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World Premier International Research Center Initiative

ICReDD
Institute for Chemical Reaction Design and Discovery
HOKKAIDO UNIVERSITY

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ICReDD-Faculty of Medicine Joint Symposium

Interconnecting chemical reaction discovery and medicine

October 15th FRI, 2021
Online

No entry fee. No prior registration required.

On-demand one month available (Oct 18 – Nov 15).

Opening remarks : Shigetsugu Hatakeyama (Dean of Faculty of Medicine)

<Session 1> 13:05-14:05

Chair : Tetsuya Taketsugu（ICReDD）

・Satoshi Maeda（ICReDD）

“Artificial force induced reaction method: A computational approach for exploring chemical reactions based on quantum chemical calculations”

・Tomohiro Onodera（Faculty of Medicine）

“Clinical development and commercialization of ultrapurified alginate (UPAL) gel transplantation　for articular cartilage injury”

・Hajime Ito（ICReDD）

“Chemistry for sensing and utilizing mechanical force”

<Session 2> 14:05-15:05

Chair : Masumi Tsuda（ICReDD/Faculty of Medicine）

・Jian Ping Gong（ICReDD）

“Challenges and opportunities of hydrogel research in ICReDD”

・Shinya Tanaka（ICReDD/Faculty of Medicine）

“Application of multi-functional hydrogel for biomedical research”

・Tamiki Komatsuzaki (ICReDD)

“Acceleration of measurements preserving diagnosis accuracy by reinforcement learning: Raman imaging and medical applications”

15:05-15:30 Intermission

<Session 3> 15:30-16:30

Chair : Yasuhide Inokuma (ICReDD)

・**Yasuchika Hasegawa（ICReDD）**

“Cancer-imaging system using luminescent lanthanide complex”

・**Kenji Hirata（Faculty of Medicine）**

“Artificial intelligence in positron emission tomography”

・**Yasuhiro Hida（Hokkaido University Hospital）**

“Expanding the application of near-infrared photoimmunotherapy of cancer”

<Session 4> 16:30-17:30

Chair: Masamichi Imajo (ICReDD)

・**Koji Taniguchi（Faculty of Medicine）**

“Inflammation-induced regeneration and cancer”

・**Ichigaku Takigawa（ICReDD）**

“Machine learning for molecules ”

・**Takasuke Fukuhara（Faculty of Medicine）**

“Establishment of a simple high-speed reverse genetics system for SARS-CoV-2”

Closing remarks : Satoshi Maeda (Director of ICReDD)

Abstracts

Artificial force induced reaction method: A computational approach for exploring chemical reactions based on quantum chemical calculations

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ABSTRACT

The motion of atoms during a chemical reaction, called reaction path hereafter, can in principle be elucidated by repeatedly performing quantum chemical calculations at all energetically feasible atomic configurations. However, the number of configurations that can possibly be involved in a reaction path can be huge. Previous studies have thus relied on assumptions (human inputs) concerning the atomic configurations along the targetted reaction path.

The human inputs may bias the results. To avoid that, we have developed an automated reaction path search method called artificial force induced reaction (AFIR) [1]. AFIR explores possible reaction paths automatically by inducing geometrical transformations in a molecule systematically using a virtual force. Combining it with a chemical kinetics method called rate constant matrix contraction (RCMC), on-the-fly kinetics simulation can be performed (FIG. 1).

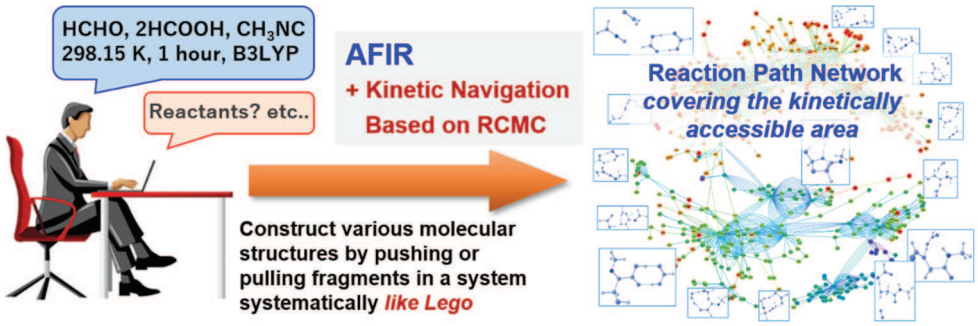

FIG 1. A schematic illustrating on-the-fly kinetics simulation by AFIR and RCMC. Based on the input, i.e., reactants, reaction temperature, reaction time, and computational level, AFIR generates many reaction paths. RCMC identifies the most feasible reaction path from the network of reaction paths.

Recently, we proposed a concept, quantum chemistry-aided retrosynthetic analysis (QCaRA) [2]. QCaRA predicts reaction paths affording a given product by an inverse reaction path search from the product toward various reactant candidates using AFIR. In a proof-of-concept study, we set difluoroglycine as the synthetic target. Then, a new synthetic route of producing a difluoroglycine derivative was proposed as the reverse process of one of the obtained reaction paths.

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Clinical development and commercialization of ultrapurified alginate (UPAL) gel transplantation for articular cartilage injury

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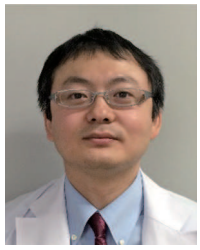

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ABSTRACT

Articular cartilage has limited healing capacity. Once injured, it cannot be repaired, typically leading to progressive joint damage and cannot be restored predictably by conventional treatments. We have developed an injectable ultra-purified alginate gel (UPAL gel) as a scaffolding material for cartilage tissue engineering (1, 2). The UPAL gel transplantation, with a simple and easy handling procedure, could provide a 1-step, minimally invasive, cost-effective cartilage tissue reparative medicine without harvesting donor cells. Since UPAL gel is safe and has a biological effect beneficial on chondrogenesis, it was applied for clinical application of cartilage regeneration. Subsequently, we have conducted a preclinical study using a large animal, the beagle dog, to demonstrate the cartilage regeneration effect (3, 4). Since the implantation of UPAL) gl enhances cartilage repair in small and large animal models, this material is expected to improve the efficacy of the current treatment strategies for cartilage lesions in human. The first-in-human trial was performed on 12 patients (12 knees) with symptomatic, post-traumatic, full-thickness cartilage lesions. This trial provided evidence for the safety and efficacy of an acellular UPAL gel transplantation to facilitate cartilage repair.

Our current aim is to the proof of the efficacy and safety of UPAL gel transplantation into articular cartilage defects in human knee joints affected by osteochondral lesions as the final step to commercialization. At this symposium, I will introduce the process of development and commercialization of this new scaffold, UPAL gel, for more than 10 years.

REF.

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2. Sukegawa A, Iwasaki N, Kasahara Y, Onodera T, Igarashi T, Minami A. Repair of rabbit osteochondral defects by an acellular technique with an ultrapurified alginate gel containing stromal cell-derived factor-1. **Tissue Eng Part A.** 2012 May;18(9-10):934-45. doi: 10.1089/ten.TEA.2011.0380.

3. Baba R, Onodera T, Matsuoka M, Hontani K, Joutoku Z, Matsubara S, Homan K, Iwasaki N.: Bone Marrow Stimulation Technique Augmented by an Ultrapurified Alginate Gel Enhances Cartilage Repair in a Canine Model. **Am J Sports Med.** 2018 Jul;46(8):1970-1979. doi: 10.1177/0363546518770436. Epub 2018 May 15.

4. Onodera T, Baba R, Kasahara Y, Tsuda T, Iwasaki N.: Therapeutic effects and adaptive limits of an acellular technique by ultrapurified alginate (UPAL) gel implantation in canine osteochondral defect models. **Regen Ther.** 2020 Feb 20;14:154-159. doi: 10.1016/j.reth.2020.01.001. eCollection 2020 Jun., PMID: 32110684

Chemistry for sensing and utilizing mechanical force

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ABSTRACT
Heat and light have long been recognized as effective ways to promote chemical reactions. On the other hand, mechanical stimulation as a method to promote chemical reactions has not received much attention for a long time. Considering the phenomena of life, even though the sensing of mechanical stimuli and their conversion into chemical reactions and the conversion of chemical reactions into forces have been done extensively, little has been done to understand this chemical reaction mechanism and to construct artificial systems. The discovery of the luminescent mechanochromism phenomenon in gold isocyanide complexes has led us to study crystals whose structure and luminescence are changed by mechanical stimuli[1]. We have also succeeded in developing new chemical synthesis methods utilizing mechanical stimuli, and have developed completely new technologies for enviromental benign chemical synthesis, such as solid-state Suzuki-Miyaura coupling reactions and mechanoredox reactions[2,3]. In this talk, I will give an overview of these studies.

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Challenges and Opportunities of Hydrogel Research in ICReDD

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ABSTRACT

A polymer gel, in general, is defined as a three-dimensional polymer network containing a large amount of solvent. Hydrogel refers to gel with water solvent. Hydrogels and biological tissues have some common features such as softness, permeability to small molecules, and are both belong to soft matter. Biological tissues possess elaborated structures and exhibit outstanding functions. On the other hand, hydrogels are usually amorphous in structure and have poor functions. Especially, conventional hydrogels have very weak mechanical strength, which limits the use of the material for applications. For a long time, the insoluble gels have been considered as side reaction products in chemical reaction. We have developed tough double network hydrogels and established a general principle for designing high strength and toughness hydrogels and soft materials. In recent years, various gels with robust functionalities along with high mechanical strength have been developed by introducing structures into the gels, which widely extends their applications in diverse fields, including soft actuators, biological sensors, and biomaterials. In this symposium, after a brief introduction of hydrogels, I will discuss challenges of hydrogels and the opportunities of the collaborative research among experimental science, computational science and information science in ICReDD.

REF.

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2. Hailong Fan, Jian Ping Gong, "Fabrication of Bioinspired Hydrogels: Challenges and Opportunities", **Macromolecules**, 53(8), 2769-2782 (2020).

Application of multi-functional hydrogel for biomedical research

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ABSTRACT

As one of the flagship projects in ICReDD, we are involving in the biomedical research using multi-functional polymer hydrogel collaborated with the information science. Polymer hydrogels mimic microenvironment and is expected to be utilized to investigate various biological reactions for applications in advanced medical care including cancer diagnosis/treatment and regenerative medicine. For cancer research, we utilized double network (DN) hydrogel composed of poly-(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS) and poly-(N,N'-dimethyl acrylamide) (PDMAAm), which possesses physical feature as soft and tough[1]. On DN gel, cancer cells rapidly formed spherical structures within 24 hours that expressed stemness markers including Sox2, Oct3/4, and Nanog. In this process DN gels can rapidly modulate cellular gene expression and facilitate the reprogramming of differentiated cancer cells towards cancer stem cells (CSCs), in a process termed HARP (hydrogel-activated reprogramming) phenomenon[2]. In ICReDD, molecular mechanism of HARP phenomenon is further analyzed[3] and collaborative with information science, we hope that DN gel will contribute to discovery of therapeutic reagents. In addition, applicability of the hydrogel is widening and currently, cartilagous[3] and neuronal tissue engineering have been achieved by using charged hydrogel. Furthermore, hydrogel may be beneficial for studies of organoids, iPS, and embryonic stem cells. In this symposium, I will introduce the merit of the collaboration research in ICReDD.

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4. Semba S, Kitamura N, Tsuda M, Goto K, Kurono S, Ohmiya Y, Kurokawa T, Gong J P, Yasuda K, and Tanaka S. Synthetic PAMPS gel induces chondrogenic differentiation of ATDC5 cells via a novel protein reservoir function. **J Biomed Mater Res A**, 109, 354-364, 2021.

Acceleration of Measurements Preserving Diagnosis Accuracy by Reinforcement Learning: Raman Imaging and Medical Applications

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ABSTRACT
In this talk, we present our recent study combined multi-arm Bandits algorithm in reinforcement learning, utilized in the Monte Carlo tree search in alpha-GO, with spontaneous Raman measurements for the acceleration of the experiments by designing and generating optimal experimental conditions "on the fly" during the measurements while keeping the accuracy of diagnosis, and *in situ* Raman imaging analysis in which single cell Raman spectra reveal the distinctive chemical features between differentiated cancer cells cultivated under the existence of heterogeneous PAMPS gels background and those on quartz.
In brief, multi-arm Bandits algorithm [1] enables us to analytically evaluate the upper and lower confidence bounds of the quantity to be quantified such as cancer index before the full distribution will be acquired, and then the algorithm feedbacks the desired experimental conditions to accelerate the diagnosis during the measurement to the Raman microscope. We present our simulation using Raman images in the diagnosis of follicular thyroid carcinoma, and show that this protocol can accelerate more than 5,000-20,000 times in speedy and accurate diagnoses faster than the standard point-scanned Raman measurement that requires the full detailed scanning over all pixels (If time allows, we will also show our developed on-the-fly Raman microscope using programmable spatial light modulator combined with our Bandits algorithm).
In ICReDD we have been collaborating with Prof. Gong and Prof. Tanaka on the acceleration of drug screening designed to eradicate cancer stem cells (CSCs) selectively by using multi-arm Bandits algorithm and *in situ* single cell Raman imaging under the existence of PAMPS hydrogels for CSCs [2]. We briefly present our preliminary result on the *in situ* single cell Raman imaging analysis based on 2D Bayesian optimization for *mouse myoblast* cells (C2C12 SS18 SSX) cultivated on PAMPS gel and quartz dish.

REF.

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Cancer-imaging system using luminescent lanthanide complex

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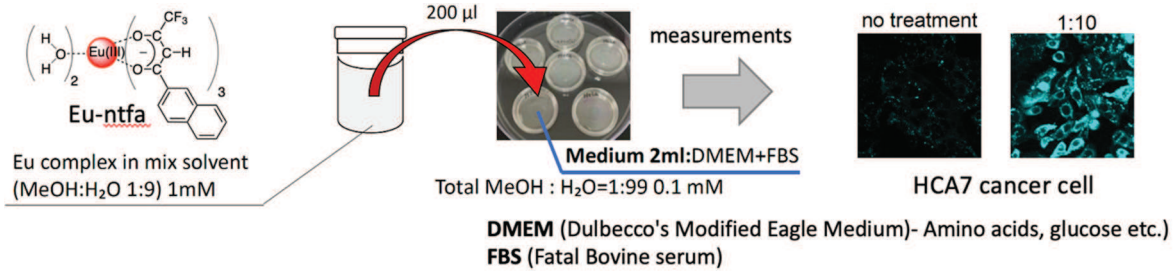
ABSTRACT

Photophysical and informatic analysis for cancer growth process and mechanism (reaction) opens up new medical science and engineering. In this study, we hope the following three points: 1) Visualization of cancer cells using water-soluble luminescent lanthanide(Ln(III)) complexes supports future medical check for understanding the growth mechanism of cancer. 2) Photophysical information and data mining of Luminescent Ln(III) micelles on cancer is useful for finding new aspects of cancer growth. 3) Using the obtained lanthanides, we hope to be able to detect cancer cells on patient's tissue specimen and finally in cancer patients *in vivo*, that is, to develop novel "cancer GPS system".

The prediction of cancer growth is one of expansion-study for chemical reaction discovery. In order to understand the cancer-cell growth reaction, we attempted to prepare the Cancer growth positioning system (Cancer-GPS) using luminescent lanthanide complex. Using water-solved Eu-ntfa complex, luminescent images of cancer cells (Hela: colon cancer, Lung cancer, Brain cancer etc.) are successfully observed. We also real-time monitored the endocytosis process in living cancer cells using luminescent images with Tanaka-group, Faculty of Medicine, Hokkaido Univ. The use of machine learning methods for images for pattern detection, prediction, and quantification is under investigation.



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Artificial intelligence in positron emission tomography

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ABSTRACT
Positron emission tomography (PET) is an in vivo functional imaging technique that can visualize biological phenomena in organs and tumors. PET using F-18 labeled fluorodeoxyglucose (FDG) is routinely used in clinical practice of oncology. Traditionally, PET images have been interpreted by trained nuclear medicine physicians or analyzed quantitatively using mathematical models such as compartmental models. More recently, artificial intelligence (AI) has been applied to different types of medical imaging, including PET. In general, AI can perform four tasks on medical images: 1) image classification, 2) object detection, 3) image segmentation, and 4) image generation. Image classification is the task of classifying an image into several categories, such as benign lesions and malignant tumors. Object detection is the task of detecting a certain lesion (e.g., lung cancer) in the whole image. These tasks are expected to help doctors diagnose and avoid oversights. Image segmentation is the task of defining the boundaries of an organ or tumor in order to measure its volume or plan radiation therapy. Finally, image generation is the task of transforming an image from a related image. For example, a noisy or low-resolution image can be converted to a normal quality image. It can also convert a PET image into a different type of image, such as CT or MRI. Image generation techniques will reduce radiation exposure and scan time. AI techniques are creating a new research area in medical image processing. Important things to remember when using AI are that 1) developing AI models requires a significant amount of training data; 2) AI does not "think", but rather uses statistical inference; 3) AI cannot replace physicians, but only assist them. In my presentation, I will introduce some examples of the use of AI for PET.

REF.

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Expanding the application of near-infrared photoimmunotherapy of cancer

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ABSTRACT
Near-infrared photoimmunotherapy (NIR-PIT) is a new cancer treatment that utilizes antibody-IRDye700DX (IR700) conjugates. IR700 changes its conformation immediately after NIR light exposure and damages the cell membrane and eventually causes rapture of the targeted cells1. Mechanisms of IR700 action were found and revealed by Dr. Hisataka Kobayashi, a senior investigator of NCI, and Professor Mikako Ogawa, in Graduate School of Pharmaceutical Sciences, Hokkaido University2. IR700 can be conjugated with any antibodies targeting various transmembrane cell surface molecules. Antibody-IR700 conjugates activated by NIR has much more potent specific cytotoxicity compared to antibody alone. Unlike radiation therapy, NIR does not affect surrounding cells because of its low energy. Moreover, it has been shown in preclinical animal models that target regulatory T cells (Treg) in tumor environment by using anti-CD25 or anti-CTLA4 antibody-IR700 conjugates to eliminate Treg and enhance cytotoxic T cell attack to tumor cells3,4. This strategy could be applied to any type of tumor regardless of histology or original organs. In September 2020, the clinical use of NIR-PIT was approved in Japan for patients with inoperable head and neck cancer-targeting epidermal growth factor receptor (EGFR). It is anticipated that NIR-PIT is applied to many other malignant tumors. To expand the application, there would be several issues to overcome. The selection of cell surface antigen for targeting is critical. High prevalence and homogeneous expression of a target molecule in the tumor cells are crucial. If there is marked heterogeneity of target molecule expression, a cocktail of antibodies conjugated with IR700 might be needed. Although immunogenic cell death following NIR-PIT might elicit adopted tumor-specific immune response against target negative tumor cells, it will be investigated in the future. Delivery of NIR to the target tumor is a complex task in human patients, especially in the case of the deep internal organs and tumors adjacent to great vessels or nerves. Although NIR is relatively safe, probes must deliver it proximate to tumors since it penetrates only about a centimeter of human tissue. Various medical, surgical and interventional radiology techniques developed for conventional therapy will accelerate safe delivery of NIR to the complex and deep anatomical area, such as intestines, liver, lungs, and central nerves. Ultimately, proofs of superiority or non-inferiority plus benefits to preexisting treatments are demanded to make NIR-PIT available for many patients. Clinical trials with an anti-EGFR antibody conjugate, an anti-CD25 antibody conjugate are ongoing for patients with head and neck, gastric esophageal, cutaneous squamous cancers and malignant melanoma5.

REF.
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https://rakuten-med.com/jp/illuminox/pipeline/

Inflammation-induced regeneration and cancer

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ABSTRACT
Inflammation is divided into two types, acute inflammation and chronic inflammation. Acute inflammation plays an important role in intestinal regeneration, while chronic inflammation plays an essential role in colorectal tumorigenesis and metastasis. In our previous study, we discovered that during intestinal epithelial regeneration, IL-6 family cytokines activate YAP via Src family kinases (SFKs) in addition to the JAK-STAT3 pathway and promotes the regeneration. We also found that loss of adenomatous polyposis coli (APC), an important tumor suppressor in colorectal cancer, induces the activation of the SFK-YAP and JAK-STAT3 pathways in addition to β-catenin. Moreover, the simultaneous activation of these signaling pathways is found in 60-70% of human colorectal cancer samples. We revealed that YAP activation, induced by loss of APC, induces upregulation of gp130/IL6ST, which results in the activation of the SFK-YAP and JAK-STAT3 pathways and establishment of an feedforward loop. The concomitant inhibition of these signaling pathways efficiently suppressed colorectal tumor growth in vitro and in vivo. In this symposium, I will introduce the recent advances of inflammation-related studies.

REF.

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Machine Learning for Molecules

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ABSTRACT

The goal of ICReDD is to innovate the way chemical reactions —chemical transformations of one set of molecules to another— are developed, and thus we hope that sharing our interests can be also beneficial to have precise understanding of biomedical phenomena in the light of molecular biology. Now that we are often overwhelmed by diverse data and facts from experiments and simulations, data-intensive technologies such as machine learning come into play in both chemistry and life sciences[1-2].

The main technical difficulties come from the fact that the molecules are combinatorial. Molecules are combinations of atoms, and chemical reactions are recombination of these atoms in molecules. Even for a very limited number of atomic elements (99% of the human-body mass is made up of 6 elements), the number of all possible combinations is astronomical due to the combinatorial explosion, and hence too huge for brute-force exploration. My long-standing interests lie in this aspect of machine learning involving combinatorial objects such as graphs. Graphs are mathematical abstractions by nodes and edges (or dots and lines), and also everyday tools for computer scientists to represent combinatorial objects. In this talk, I will briefly give an overview on recent excitement, advances, and implications on machine learning over graphs[3-9] for molecules and molecular networks.

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Establishment of a simple high-speed reverse genetics system for SARS-CoV-2

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ABSTRACT
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of a global pandemic of coronavirus disease 2019. We know various mutations have been accumulated on the genome of SARS-CoV-2. To understand the effects of those mutations in the viral life cycle of SARS-CoV-2, it is essential to generate recombinant viruses with each mutation. However, classical reverse genetics systems of SARS-CoV-2 are very complicated and time-consuming due to the large genome size of coronaviruses. To overcome the problem, we attempted to establish a quick reverse genetics system to generate recombinant SARS-CoV-2 using circular polymerase extension reaction (CPER).
We generated 9 cDNA fragments (~5,000 base pairs) covering the full-length of SARS-CoV-2, and a linker fragment encoding the sequences of cytomegalovirus promoter, hepatitis delta virus ribozyme, and bovine growth hormone polyA signal. All of these fragments were designed to possess complementary ends with 25 to 452 overlapping nucleotides with adjacent fragments. The amplified 10 fragments were assembled by CPER and transfected into the tetracycline-inducible ACE2 and TMPRSS-expressing HEK293 cells.
Cytopathic effects were observed in cells at 7 days post-transfection of the CPER products. We confirmed that the recovery of recombinant SARS-CoV-2 at high titer with high accuracy by titration and genome sequencing. The recombinant SARS-CoV-2 exhibited similar propagation property with the parental virus in the Vero cells expressing TMPRSS2. We also applied the CPER method for the construction of SARS-CoV-2 carrying reporter genes or mutants. The infectious clones of the recombinant viruses could be completed in the following steps: introduction of reporter genes or mutations into the desirable cDNA fragments by PCR and assembly of cDNA fragments by CPER. In this study, we establish a PCR-based and bacterium-free method to generate recombinant SARS-CoV-2. We hope that our reverse genetics system can contribute to a further understanding of the molecular characteristics of SARS-CoV-2.

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