Innovation Polymers (GRIP2020)

Presentation Title: “Lithium-Ion Secondary Batteries with Silicon Based Anode Highly Stabilized with Self-Healing Polymer Binder Matrices”

2. Excellent Poster Award at the 70th Annual Meeting of the Society of Polymer Science, Japan (May 26-28, 2021).

Poster Number: 3Pc063
Poster Title: “Crosslinked BIAN Polymer Matrices to Stabilize Silicon Anode in Lithium Ion Secondary Batteries”

Conference Participation (Oral)

1. “BIAN-paraphenylene Type Polymer Binder for Ultra-long Cyclable Lithium Rechargeable Battery”.
Agman Gupta, Rajashekar Badam and Noriyoshi Matsumi
The 60th Battery Symposium in Japan, Kyoto International Conference Centre, November 13-15, 2019

2. “Lithium-Ion Secondary Battery with Silicon Based Anode Highly Stabilized with Self-healing Polymer Binder Matrices”.
Agman Gupta, Rajashekar Badam, and Noriyoshi Matsumi
69th Symposium on Macromolecules, SPSJ (Society for Polymer Science Japan), Presentation Number 2ESB02, Web conference (online), Japan, September 16-18, 2020

3. “Polymerized BIAN/Poly(acrylic acid) Based Composite Binder for High Capacity Silicon Anodes in Lithium Ion Secondary Batteries”
Agman Gupta, Rajashekar Badam, and Noriyoshi Matsumi
The 61st Battery Symposium, WEB conference (online), Japan, November November 18-20, 2020
Abstract of Dissertation

Thesis title: Effect of salt addition on the properties of Poly(vinyl alcohol)

Doctoral of Degrees: Material Science

Laboratory: Masayuki Yamaguchi Laboratory

Student number: 1820412

Name: Riza Asma A Binti Saari

Research Content

Poly(vinyl alcohol) (PVA) is one of the most well-known water-soluble polymers. It is a semi-crystalline polymer, used in various applications, because it shows high mechanical strength and good biocompatibility. PVA exhibits strong intermolecular hydrogen bonding between hydroxyl groups in each chain, which is responsible for the crystallinity, and therefore, various properties. However, PVA fibers exhibit a low degree of molecular orientation. Because of the strong intermolecular hydrogen bonding between hydroxy groups, the chain orientation is prohibited at spinning process. Therefore, the modulus and strength are much lower than those predicted theoretically. In other words, the fiber with a high degree of orientation is difficult to be produced for PVA. One of the objectives of this research is to improve the molecular orientation in the PVA fiber to increase the modulus and strength. For the purpose, the effect of salt addition on the structure and properties of PVA aqueous solution, films and fibers were investigated in detail. The main aim of this research is to understand the effect of the salt addition on the hydrogen bonding to control its strength.

The present study focused on the effects of the addition of potassium, sodium, magnesium, and lithium salts on the rheological properties of PVA aqueous solutions, solid state of PVA films and also fibers.

A plateau of shear storage modulus was detected in the low frequency region for PVA aqueous solution. This phenomenon demonstrates that the solution has a well-developed network structure owing to the hydrogen bonding. The addition of lithium salts evidently decreased the value of the plateau modulus, which became obvious with temperature. The anion species in the salt plays an important role in determining the rheological properties, including
the magnitude of the plateau modulus as demonstrated by the experimental results. In the Hofmeister series (HS), the iodide anion was classified as a “water-structure-breaker” ion, which decreased the plateau modulus with the shear viscosity effectively. Besides, at high temperatures, the modulus decreased with the LiI addition owing to the reduced extent of hydrogen bonding.

The data obtained using films demonstrated that the strong ion-dipole interactions between anions and PVA chains also have a significant impact on the glass transition temperature and crystallinity. This is the first study to reveal that the impact of the salts addition follows the Hofmeister series. The experimental results using various bromine salts clarified that Li⁺ is more effective at disrupting the water structure than other cations such as Na⁺, K⁺ or Mg²⁺. Furthermore, further experiments using lithium salts with various anion species verified that lithium salts are responsible in determining the hydrogen bonding in aqueous PVA as well as the crystallinity in the solid state which is responsible for the mechanical properties of films. This phenomenon clearly follows the HS in the order of LiClO₄ > LiI > LiBr > LiNO₃ > LiCl. Besides, magnesium salts also show an interesting result as the glass transition temperature of the PVA films was enhanced. This result was attributed to the strong ion-dipole interactions between magnesium salts and PVA chains.

In case of PVA fiber, the addition of LiBr in the spinning solution greatly reduced the inter- and intramolecular hydrogen bonding which results in the higher level of molecular orientation after wet-spinning process. Furthermore, the salt was removed from the fibers in the coagulation bath and/or washing process. As a result, the modulus and yield strength were greatly improved.
Research Purpose

Although much research has been conducted on PVA, there are still limited reports on the rheological properties of its aqueous solution because of the difficulty in the measurements. In this study, the rheological properties obtained by an appropriate measurement method that was established in this study were discussed in detail to clarify the role of anion and cation species, respectively.

Besides, there was no study on the development of PVA fiber with the addition of a salt. Since PVA has a strong hydrogen bonding, it is difficult to obtain a highly oriented state, and the resulting defects cause a decrease in strength and elastic modulus. Therefore, the mechanical properties are poor compared to the theoretical values. The addition of an appropriate salt reduced the hydrogen bonding in aqueous solution and made it possible to show a high degree of orientation at wet-spinning process. This is the first report on the modification of mechanical properties of PVA fibers by the salt addition.

Water-soluble polymers such as PVA have a significant impact on our lives because of their versatility and a high demand due to SDGs. The findings in this thesis on the modification of structure and properties of PVA by the salt addition would be very useful for new material design in the future. Since this systematical study on PVA was firstly summarized in relation to the HS, it will be easier to understand and also applicable to not only PVA but also various hydrophilic polymers. Besides, there are a plenty of possibilities to control the characteristics of a single polar polymer by the addition of different types of salts, which provide a new specific function. It is expected that further interesting findings can be obtained, not only in basic research but also for the industrial aspect.

Research Achievements

Paper publication (peer-reviewed)

2. **R.A. Saari**, R. Maeno, W. Marujiwat, M.S. Nasri, K. Matsumura, M. Yamaguchi,
   Modification of poly(vinyl alcohol) fibers with lithium bromide, Polymer 213 (2020) 123193.


**Conferences – Poster session (peer-reviewed)**

**International Conferences**


**Domestic Conferences**


**Awards**


2. Receiver of Japanese Government (Monbukagakusho) MEXT Scholarship.
A Programmable RNA Knockdown Using CRISPR-Cas13 Ribonuclease
Towards Gene Therapy

Materials Science, Tsukahara Laboratory
Student Number: 1820413
Name: Saifullah

Abstract

Research Content:
Recent approvals in gene therapy have paved the road for an extensive second upsurge of therapies and forefront the groundwork for next-generation treatment strategies. CRISPR-Cas effectors have flourished as an overwhelming tool that can potentially endeavor future genetic medicine. In this doctoral thesis, I investigated the therapeutic potential of anaplastic lymphoma kinase (ALK) and developed optimal parameters for programmable RNA knockdown using CRISPR-Cas13a ribonucleoprotein. Furthermore, I applied the optimized protocol for echinoderm microtubule-associated protein-like 4 (EML4)-ALK transcript knockdown as a proof-of-principle for RNA-based cancer therapy. More explicitly, this dissertation consists of five chapters. Where, chapter one summarizes the recent development in RNA-targeted therapeutics including RNAi and CRISPR-Cas systems, and discusses the landscape of EML4-ALK-positive lung cancer treatment. This summarization of recent work will assist to understand the recent advancement of RNA-targeted CRISPR-Cas technologies and underpinning the contemporary ALK-positive cancer therapeutics.

In the second chapter, I explored the clinical outcome and associated genes of ALK expression using integrative bioinformatics. ALK is a tyrosine kinase receptor that is genetically altered in several cancers, such as non-small cell lung cancer (NSCLC), melanoma, lymphoma, and other tumors. Although ALK is associated with various cancers, the relationship between ALK expression and patient prognosis in different cancers is poorly understood. Here, I show a correlation between ALK expression and its clinical outcome in patients with lung adenocarcinoma (LUAD), melanoma, ovarian carcinoma (OV), diffuse large B-cell lymphoma (DLBC), acute myeloid leukemia (AML),
and breast cancer (BC) using different computational assessments. I analyzed ALK transcriptional expression, patient survival rate, genetic alteration, protein network, and gene and microRNA (miRNA) co-expression. I found that deregulated expression of ALK is associated with a high mortality rate in ALK-positive cancers. I identified 214 missense mutations, 24 truncating mutations, seven fusions, and two in-frame mutations, with the highest alteration of ALK in melanoma. I further showed that 17 genes and 19 miRNAs were exclusively co-expressed and found that EML4 was the most positively correlated gene. The gene ontology and signaling pathways of the genes co-expressed with ALK involved in these six cancers were also identified. My findings offer a basis for ALK as a prognostic biomarker and therapeutic target in cancers, which will potentially contribute to precision oncology.

In the third chapter, I developed optimal parameters for programmable and effective RNA knockdown using marker genes including firefly luciferase and mCherry transcripts. RNAi technology has noteworthy potential as a future medicine and could ideally be used to knock down disease-related RNAs. However, owing to frequent off-target effects, limited accessibility of nuclear transcripts, and low efficiency, the medical application of the technique remains challenging. Here, I first evaluated the stability of the Cas13a transcript and guide RNA. Next, I optimized the Cas13a and guide RNA expression vectors to achieve effective knockdown of firefly luciferase (FLuc) transcript, used as a target RNA. The knockdown specificity of Cas13a on target-search was next examined. I found that the 1:3 molar ratio concentration of Cas13 and guide RNA vector is preferable for effective knockdown than the vector amount. Based on the Cas13a selectivity results, I observed restricted endonuclease activity in 3′ crRNA–gRNA orientation. I also found the highest activity between 24–30 bp long gRNAs with limited mismatch tolerance. Cas13a could effectively knock down FLuc luminescence (70–76%), and mCherry fluorescence (72%). Accordingly, Cas13a has strong potential for use in RNA knockdown and regulation.

Next, chapter fourth showed the feasibility of Cas13a ribonuclease in downregulation of oncogenic driver EML4-ALK expression in human disease model lung cancer cells. ALK tyrosine kinase inhibitors provoke a significant anti-tumor response; however, they inevitably succumb to the acquired resistance. Therefore, an alternative
therapeutic strategy that limits ALK over-activation is necessary for the treatment of this lung cancer. Here, I show that the CRISPR-Cas13a effector possesses effective knockdown potency for oncogenic driver EML4-ALK transcript in lung cancer cells. I found the EML4 transcript was not substantially expressed but ALK expressed 80–100 fold higher in ALK-positive lung cells compared to a non-fusion transcript in HEK293T cells. I also found that the EML4-ALK oncoproteins were robustly down-regulated (>80%) by employing Cas13a in those lung cancer cells based on western blot results. Consequently, the tyrosine kinase phosphorylation (50–70%) and cell growth (up to 40%) were inhibited. Overall the obtained data demonstrated that the CRISPR-Cas13a protein downregulated the ALK expression in the lung cancer cells. Thus, CRISPR-Cas13a mediated EML4-ALK RNA knockdown devises a potential therapeutic strategy for treating ALK-positive lung cancer.

Finally, chapter V recapitulates the total work and discusses the benefits, challenges, and future directions. In conclusion, Cas13a has strong potential for use in RNA regulation and could contribute to the development of next-generation genetic medicine.

**Research Purpose:**

In the course of my doctoral research, I have identified ALK gene as a prognostic biomarker and therapeutic target in cancer using bioinformatics analysis and developed a potential therapeutic strategy for the ALK-positive lung cancer using the CRISPR-Cas13a system in disease model human cells. The principal objectives of the current doctoral thesis are given below:

- Identification of ALK expression as a prognostic biomarker and therapeutic target in ALK-positive cancers using integrative bioinformatics approach
- To break down of firefly luciferase RNAs using the CRISPR-Cas13a machinery as a target model for the further application in disease-relevant RNAs, which helps to comprehensively understand how is the efficiency, specificity, and functionality of Cas13a ribonuclease on a target-specific RNA.
- To employ the CRISPR-Cas13a machinery in downregulation of EML4-ALK oncofusion RNAs for the development of novel treatment system for Non-small-cell lung carcinoma (NSCLC)
Research Accomplishment:

❖ **Original Research Article**


❖ **Abstract publication (International Conference)**


9. **Saifullah**, Nagasawa H, Tsukahara T. Proposing a probes-on-carrier based oligo-DNA microarray method for SNPs detection. JAIST Japan-India Symposium on Materials Science (JISMS), March 5-6, 2018 (Poster). (Peer-reviewed)
Abstract

Study of strain dependence of physical properties in MoS$_2$ layers by in situ transmission electron microscopy observation

XIE Lilin (1820416) Oshima Lab
Doctor of Science (Materials Science)

1. Research Contents

Background

Owing to the ultrathin crystal structure, two-dimensional (2D) materials, such as molybdenum disulfide (MoS$_2$), exhibit unique mechanical and electronic properties that make them promising for the application in electronic devices. Applying strain is an effective method to modify its physical properties since strain can modulate not only atomic structure but also electronic structure. Theoretically, such strain dependence of structure or electronic structure have been investigated by the first principle calculations and molecular dynamic calculations, experimental results have been rarely reported. I developed in situ transmission electron microscope (TEM) holder equipped with stretching device and investigated the strain dependence of the atomic structure of MoS$_2$ nanosheets at an atomic scale. I found that the MoS$_2$ nanosheets formed ripple structure at subnanometer scale in amplitude. The ripple structure was explained by nonlinear mechanical response of MoS$_2$ nanosheets, since the Poisson’s ratio was not constant with increasing the applied strain.

Aim

In this study, transmission electron microscopy is used to characterize MoS$_2$ layers at the atomic scale, and the in situ TEM holder equipped with a stretching function has been developed to investigate the influence of strain on the properties of MoS$_2$ layers under tensile strain. The design of the stretching TEM holder is shown in Fig.1(a), and a Si chip is introduced to mount the 2D MoS$_2$ layers, as shown in Fig.1. (b). By applying the bias voltage on the piezo actuator, a displacement will be generated and drive the motion of the titanium plate that stretches the Si chip, thus the sample mounted across the trench of Si chip will be stretched. Therefore, the in situ TEM observation of the MoS$_2$ layers under strain conditions can be achieved, correspondingly, the atomic-scale structure can be obtained from the high-resolution TEM (HR-TEM) image.

Figure 1. (a) Design of the stretching TEM holder. (b) The optical image of Si chip with the MoS$_2$ layers suspended on the trench. The red arrow indicates the stretching direction.
As mentioned above, the atomic-scale structure of MoS$_2$ layers can be obtained using the developed in situ TEM holder. By analyzing the atomic-scale TEM image of MoS$_2$ layers under strain conditions, I aim to investigate the strain dependence of physical properties in MoS$_2$ layers, such as the structural evolution of the ripple structure under strain condition, the bending behavior of multilayer MoS$_2$, and also the strain dependence of its Poisson’s ration.

**Experimental results**

With our newly developed TEM holder, the suspended MoS$_2$ nanosheets were observed. In the TEM observation, I found that the contrast in the HR-TEM image gradually modulated along the armchair direction (y-direction), indicating that the ripple structure was formed parallel to the armchair configuration of MoS$_2$. Owing to the wavy pattern, the projected lattice spacing of the rippled MoS$_2$ nanosheet was apparently modulated along the ripple direction, as shown in Fig.2 (a). By applying geometrical phase analysis (GPA) to the HR-TEM image, the lattice modulation can be obtained as the apparent strain ($\varepsilon_{GPA}$), as shown in the map of Fig. 2 (b). The distribution of corresponding apparent strain, as shown in Fig. 2 (c), is sensitive to the geometric structure of ripple. Therefore, the rippled structure of MoS$_2$ nanosheet can be estimated at the sub-nanometer scale based on its apparent strain. The observed ripple structure in Fig.2 (a) was estimated to be an inclined sinusoidal pattern ($z = A\sin\left(\frac{2\pi y}{\lambda}\right)$, where $Z$, $A$, and $\lambda$ are the out-of-plane displacement, amplitude, and period, respectively), and the $\lambda$ and $A$ were estimated to be 5.5 and 0.3 nm, respectively.

![Figure 2](image-url)

**Figure 2.** (a) Filtered HR-TEM image of the ripple structure in MoS$_2$ nanosheet. (b) Corresponding apparent strain map ($\varepsilon_{GPA}$), inset is the overlapping of TEM image and corresponding apparent strain map. (c) The apparent strain profile of the region marked with black frame in (b).

Additionally, I experimentally investigated the in situ mechanical response of MoS$_2$ layers under strain conditions. The geometric evolution of ripple structures in MoS$_2$ layers under tensile strain was revealed. It was found that the relationship between the tensile strain ($\varepsilon_x$) and the amplitude/period was not followed the 1/4 power scale law, as shown in Fig.3. (a), indicating the observed ripple structure at the atomic scale does not follow the classic continuum mechanics. As the bending in continuum mechanics is described by the plate model, in which the expansion and contraction occur on either side of the bent layers by assuming a strong interlayer interaction. Instead, our results can be explained by the layer model, in which the bending of each layer does not involve
such expansion or contraction, due to the weak interlayer interaction. Further, By correlating the theoretical calculations with experimental results, where the width of the non-ripple region and the ripple structure are same due to their smooth connection, I retrieved the relationship between the tensile strain and geometric structure of the ripple structure, which is $\varepsilon_{\chi} = \frac{\pi^2 \lambda^2}{(2\lambda^2)}$, where $\nu$ is the Poisson’s ratio. The values of Poisson’s ratio can be calculated by substituting the experimental values, as shown in Fig. 3 (b). I found a nonlinear mechanical response of the MoS$_2$ layers: Poisson’s ratio was constant below the tensile strain of 1%, while it gradually increased for the tensile strain higher than 1%. This nonlinearity can be explained by the structural model that the bond length does not change in proportion to the tensile strain above 1%.

Figure 3. (a) Relationships of the period and amplitude of ripple structure with $\varepsilon_{\chi}^{1/4}$ ($\varepsilon_{\chi}$: the tensile strain). (b) Relationships between the $\varepsilon_{\chi}$ and estimated Poisson’s ratio.

2. Research significance

2D materials have attracted abroad research interests due to their ultra-thin layer structure that presents unique physical properties. However, the experimental report on the mechanical behavior of 2D materials at the atomic-scale is rarely reported. Therefore, it is important to clarify the strain dependence of the mechanical response of 2D materials on atomic scale, since the physical properties occurring on the nanoscale are sensitive to the strain.

In this study, I developed an in situ stretching TEM holder, which realized the atomic-scale resolution and displacement rate, for investigating the physical properties of 2D materials. By using the in situ TEM holder, the tensile strain can be applied on 2D materials simultaneously during the TEM observation. Furthermore, I proposed a strain-based analysis method that can estimate the ripple structure of MoS$_2$ layers on sub-nanometer scale. Since the modulation of projected lattice spacing of the ripple structure, which is related to the geometric parameters, can be retrieved by applying GPA on the HR-TEM images. Our approach provides an effective method that contributes to a better understanding of 2D materials.

Additionally, I investigated the mechanical behavior of MoS$_2$ layers under strain conditions. I experimentally revealed the strain dependence of the ripple structure, it showed that the period and amplitude depended on the tensile strain. These experimental results could not be explained by
continuum mechanics and showed that Poisson’s ratio was varied depending on the tensile strain. By correlating the theoretical calculations with experimental results, I proposed a structural model that such a nonlinear response was explained by the mechanical response that the bond length in MoS$_2$ structure did not change in proportion to the tensile strain. Such a study using in situ techniques to understand the mechanical behavior of 2D MoS$_2$-based materials on atomic scale has not been previously conducted. Our results clarified the inherent nonlinearity, which is important in understanding the specific mechanical properties of 2D materials.

This study proposes an experimental method to reveal the strain dependence of physical properties in 2D materials on the atomic scale. Such new characterization and analysis methods present a new field for investigating atom-scale properties of 2D materials under strain conditions. I believe that this study will greatly advance the understanding of the mechanical reactions of 2D materials. In this regard, the conducted research is novel and holds immense potential to better understand the atomic-scale materials.

3. Research Accomplishment

- **List of Publications**
  2. L. Xie and Y. Oshima, “Nonlinear mechanical response of rippled MoS$_2$ nanosheets evaluated by in situ transmission electron microscopy”. (Submitted, peer reviewed)

- **Presentation**
Synthesis of weak acid polymers and investigation of electrical properties toward field-effect transistors (FETs)

Doctor of Science (Material Science)

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Part 1: Research content

Background and aims

Proton transport acts as an essential role in various systems, including biological systems. Many carboxylic acid-functionalized materials were reported to act as a proton conductor. Although the proton conductivity of materials or biopolymers containing weak acid groups is rarely comparable to polymers with strong acid groups, the study of proton transport in these materials is necessary to understand the working mechanism in many biological systems. As commonly found in biomaterials, it is still unclear how carboxylic acid concentration affects proton conduction. This research mainly aims to synthesize polymers with well-defined weak acid concentrations and use them as proton conductive materials, as shown in Figure 1a.

Figure 1. The schematic represents the research strategies.

Moreover, to understand more about proton transport in biological systems, the use of protonic field-effect transistors (H⁺-FETs) is a promising candidate for bridging biological and electronic systems. In previous H⁺-FETs, the protonic current was generated from a palladium hydride (PdHₓ) electrode under the H₂ atmosphere and a direct current (DC). This Pd electrode reaction affects proton-conductive materials' electrical properties and should be avoided. Therefore, the another objective is to develop the thin film’s ion conduction in the devices with gold FET electrodes and alternative current (AC) impedance measurement, named PFET and CFET, to prevent the electrode reaction, as shown in Figure 1b-c.
This dissertation consists of 5 chapters: 1. General introduction of proton conductive polymers and FETs, 2. Proton transport pathways of weak-acid functionalized styrene-based copolymers thin films, 3. The development of the parallel-shaped FET electrodes for alternative current impedance measurement, 4. The development of the comb-shaped FET electrodes for low conductivity polymer thin films investigation, and 5. General conclusion.

**Experimental results**

Chapter 2 of this dissertation examined the effect of carboxylic acid concentration on both interfacial and internal proton transport since protons can transport across both the materials’ internal and the surface. Several styrene-based polymers containing well-defined carboxylic acid concentrations were synthesized to explore the influence of weak acid concentration on polymer thin films’ electrical properties. The IR p-polarized multiple-angle incidence resolution spectrometry (pMAIR) spectra show the higher ratio of the free carboxylic acid rather than cyclic dimer carboxylic acid form in the high carboxylic acid concentration polymer thin films, facilitating the efficient hydrogen bonding networks and proton transport. Furthermore, the water uptake of thin-film result reveals the relatively low and similar number of adsorbed water molecules per carboxylic acid group regardless of acid concentration, suitable for applications since the applied devices will less suffer from the swelling effect. Remarkably, as shown in Figure 2, polymer thin films with high carboxylic acid concentrations allow internal proton conduction because of the efficient hydrogen bonding network. In contrast, interfacial proton conduction was found in low carboxylic acid concentration polymers because the free acid groups were more located at the film interfaces.

![Figure 2](image)

**Figure 2.** Schematic representation of the dependence of main proton transport pathway in polymer thin film on the carboxylic acid concentration.

In chapter 3, the devices with Au electrodes and AC measurement were newly developed to investigate the low proton conductivity polymers’ electrical properties without the effect of electrode reaction. Compared to the DC method, the AC method gives us more information and separation of each component depending on the frequency. The devices were designed as a small parallel-shaped of several micrometers channel size, named PFET. The previously fabricated weak-acid functionalized styrene-based copolymer’s electrical properties were successfully investigated using Au electrodes and AC-impedance measurement. However, due to the relatively low proton conductivity and high resistance of the fabricated polymer thin film,
the electrical response of polymer thin film was dominated by the double layer capacitance response. Furthermore, the electrical properties of the film cannot be estimated.

Therefore, in chapter 4, the devices with highly extended channel width and short channel length comb-shaped gold electrodes (CFETs, as shown in Figure 3) were fabricated to reduce the resistance of polymer thin films. The channel length of CFETs was varied to study the effect on the polymer thin film’s resistance reduction. The low proton conductivity polymer thin film was applied to the designed CFET, and its electrical properties were successfully investigated using the AC-impedance measurement. The polymer thin film’s impedance response obtained from CFETs was clearly shown without the dominated by the double layer capacitance response, as shown in Figure 4. The semicircle and relative humidity (RH) dependence responses were also observed, confirming the response of polymer thin film’s proton conduction, and the electrical properties of low conductive polymer thin film can now be estimated. The influence of channel length shows that CFETs with shorter channel lengths are better in the polymer thin film’s resistance reduction. Furthermore, the polymer thin films’ proton conductivity and normalized resistance on CFETs were differed from that on the quartz substrate, indicating the influence of the film/substrate interface on the film’s electrical properties.

![Figure 3](image3.png)

**Figure 3.** a) The image of prepared CFET with 10 µm channel length. And the optical microscopic images of b) 5 µm, c) 10 µm, and d) 15 µm channel length CFETs.

![Figure 4](image4.png)

**Figure 4.** a) The Nyquist plots of carboxylic acid-functionalized styrene-based polymer thin film, P4VBA100, at various RH on the CFETs with the channel length of 15 µm, and b) the zoom-in image of the low resistance region.
Conclusion

First, the carboxylic acid concentration was found to influence the proton transport pathways of the St-based polymer thin films. The high acid concentration films dominantly provide internal proton conduction, while low acid concentration films prefer interfacial proton conduction. Second, there are two pathways for proton transport. One is the interfacial region between substrate and polymer thin film. Another is the interfacial region between polymer thin film and air. I found the strong effect of the substrate surface on the polymer thin film’s electrical properties. Third, the electrical properties of low proton conductive materials were successfully investigated using the developed CFETs and the AC-impedance measurement method without affected by electrode reaction.

Part 2: Research purpose

This study provides insight into the proton transport behavior that differs depending on the weak acid concentration and the substrate surface, which could support the explanation of proton transport in many systems. Moreover, the developed devices overcome the drawbacks of dominating double layer capacitance response for weak acid polymer thin films and the short circuit between two electrodes through the thin dielectric layer in the AC-impedance measurement method toward FETs. Notably, the electrical properties of relatively low proton conductive materials can now be estimated without being affected by electrode reactions.

Part 3: Research accomplishments

“Interfacial and Internal Proton Conduction of Weak-acid Functionalized Styrene-based Copolymer with Various Carboxylic Acid Concentrations”

Athchaya Suwansoontorn, Katsuhiro Yamamoto, Shusaku Nagano, Jun Matsui, Yuki Nagao


Keywords: Carboxylic acid; FETs; Interfacial proton transport; Internal proton transport; Impedance measurement
Abstract of dissertation

Part 1: Research Content

Background:
The sense of touch allows humans to physically perceive and interact with their environment. Touch is even crucial for robots, as robots equipped with tactile sensation can more human-like intelligently interact and manipulate with the surroundings and real-world objects because touch sensing could perceive and provide direct feedback of diverse information/characteristics of the contact (such as contact force/pressure, sense of pain, shape, size, and stiffness). Unfortunately, most of the current robot is still missing the sensitive artificial skin. The challenges of touch sensing technology relate to mimicking the inherent complexity of natural skin structure that has a particularly high density of various types of sensory receptors.

Although in recent decades, tactile sensor technology has shown great advances in design [1,2]. The common techniques currently being used for the tactile sensors are based on capacitive, piezoresistive, piezoelectric, magnetic, and optical transduction methods. Each of these transduction principles is employed to convert physical stimuli from the environment into an electrical signal for a data acquisition system. The previous approach focused mostly on developing a skin-like structure with a matrix of sensing elements at small-scale (fingertips/grippers) without considering the difficulty in the level of system design, such as complexity of wiring, the bulk of electronics, etc. for the deployment of a large-scale tactile sensing system, as well as requirement of highly flexible/soft and durable. At present, typical skin designs involve integrated electronic circuits formed by many spatially distributed modular sensing points, allowing data to be processed locally, and sent through a serial bus to reduce the number of wires [3]. However, this design based on the integration of various electronic components is low spatial resolution and very expensive, the embedded electronic sensors inside are not durable enough for frequent collisions.

Fig. 1. The proposed TacLINK with tactile force sensing for robot links (A demonstration video and open source at https://github.com/lacduong/TacLINK).
**Research Aim:**

The research aim is to develop a vision-based artificial sensory system for robotic links, called TacLINK, which can be assembled to form a whole-body tactile sensing robot arm. The research contents ranging from developing sensing principles, mechanical design, fabrication, modeling, algorithms to implementation and experimental evaluation, could be summarized and introduced as

- **Mechanical Design:** TacLINK was designed as an elongated structure [see Fig. 1(a)], consisting of a rigid transparent tube covered by continuous artificial skin, made by a soft silicon rubber which is very safe, and comfortable to touch, as well as durable and inflatable to change its form and stiffness. The deformation of the skin is tracked by two cameras installed at both sides of TacLINK to capture an array of marker points sit on the inner wall of the skin.

- **Vision System Model:** The vision system with two facing cameras is successfully modeled. The mathematical model and analysis of the effect of vision parameters on the characteristics of 3-D measurement are provided. The robust techniques for image processing, stereo registration, and 3-D reconstruction are developed. The proposed tracking algorithms could track efficiently all the markers locating on the inner wall to provide detailed deformation of the skin surface [see Fig. 1(b)]. TacLINK perceives tactile information through the three-dimensional (3-D) deformation of the skin that results from the tracking of an array of marker points sitting on its inner wall by two cameras installed at both sides of the transparent tube.

- **Finite Element Model:** The research used the finite element method (FEM) to establish the relationship between force and deformation of artificial skin. The analytical formulation of flat shell elements is formulated, including defining element geometry, formulating elemental displacement, strain, and stress fields, resulting in the structural stiffness matrix of the skin is obtained that is a key to calculate the contact force which is a great difficult in the previous researches. Based on FEM, we could also characterize the skin to evaluate and optimize the design for specified applications.

- **Tactile Force Computation:** This research utilizes the advantage of the finite element (FE) model, the contact forces can be treated as the acting concentrated force resulting from the nodal forces of the FE model. The distribution of applied forces, therefore, can be calculated easily based on a structural stiffness matrix and extracted displacements of the markers.

**Experimental result:**

The design aim of TacLINK is to sense various contacts with real-world objects and humans. Thus, we performed interactive experiments in which TacLINK contacts real objects, and humans. Fig. 2 (a)-(b) shows the sensing performance of TacLINK contacting with cylindrical and flat surfaces, respectively. We can observe that not only the shape of the contacted surface is robustly reconstructed, but also the location and intensity of contact force are provided in detail on the whole skin surface. Tactile forces are distributed only in contact regions but are absent from other regions, even those that showed deformation.

Similarly, although diverse actions could be acted from human fingers and hands, such as touch, press, poke, tickle, rub, pinch, etc. TacLINK could efficiently sense these various contact scenarios. For example, press and tinkle with a finger and multiple fingers are shown in Fig. 2 (c) and (d), respectively. These results indicate that TacLINK can robustly reconstruct both 3-D deformation and external acting forces in large and multi-contact regions. Generally, under conditions of physical interaction, TacLINK documents detailed 3-D deformation of the skin surface caused by external forces, of which location and intensity are represented by the distribution of tactile force.

It is noteworthy that previous vision-based tactile force sensing techniques with markers [4], such as machine learning and experimental calibration, could estimate the total force applied, but were not able to determine the distribution of applied forces in scenarios such as under large area and multiple point contacts. Besides, tactile skin designs with discrete electronic sensing elements can not detect a touch in regions not occupied by such sensing elements. However, using the proposed FEM approach to reconstruct contact forces over a large and continuous area, the proposed vision sensing system can simultaneously perceive contact forces throughout the skin surface efficiently.
Part 2: Research Purpose

Originality:

a) It is rather challenging to develop the robotic tactile skin by the conventional transduction methods. However, this research utilized vision technology to develop a large-scale tactile force sensing system. The developed system has many advantages, such as a miniature hardware platform, that cameras could be installed to track the deformation of a large skin surface without the need of embedding any sensing elements, resulting in eliminating the challenges in wiring, the bulk of electronics.

b) Previous vision-based force-sensing research has met difficulty in finding an efficient method to determine the relationship between force and displacement, such as analytical methods, experimental calibration, or machine learning. However, these techniques could provide the estimation of the total applied force, but could not derive the location and force distribution of multiple contact points or surface contact. The research is the first time utilizing the finite element model (FEM) to determine the force from the displacements of artificial skin by using a structural stiffness matrix of the skin.

c) The artificial skin of TacLINK not only provides tactile force feedback but can change its form and nominal stiffness by providing air to inflate at low pressure (0-2.5 kPa).

Novelty:

a) A novel large-scale tactile sensing system at low cost for robot links: The research proposes an accomplished tactile sensing system for robot links with a large sensing area ~500cm² at very low cost (about 150$). TacLINK is high sensing performance in both contact geometry and contact force upon interactions between the robot and surroundings.

b) An efficient vision-based tactile sensing system: The proposed vision system comprising two cameras with proposed algorithms tracks efficiently displacements of all the markers locating on the inner wall of the elonged skin surface. The proposed vision-based sensing shows the advantages for developing large-scale tactile sensing systems, such as minimizing the challenges in wiring, the bulk of electronics, and the risk of damage.
The finite element method (FEM) for tactile force sensing: Previous research on vision-based force-sensing faced difficulty in establishing the relationship between force and displacement/deformation. This research utilized the FEM to derive the structural stiffness of the skin that is a key to calculate the contact force straightforwardly.

Possibilities:

a) TacLINK is scalable in size, durable in operation, and low in cost, as well as being a high-performance system, that can be widely exploited in the design of robotic arms, prosthetic arms, and humanoid robots, etc. For instance, the intelligent robot, such as a humanoid robot, service robot, therapist robot, frequently interacts with the human at close distance, as well as these robots are expected to have the ability to manipulate objects dexterously. Thus, equipping and deploying the robot with the sense of touch enables safe and intelligent interaction and better manipulation. Besides, the generation of human-like prosthetics with artificial sensing and nerve is very promising in practice. Smart prosthetics can help the handicapped person perceiving pain, sense real-world objects, etc. as the sensory function of a normal person. This approach is very practical and expected to realize in near future.

b) The research introduces a highly scalable structure for the development of vision-based tactile sensing devices: The proposed artificial skin with markers is very simple and easily fabricated by the casting method, the shape and size are customizable for the specified application. The finite element method (FEM) provides the structural stiffness of the skin. Vision technology is now powerful and small in size, thus a set of cameras can be set to track the displacements of the skin. Thus, future works will continue to use this technique for other parts of a robot, such as the head, hands, fingers, chest, legs, etc., and various sensory devices in the industry, medical, and healthcare. The research expects to contribute significantly to the field of tactile sensing technology with a generalized method for designing vision-based tactile devices. Besides, the deployment of the tactile devices on the control tasks, such as safe reaction, interactive action, is also an essential research direction for future works.

Part 3: Research Accomplishment

Journal Papers (peer review):

International Conference Papers (peer review):

Patent:
1. ホアンヴァン, Duong, 朝比奈, 触覚検知装置及び触覚検知方法, 出願番号: 2019-018391 (2019/02/05)

Reference

1: Eigenfactor: 0.01428 (#1 in robotics), total citations: 14,259 (#1 in robotics), impact factor: 6.123 (#2 in robotics)
ABSTRACT

Study of Capacitive Coupling via Mica Nanosheets through Measurement of Mechanical Energy Dissipation using Frequency Modulation Atomic Force Microscopy

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Research Content

The invention and advancement of atomic force microscopy (AFM) [1] broadened the area of investigation for exotic materials varying from metallic to semiconducting and insulating as well as biological materials with crucial functionalized properties. Through measurements of atomic-scale force interactions between an AFM tip and a sample, such exotic materials started to reveal their atomic arrangements, defects, and reconstructions which are the prerequisite bases for their applications and device fabrications. Besides atomic-scale imaging, the demanded surface nanoscale properties, for example, current tunneling, surface–interface resistances, capacitances, mechanical responses, energy dissipations, etc. have also been examined simultaneously. In addition, to minimize the mechanical energy dissipation due to stick-slip occurring in a contact AFM [2] and to preserve the natural environmental characteristics especially for soft polymer or biological samples, a novel AFM method known as dynamic AFM (d-AFM) or frequency modulated atomic force microscopy (FM-AFM) had been developed by T. Albrecht et al. [3]. Subsequently, it took the attention of most surface science researchers due to its non-destroying imaging capability to reveal various additional unexpected phenomenon such as nanoscale capacitance between the tip and the sample as well as mechanical and electrical energy dissipation. Among those, the energy dissipation measured in the FM-AFM has been attracted with intense curiosity for nearly the last 30 years after the declaration of FM-AFM being able to separately measure the conservative and nonconservative interactions [4].

The mechanical dissipation is related to the change in oscillation amplitude of an AFM cantilever at its resonance that periodically interacts with the sample surfaces under a constant resonance frequency shift (\(\Delta f\)), typically comprises supplementary information for the entire characterization of the force interactions. Such experimental observations often convey the possibility to reveal the extreme local properties of a sample surface which may vary for conductors, semiconductors, and insulators. Nowadays, several analytical and numerical methods have been established to interpret the results obtained through spectroscopic force–distance curves as well as the dissipation–distance curves under various attractive and repulsive interactions which are directly connected to the frequency shift (\(\Delta f\)), amplitude change, and phase change of the cantilever oscillation [5–8].

Even though there are persistent studies on the force and dissipation measured in the FM-AFM, however, it is neither fully understood nor straightforward for explanation due to its nonlinear distance dependence variation within a very small tip–sample separation and therefore still attracts much attention. Hudlet et al. analytically described the capacitive force between a metallic tip and sample by approximating the probe–sample model as a dihedral capacitor [5]. Their results showed that the tip apex contribution was dominant for imaging and force analysis. On the other hand, Gotsmann et al. discussed conservative and dissipative interactions between a Si tip and a mica surface briefly with the help of numerical simulation.
technique [7]. Models of force and experimental Δf–distance were used for numerical simulation to simplify the complexity regarding various force interactions between the tip and the sample along with good use of dynamic spectroscopy. Recently, Arai [9] showed a proportional relationship between the measured dissipation and the frequency shift of the cantilever. All those literature shows the importance to conduct the researches using FM-AFM for the unresolved phenomenon.

We simultaneously observed the topographic and energy dissipation images using two output channels of the respective signals of our FM-AFM system. The spectroscopic measurement was performed concurrently with the topographic imaging to some preselected points over the mica nanosheet and the bare Ir/Si substrate by varying the cantilever height in defined ranges. We chose two different thinly cleaved mica regions corresponding to 3 nm (3-layer mica) and 5 nm (5-layer mica) to compare the differences in dissipation and forces on the 3- and 5-layer mica nanosheets and the bare Ir/Si substrate. At a positive Δf, we primarily confirmed the FM-AFM images, where the repulsive force acted. The interaction force curves were numerically calculated from the Δf–distance curves using Sadar method [10]. From the force–distance curve, we found long-range electrostatic and van der Waals forces in the attractive regime with decreasing tip-sample separation, followed by short-range repulsive interactions at closer separations. The force was slightly greater on the mica surface, easily noticeable from the force–distance curve. Simultaneous measurement of energy dissipation both on the mica nanosheet and the bare Ir/Si substrate showed higher dissipation on the mica region. Due to the electrostatic interactions, Joule heat energy dissipation dominated between the smaller tip–sample separation. Such a phenomenon can be described by utilizing a parallel plate capacitor where the displacement current can pass through the metallic plate under some moderate separations. Additionally, when this displacement current passed through any resistance in any of the two plates, dissipation may occur through Joule heat. In our model, we inserted a dielectric thin mica in between the metallic tip and bare Ir/Si which is also metallic. Therefore, the insertion of mica increased the capacitance through which displacement current passed, resulting in a higher dissipation on the mica nanosheet. From the formula of dissipation, derived by Arai [9], the slope of dissipation–Δf curve showed the resistance value of the GΩ order through which displacement current passed. Such higher dissipation may also contribute to the Joule heat dissipation.

To measure the CPD, we considered the Δf–bias curve under a fixed bias voltage, separately on the mica nanosheet and the bare Ir/Si substrate. From the parabolic behavior of the distance curves, we confirmed two different CPD values corresponding to the mica nanosheet and the bare Ir/Si substrate. We used those CPD values to compensate the electrostatic potential for the respective surfaces and to exclude the electrostatic force interaction, that is, only the van der Waals interactions. From our force–distance curves, we found the force curves were minimized after using the CPD value for the respective surfaces. We also constructed an equivalent circuit model based on our findings. Therefore, we found, our present work possibly shows some nanoscale mechanical and electrical changing properties that are related to the forces and energy dissipation characterized by the FM-AFM method.

**Research Purpose**

The purpose of this study lies in the characterization of the nanoscale mechanical and electrical energy dissipation under force interactions in an FM-AFM system. Based on our primary focus on energy dissipation regarded as Joule heat due to displacement current, we chose a metallic tip and an iridium (Ir) coated n type-Si(111) (Ir/Si) substrate with a dielectric
thin film between them. Therefore, the entire arrangement will act as a parallel plate capacitor through which displacement current can pass under a bias voltage. We used artificially synthesized phlogopite mica \((\text{KMg}_3\text{AlSi}_3\text{O}_{10}\text{F}_2)\) as the dielectric film that is thinly cleaved and pasted on the Ir/Si substrate. The mica nanosheet can change the capacitance between the tip and the sample. The forces and energy dissipation will be compared over the mica nanosheet and on the metallic Ir/Si substrate. We also measured the contact potential difference (CPD) and frequency shift \((\Delta f)\) with respect to the sample bias voltage. Moreover, we used the CPD value to compensate for the long-range electrostatic force and to image the surface structure under van der Waals forces. A model for the dissipation mechanism is proposed, leading to the basis of designing future nanoelectromechanical systems having a capacitive component of thin insulating materials.

Through our present research work, we have tried to explain the forces and energy dissipation phenomena for two different surfaces, i.e., a dielectric thin film (mica) with varying thicknesses and the Ir-coated Si substrate (Ir/Si) using the FM-AFM method. We used our developed mechanical exfoliation technique through which we prepared a thin mica nanosheet from bulk artificially synthesized mica. The forces and dissipation were comparatively higher on the 3- and 5-layer mica nanosheets than the Ir/Si substrate. The resistance corresponding to the Joule heat was measured to GΩ order. Up to now, this higher resistance is still mysterious, however, the dissipation measurement apparently shows its potential to characterize the energy dissipation using the non-contact probing system.

References:

Research Accomplishment

List of publications:

i) **M. M. Hasan** and M. Tomitori, “Characterization of mechanical energy dissipation between a conductive tip and a thin dielectric film on a metal-coated Si-substrate by frequency modulated atomic force microscopy”. Submitted to JJAP (Under review).

Domestic conferences: (Oral presentation)

i) M. M. Hasan and M. Tomitori, “Dissipative interaction via capacitive coupling through mica nanosheets examined by non-contact atomic force microscopy”, the 81st JSAP Autumn Meeting 2020, September 8–11, 2020, Online virtual meeting, Japan.


International conferences: (Oral presentation)

i) M. M. Hasan and M. Tomitori, “Mechanical Energy Dissipation via a Thin Mica Film on a Metal-Coated Si Substrate Measured by Frequency Modulation Atomic Force Microscopy”, the 28th International Colloquium on Scanning Probe Microscopy (ICSPM28), December 10–11, Online virtual meeting, Japan.
Abstract

Morphological control of self-organized body on supergiant polysaccharides

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Graphical abstract

In materials design, the relationship between polymer morphology and physical properties is crucial. The researchers are attempting to utilize alternative materials such as natural biopolymers to limit the consumption of non-renewable resources produced by synthetic materials. In recent decades, there has been an increased interest in the utilization of polysaccharides, particularly bioactive ones, for various novel applications owing to their biocompatibility, non-toxicity, and
some specific therapeutic activities. The biological activities of polysaccharides are strongly affected by their chemical structure and chain conformations. The morphological control of polysaccharides from nano to micrometer scale has seldom been utilized in vitro because of the difficulties in regulating self-assembled structures. Herein, it is demonstrated that a cyanobacterial polysaccharide, *sacran*, could hierarchically self-assemble as twisted fibers from the nanoscale to microscale with diameters of \(~1 \mu m\) and lengths \(>800 \mu m\), that are remarkably larger than polysaccharides previously reported. Unlike other rigid fibrillar polysaccharides, the *sacran* fiber was capable of flexibly transforming into two-dimensional (2D) snaking and three-dimensional (3D) twisted structures at an evaporative air-water interface. It was determined that the microfiber had a diameter of \(~1 \mu m\) and formed of a self-assembled torsional structure of nanofibers with diameters of \(~50 \text{ nm}\). This structure was stable in pure water. Upon adding monovalent cations such as Na\(^+\) from NaCl, the microfiber is capable of disassembling into submicrometer-scale particles. The microfibers tended to integrate into a uniaxially oriented state along the contact line of the evaporative air-water interface. When one side of the microfibers was fixed, they drastically transformed into 2D snaking and 3D twisted structures. The velocity of the contact line movement during drying affected the structure formed because of the capillary forces at work between the microfibers. The evaporative air-water interface also induced multi-twisted structures to form from multiple microfibers, thereby showing that this material has self-similar nano and microstructures. The snaking and twisted fibers made possible a film exhibiting millisecond-response repulsive motion to water vapor. By introducing functional molecules into the microfiber, it would be possible to prepare a variety of soft actuators responding to other changes in the external environment, such as light, pH, and temperature. The method for preparing vapor sensors developed by this study not only improves understanding of how the motion of self-assembled...
structures responds to stimuli but also contributes towards the design of environmentally adaptive materials with a high potential for sustainable use. We envision that this approach is capable of reconstructing additional natural materials towards developing advanced microsensors. Moreover, the scaffold of highly oriented M-fibers was successfully prepared. This could be an excellent place to start looking into cell orientation in relation to M-fibers for the new material design.

Moreover, the deformation process of *sacran* microfiber to nano-micro particles was also clarified in vice versa. The interaction between polymer chains is an essential component in studying *sacran* morphological change. The microfibers deformed to particles by reducing the intermolecular interaction, for example, the dilution and the salination. In case of decreasing concentration to the semi-diluted state ($c < c_e 0.015$ wt%), the polymer would be in the unentangled state which is unable to form microfiber. At the concentration of 0.010 wt%, the microfibers significantly deformed into the nanofibers and nanofragments. This could be the boundary concentration of nano to micro assembling process. After that, the salination effect was studied as another factor that suppresses the interaction between the polymer chains. Sacran at a diluted state (0.001 wt%) with 10 mM of NaCl added, the salting-in affected on higher solubility of polymer chains. Then, 100 mM of NaCl was added, the salt affected on less soluble of the polymer referred to the salting-out. This effect could produce the *sacran* particles with a very narrow size distribution. However, the dilution and the salination method conferred a small quantity of particles because the particle would be formed with narrow size distribution under a very low polymer concentration or with a crucial high concentration of salt. That problem could be overcome by the ultrasonication technique. At polymer concentration 0.010 wt%, the *sacran* microparticles are successfully prepared. Sonication plays a vital role in polymer scission and spherical shape formation through cavitation. The overall treated ultrasonic energy directly affects the size of the particles.
Consequently, the polymer particles were annealed, aiming to get microparticles gel for further applications. For instance, the sacran microgel represented super-moisturizing ability comparative to other currently used materials. This would be a significant investigation of a new polysaccharide particle because the preparation processes are ultimately simple and non-necessity of organic solvents nor artificially synthetic molecules, they would be the alternative option in the green chemistry. Furthermore, the Sacran microparticles could be modified for additional applications such as microgel drug carrier or intranasal spray for moisture maintaining. Based on the outstanding properties of the prepared, this study could lead to a new material design for medical purposes in the future.

**Keywords**: Polysaccharides, self-assembly, fibers, particles, drying.

**Journals:**

論文概要

論文題目: 長尺形状の結晶性プラスチック材料が曲げ変形下で示す力学特性に関する研究
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1. 研究内容

近年、様々な分野で多種多様な結晶性プラスチック材料が用いられている。特に日用品や家電製品は身体に近い状況で長期間使用されるため、安全性が重要視される。具体的には、曲げ変形下における破壊抑制であり、産業界から強く求められている。

そこで本研究は、結晶性プラスチック材料の中でも日用品の分野で広く使用されているポリプロピレンとポリオキシメチレンを主対象とし、長尺形状の射出成形体の曲げ変形下における破壊抑制を実現する新たな設計手法の確立を目的とした。特に成形体の物性を損なわずに力学特性を改質できる“成形体の構造制御”の観点から学術的に提案する。以下に各章の主要な成果を記す。

1) 積層構造を利用した結晶性プラスチック材料の破壊靭性向上（第2章）

本章では、ポリプロピレンを対象として曲げ変形下における射出成形体の破壊靭性向上について検討した。分子量の異なる二種のアイソタクチックポリプロピレンを用いて、射出成形によって三種の長尺形状（長さ100mm×幅5mm×厚み1、2および3mm）の試験片を合計6種作製し、三点曲げ試験による曲げ荷重を試験片に負荷した。試験後に試験片の表面をマイクロスコープで観察した結果、分子量によらず厚みが3mmの試験片には、クレイズ形成の発生源である応力白化が発生したが、厚みが1および2mmの試験片には、応力白化が生じなかった。これは試験片の厚みが薄くなることで、曲げ変形下において膨張応力を生じやすい平面ひずみ状態から膨張応力を生じにくい平面応力状態になったためと考える。

次に長さ100mm×幅5mm×厚み2mmのポリプロピレン試験片に長さ100mm×幅5mm×厚み4mmの熱可塑性エラストマーを二色成形した合計厚み6mmのラミネート試験片を作製し、30mmの変位を負荷して5分保持する条件で三点曲げ試験を行った。試験後の試験片表面をマイクロスコープで観察した結果、長さ100mm×幅5mm×厚み2mmのポリプロピレン試験片には応力白化が発生したが、ラミネート試験片には応力白化が生じなかった。熱可塑性エラストマーは引張弾性率が低いものの、圧縮弾性率はプラスチックに近い値を示す。そのた
め、曲げ変形下において圧縮ひずみが生じても体積収縮しにくい。すなわち、二色成形した熱可塑性エラストマーが曲げ変形下において試験片に生じる圧縮応力に抵抗したため、ラミネート試験片には応力白化が生じなかったと考える。

以上の結果から、試験片の厚さや熱可塑性エラストマーとの二色成形といった“成形体の構造制御”によって、曲げ変形下でのポリプロピレン成形体のクレイズ発生を抑制することができた。本手法は過剰な外力が負荷しても成形体の破壊を防ぐことが可能となることから、安全性を必要とする様々なプラスチック製品に応用できる。

2）ヒンジ構造を利用した結晶性プラスチック材料の可聴音発生（第3章）

本章では、ポリオキシメチレンを主な対象とし、曲げ変形下における射出成形体の音響特性を調査した。結晶性プラスチック材料として、ポリオキシメチレンに加えて高密度ポリエチレンとポリプロピレンを用い、射出成形によって内部にヒンジ部を有する長さ63 mm×幅10 mm×厚み2 mmの長尺形状の試験片を作製した。音圧計を設置して試験片の片端を固定した状態で曲げ試験を行い、ヒンジ部の飛び移り座屈によって発生する音の音圧レベルを測定した。その結果、高密度ポリエチレンは検出限界の30 dB未満であったが、ポリプロピレン及びポリオキシメチレンは、いずれも可聴音を発生することが明らかとなった。その中でも弾性率が最も高いポリオキシメチレンが発生する可聴音は70 dBと一番高い結果であった。これは、弾性率が一定の値より高くなると可聴音に相当する力学エネルギーを放出するためと考える。

次にポリオキシメチレンを用いてヒンジ高さの異なる三種の試験片を射出成形によって作製した。上記と同様の曲げ試験を実施し、ヒンジ部が飛び移り座屈した時の音圧レベルを測定した結果、ヒンジ高さ1.00 mmの試験片は検出限界の30 dB未満だったが、ヒンジ高さ1.10 mmの試験片は59 dB、ヒンジ高さ1.22 mmの試験片は70 dBの可聴音を発生することが分かった。これは、ヒンジ高さが高くなることで飛び移り座屈した際により大きい力学エネルギーを放出したためである。

以上の結果から、曲げ変形下における飛び移り座屈現象を利用して可聴音を発生させることができることを見出した。この機能はポリオキシメチレンのような弾性率が高い結晶性プラスチック材料を用いて、ヒンジ高さを調整するという“成形体の構造制御”によって設計可能となる。本手法は成形体に音響特性機能を付与することが可能であり、負荷できる外力の臨界値を使用者に聴覚で認識させることができる。様々なプラスチック製品に適用できるため、新たな破壊抑制の手法としての応用が期待できる。
2. 研究の意義

プラスチック製品は人々の豊かな生活を実現する上で必要不可欠となっている。特に日用品や家電製品等の分野において、様々なプラスチック製品が上市されている。身体の近くで長期間使用される日用品や家電製品は、安全性の確保が必須である。具体的には、“曲げ変形下における破壊抑制”である。曲げ変形下において射出成形体をミクロな視点で観察した場合、成形体は場所によって引張ひずみ、圧縮ひずみ、せん断ひずみを生じる。その結果、結晶性プラスチック材料は、引張、圧縮、せん断の最も弱い部分でクレイズを形成し脆性破壊を引き起こす。昨今の高分子化学において、材料の力学特性に関しては曲げ強度や曲げ弾性率の議論に留まることが圧倒的に多く、この曲げ変形下における破壊抑制については、研究例がほとんどない。

プラスチック材料の機能や力学特性の向上が求められる状況にある中で、新規の一次構造を有するプラスチック材料を設計して工業化まで実現することは難易度が非常に高く非現実的である。そのため、昨今では“ポリマーブレンド”，可塑剤やフィラーなどの“副資材の添加”といった手法が精力的に検討されている。しかし、これらの手法では、狙った力学特性以外の力学特性を低下させるというトレードオフが生じる。

そこで、本研究では、結晶性プラスチック材料を用いて、新規の一次構造等の“マテリアルの構造”ではなく“成形体の構造”の視点から曲げ変形下における破壊抑制について詳細に検討し、新たな設計手法を提案した。特に“成形体の構造”からのアプローチについては、精密な理論に関する研究は散見されるものの、力学特性の改質手法を調査した具体的な研究例は極めて少なく、確立されているとは言い難い。成形体の構造を制御することで狙った力学特性以外の物性を損なわず、且つ実用的に力学特性を改質できることが期待できる。本研究における独創性はこの点にある。

本研究で得られた成果や知見は、特に曲げ変形下における破壊抑制が求められる日用品や家電製品等のプラスチック製品において、安全性の観点から非常に有意義であると確信している。本手法を活用した新たなプラスチック材料が設計され、日用品などの実際の工業製品を通じて全世界の人々の更なる幸福に寄与することを強く願う。
3. 研究業績

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