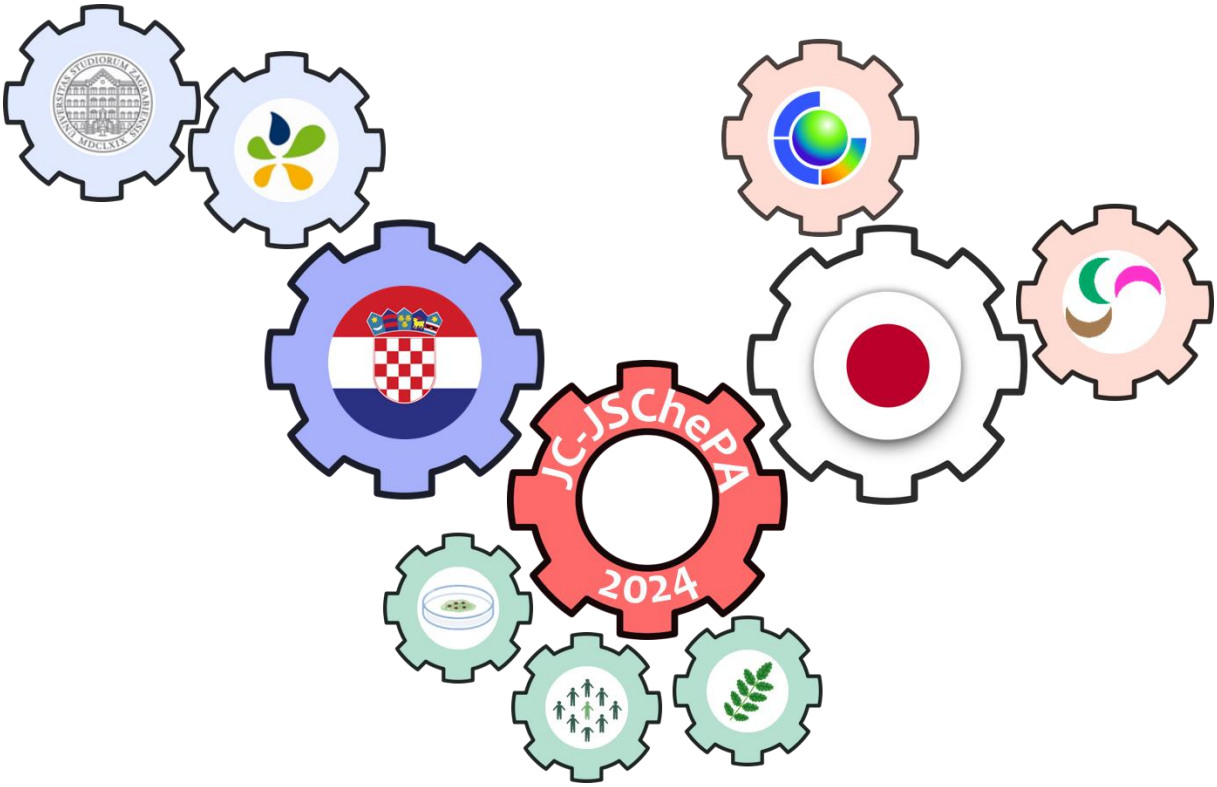
Japan-Croatia Joint Symposium on Chemical Engineering & Plasma Agriculture for Young Scientists Global Networking (JC-JSChEPA) : Book of Abstracts

Japan-Croatia Joint Symposium on Chemical Engineering & Plasma Agriculture for Young Scientists Global Networking (JC-JSChEPA)

Zagreb, Croatia, October 15 – 17, 2024



BOOK OF ABSTRACTS

https://www.fkit.unizg.hr/JC-JSChEPA

jcsymposium2024@gmail.com

BOOK OF ABSTRACTS

Japan-Croatia Joint Symposium on Chemical Engineering & Plasma Agriculture for Young Scientists Global Networking (JC-JSChEPA)

Zagreb, Croatia, October 15 – 17, 2024

Organized by:

- University of Zagreb Faculty of Chemical Engineering and Technology
- Kyushu Branch, The Society of Chemical Engineers of Japan (SCEJ-KB - Kyushu branch)
- COI-NEXT Research Center for Plasma Agriculture Science (Center of Innovation NEXT, Japan Science and Technology Agency)

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Trg Marka Marulića 19
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Croatia

Zagreb, Croatia, 2024

FOREWORD

Being the first event of its kind, the Japan-Croatia Joint Symposium on Chemical Engineering & Plasma Agriculture for Young Scientists Global Networking (JC-JSChEPA) is organized to give a unique opportunity for students and young scientists to connect on an international scale with keyword of "Chemical Engineering".

Chemical engineering, which was initially aimed at chemical factories, has expanded into many fields such as environment sciences, energy sciences, the new materials, the biotechnology, the plasma technology, and so on. Today, in all technical fields, the major issue is how to rationally develop complex systems, and the scope of problems and issues have expanded from a very limited scope, such as a single factory, to a region that transcends national borders and the entire globe. The methodologies and achievements in each field that chemical engineering has cultivated are expected to continue to develop and contribute to the development of science and technology.

The Symposium offers insightful lectures by invited speakers and a chance for students and young scientists to engage in expanding their network and collaborate with peers and experts in the field of chemical engineering and plasma agriculture.

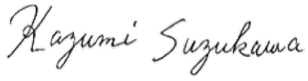
We are excited about state-of-the-art lectures from the experts as well as rapid-fire and poster presentations from young scientists and students.

We are deeply grateful to everyone who has helped bring this symposium to life – the organizers, sponsors, speakers and attendees.

Thank you for joining us in what we hope will be the start of a lasting tradition. We look forward to the ideas you will share and the discussions you will spark from this meeting.

Prof. Kazumi Suzukawa, PhD

Chair of the International Organizing Committee



Assoc. Prof. Anamarija Rogina, PhD

Chair of the Local Organizing Committee

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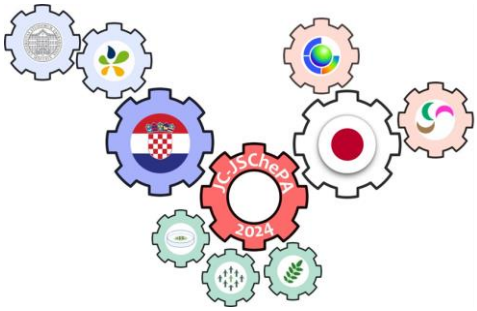


Poster presentations

Invited lectures

APPLICATION OF ULTRASOUND TO CHEMICAL ENGINEERING

Susumu Nii*

Department of Chemical Engineering, Kagoshima University, Kagoshima, Japan

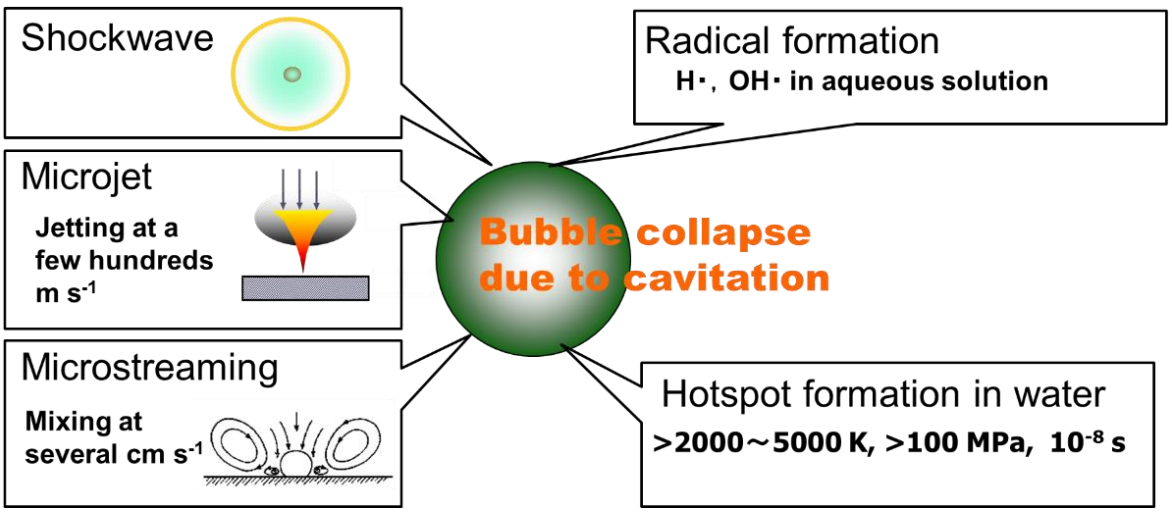
ABSTRACT

Ultrasound provides not only vibration but also triggers various changes in liquids. Once the ultrasound is irradiated to liquids, ultrasound drives both physical and chemical effects to the liquid itself, as well as to particles dispersed in the liquid. The prime effect is known as ultrasonic cavitation. Ultrasound, as pressure fluctuation, causes the formation of bubbles in liquid. These bubbles contain gases dissolved in liquids and also vapors of liquids. They oscillate right after the occurrence. Some bubbles dissolve and disappear, while some coalesce into bigger bubbles. Others take a unique path of rapid collapse after sudden expansion. Such a collapse is called implosion of bubbles and leads to microjet, shock wave and formation of hydroxyl radicals when those bubbles are in aqueous solutions, as illustrated in the graphical abstract. Such physical and chemical effects provide unique fields within a liquid and also interface between solid and liquid. We have applied ultrasound to enhance the size uniformity of glycine crystal[1], to speed up emulsion splitting[2], to help dissolution of solids and also to achieve fractionation of submicron particles from suspensions[3].

Keywords: ultrasound, cavitation, mass-transfer enhancement, radical.

References: 1. 10.1016/j.ultsonch.2014.03.033; 2. 10.1016/j.ultsonch.2008.07.005;
3. 10.1016/j.ultsonch.2013.11.009.

GRAPHICAL ABSTRACT



* Correspondence: Susumu Nii, niisus@cen.kagoshima-u.ac.jp

DEVELOPMENT OF BIOCATALYTIC PROCESSES – AN ENGINEERING APPROACH

Zvjezdana Findrik Blažević*

University of Zagreb Faculty of Chemical Engineering and Technology,
Trg Marka Marulića 19, 10000 Zagreb, Croatia

ABSTRACT

Due to its numerous benefits, biocatalysis is considered a transformational technology for chemical production.[1] This does not surprise considering the mild process conditions and high catalytic power of enzymes. Still, when it comes to its application in industry, not all goes always as well as planned. Industrial biotransformations need to be set up in a way to produce a significant amount of product[2] with high product yield, excellent enantioselectivity, volume productivity, substrate conversion, etc. Thus, a careful choice of reactor set-up, facilitated by a detailed kinetic analysis[3,4], or some other engineering approach[5], is important. This is not only essential for complex reaction systems, but also for relatively simple ones. For an efficient enzymatic process in general, it is important to combine the knowledge of enzyme kinetics, enzyme operational stability, as well as reaction equilibrium, or determine the process parameters with the greatest impact on the process outcome. This enables defining the dependence of crucial process variables on designated goal functions and enables deep analysis by using models and software. In this talk, such methodologies will be demonstrated by the examples of research done in our group.

Keywords: biocatalysis, process metrics, kinetic parameters, optimization, enzymes.

References: 1. 10.1039/B716045C; 2. 10.1016/j.tibtech.2008.03.004; 3. 10.1002/adsc.202000984; 4. 10.1021/acs.iecr.1c02343; 5. 10.1039/C5RA14414K.

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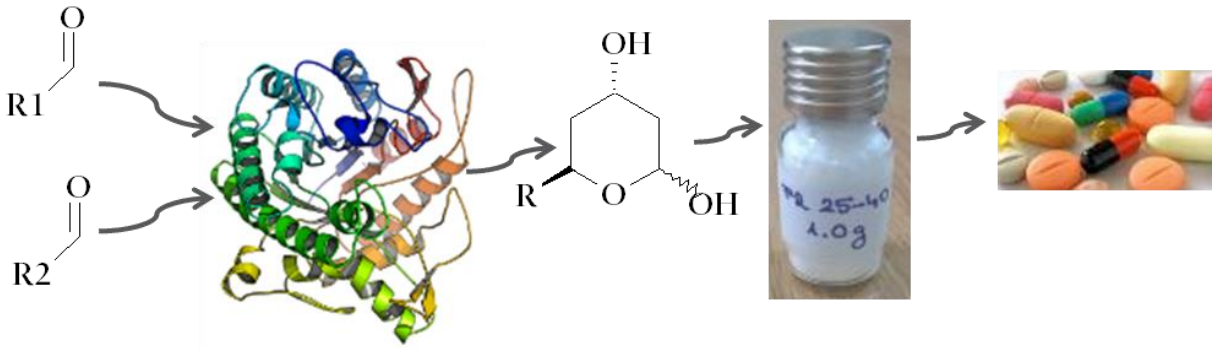


From reaction investigation to final product

* Correspondence: Zvjezdana Findrik Blažević, zfindrik@fkit.unizg.hr

LIVER RECONSTRUCTION FOR REGENERATIVE MEDICAL SCIENCE

Yusuke Sakai*, Hiroyuki Ijima

Department of Chemical Engineering, Kyushu University,
744 Motooka, Nishi-ku, Fukuoka, 819-0395, Japan

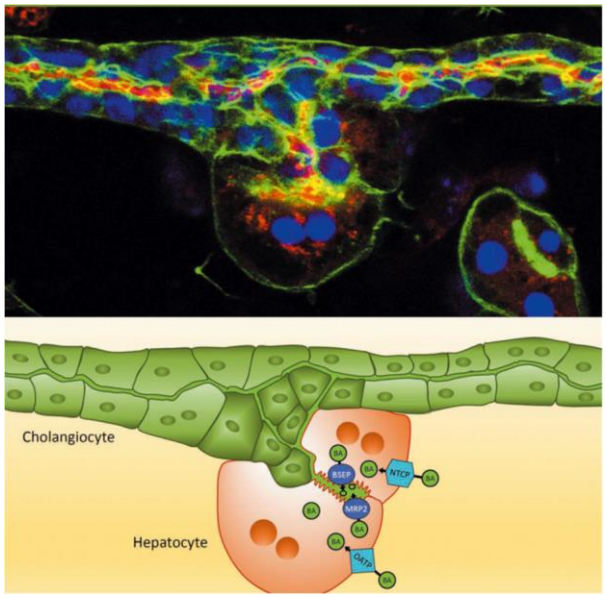
ABSTRACT

The liver is a main organ of metabolism and has been attracting attention as a target for regenerative medicine and drug screening. It is known that the liver in the living body can regenerate after resection or injury, but this is difficult to do *in vitro*. Many researchers are challenging the reconstruction of liver tissue in regenerative medical science. In particular, the reconstruction of the bile excretion system of liver metabolites has been of interest to many researchers in recent years. In this presentation, we will report on the formation of spherical cell tissues and the construction of a human liver with a vascular network that can lead to regenerative medicine, focusing on liver tissue culture technology.[1,2] On the other hand, there is concern that the loss of the bile duct network will lead to a decrease in function due to cholestasis. In order to reconstruct the bile duct, which is one of the important metabolic product excretion pathways, we used chemically-induced liver progenitor cells (CLiPs).[3] The composite hepatic tissue of hepatocytes and cultured bile ducts normalizes hepatocyte polarity and hepatic transporters, and maintains liver-specific functions for a long period of time. We expect this to be an organ model for drug screening and liver disease treatment.

Key words: Liver, Tissue engineering, Hepatocyte, Bilecanaliculi, Bile duct.

References: 1. 10.1159/000272316; 2. 10.1016/j.biomaterials.2015.06.046; 3. 10.1002/bit.27773; 4. 10.1002/bit.27410

GRAPHICAL ABSTRACT[4]



* Correspondence: Yusuke Sakai, y.sakai.bioeng@gmail.com

ADVANCED HYDROGELS FOR LIVER TISSUE ENGINEERING AND *IN VITRO* HEPATIC MODELS

Gloria Gallego-Ferrer[1,2*]

[1] Center for Biomaterials and Tissue Engineering (CBIT), Universitat Politècnica de València, Valencia, Spain
[2] Biomedical Research Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Carlos III Health Institute, Valencia, Spain

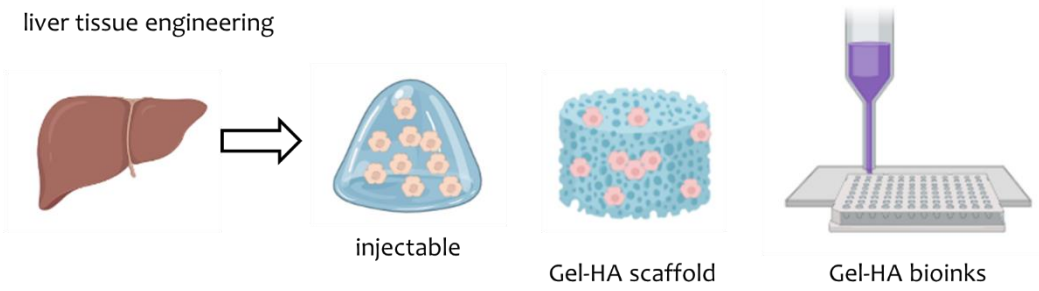
ABSTRACT

Liver tissue engineering using biodegradable hydrogels with hepatocytes has been proposed as an alternative to liver transplantation to treat end-stage liver diseases.[1] Hydrogels have been also proposed as biomimetic systems of the extracellular matrix of the liver to create 3D culture systems. Encapsulated hepatic cells can generate biomimetic systems for liver disease-modeling or to perform hepatotoxicity studies.[1] In our group we have developed hybrid injectable gelatin and hyaluronic acid hydrogels (Gel-HA) of different compositions and optimized the proportion for the culture of HepG2 cells, a hepatoma cell line widely used in Hepatology.[2] The selected composition was produced in the form of a scaffold for the culture of primary human hepatocytes (PHH). When PHH were cultured in the scaffold they exhibited increased albumin and urea secretion and metabolic capacity (cytochrome P450 and UDP-glucuronosyltransferase activity levels) compared to standard monolayer cultures. The transplant of the scaffold containing human hepatocytes led to an improvement in liver function (transaminase levels, necrosis) and ameliorated damage in a mouse model of acetaminophen-induced liver failure. Additionally, the *in vivo* study also provided a mechanistic understanding of acetaminophen-induced liver injury and the impact of transplantation by analyzing cytokine production and oxidative stress induction to find suitable biomarkers of cell therapy's effectiveness.[2] The injectable hydrogels were also proposed as bioinks to build complex structures that can be used in the future in the performance of co-culture experiments. Bioprinting of HepG2 cells demonstrated the suitability of the selected bioinks for the culture of hepatic cells.

Keywords: liver tissue engineering, hydrogels, scaffolds, *in vivo* study, bioprinting.

References: 1. 10.36922/ijb.2706; 2. 10.1016/j.bioadv.2023.213576.

GRAPHICAL ABSTRACT



* Correspondence: Gloria Gallego-Ferrer, ggallego@ter.upv.es

TOWARDS WEARABLE CHEMICAL OPTICAL SENSORS FOR MULTIMODAL DETERMINATION OF BIOMARKERS

Ivana Murković Steinberg

University of Zagreb Faculty of Chemical Engineering and Technology,
Trg Marka Marulića 19, HR-10000 Zagreb, Croatia

ABSTRACT

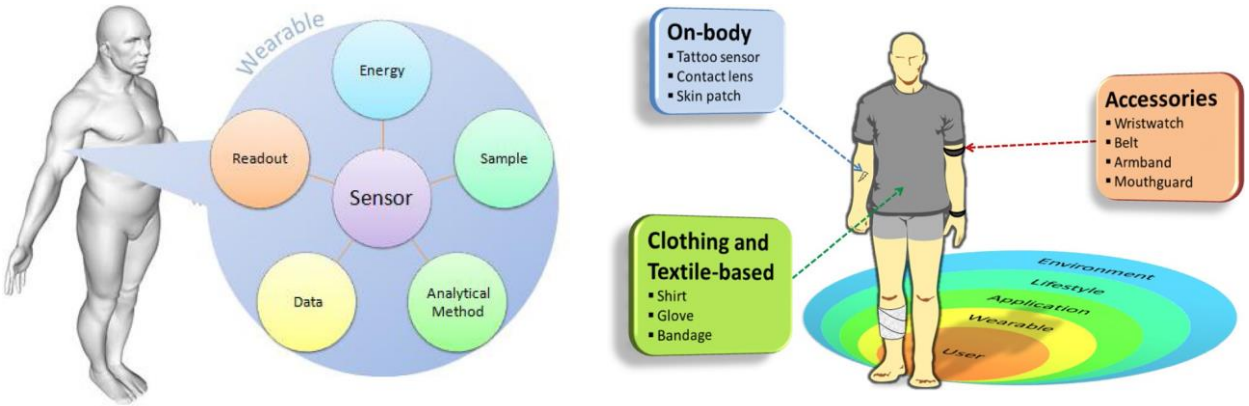
Design of wearable (bio)chemical sensors brings many challenges in device ergonomics, chemistry, physics, and electronics, particularly for material/chemistry architecture which should ideally be reversible, reproducible and biocompatible.[1] This talk will present our current research on the development of optically sensitive (nano)materials and sensing schemes targeted at implementation on low-cost wearable devices. Our research aims to adapt natural and biocompatible materials using appropriate chemical modifications to design optical sensing/sampling building blocks for integration with wearables. Initial work has focused on wound and sweat monitoring [2,3], and an overview of optically responsive sensing chemistries for pH, electrolytes and redox-active analytes designed for immobilization on textiles and cellulose derivatives will be presented. Lastly, the talk will introduce a new direction of research in which we intend to implement multimodal biomarker monitoring combining physical and biochemical parameters from the skin surface (on-skin patches) and under the skin (dermal tattoos) using wearable optoelectronic devices.

Acknowledgement: this work was partly supported by the Croatian Science Foundation under the project *WearSense* HRZZ IP-2022-10-2595.

Key words: chemical sensors, biosensors, wearables, cellulose, pH sensing, biomarkers.

References: 1. 10.1002/elan.201600094; 2. 10.1016/j.snb.2017.02.095; 3. 10.1016/j.talanta.2018.09.031

GRAPHICAL ABSTRACT



* Correspondence: Ivana Murković Steinberg, ivana.murkovic@fkit.unizg.hr

MICROALGAE CELL ENGINEERING TECHNOLOGY TOWARD SUSTAINABLE GREEN CELL FACTORIES

Yoshinori Kawabe

Department of Chemical Engineering, Faculty of Engineering, Kyushu University, Kyushu, Japan

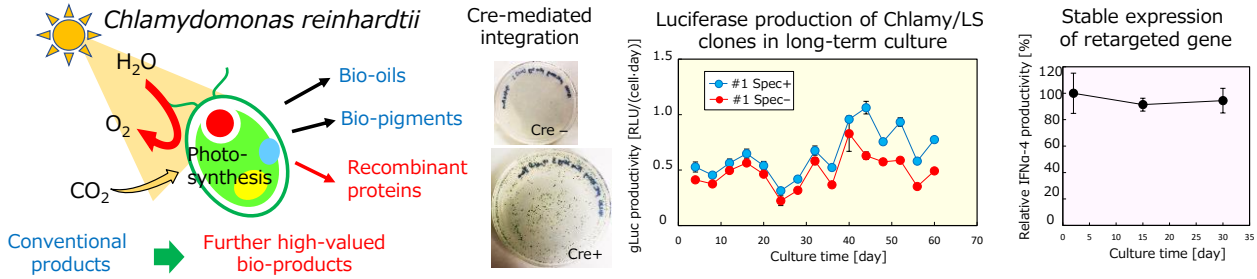
ABSTRACT

The unicellular photosynthetic eukaryote *Chlamydomonas reinhardtii*, a microalgae species, serves as an ideal model for developing biofuels, recombinant proteins, and bio-pigments production technologies. While advances in genetic recombination have been anticipated to enhance the production of high-value-added substances, challenges remain in overcoming the suppression of exogenous gene expression, particularly in nuclear genome modifications. Thus, improving transgene expression is crucial. Targeted transgene integration into the cellular genome offers a promising solution by providing stable and predictable gene expression, with precise control over copy number and expression levels at specific loci. Among the site-specific gene recombination systems, the Cre/loxP system has been widely studied, allowing efficient gene recombination between two loxP sites without additional cellular factors. In our study, a donor plamid harboring reporter genes and a loxP site was introduced into *Chlamydomonas* cells. Screening of over 200 transformants based on reporter gene expression resulted in the creation of transgenic Chlamydomonas lines capable of stable and high-level foreign gene expression without drug selection.[1,2] These cells containing a target loxP site within the transgene can serve as founder cells for further insertion of transgenes using the Cre/loxP system, facilitating the production of valuable bio-products. When Cre-mediated retargeting of the IFN expression cassette at the designated locus was carried out, the newly introduced transgene also demonstrated long-term expression in the absence of selective drug. This confirms the potential application of the cells for the production of high-value bio-products. In this presentation, we will introduce these technologies and our recent advancements, and together discuss how they could improve recombinant protein production, contributing to the development of sustainable green cell factories.

Key words: Microalgae, *Chlamydomonas reinhardtii*, Cre-loxP system, Gene retargeting.

References: 1. 10.1051/matecconf/202133307003; 2. 10.1016/j.jbiosc.2021.07.006.

GRAPHICAL ABSTRACT



* Correspondence: Yoshinori Kawabe, kawabe@chem-eng. kyushu-u.ac. jp

Invited lectures

GREEN PLASMA TECHNOLOGY FOR SUSTAINABLE AGRICULTURE

Kazunori Koga*, Pankaj Attri, Takamasa Okumura, Masaharu Shiratani

Kyushu University, Kyushu, Japan

ABSTRACT

The collapse of the nitrogen cycle is one of the most important issues in the Planetary Boundary.[1] It is partly due to the excessive installation of nitrogen fertilizers in arable lands, resulting in accumulating nitrate in soil and groundwater. Since the Green Revolution in the 1970s, crop productivity has continuously increased through fertilizer administration. The nitrogen fertilizer has been produced using the Haber-Bosch process which emits CO_2. The nitrogen fertilizer production process produced 700 million tons of CO_2 in 2014, which is 1-2% of global CO_2 emissions. It is predicted to increase by 23% by 2050 because of climate change. Climate change accelerates food production decline leading to excessive fertilizer administration. This situation is creating pressure in the direction of environmental change combined with agricultural production. To overcome the issue, innovative plant growth enhancement technology should be developed in an environmentally friendly way.

Plasma is promising to solve the issue. It generates chemically active molecules at room temperature. In air plasma, chemically active molecules such as OH, NO, O, O_3, H_2O_2, NO_3^-, and NO_2^- are produced. These are so-called reactive oxygen nitrogen species RONS. The RONSs regulate plant response and produce nitrogen fertilizers, leading to improved plant germination, growth, and harvest. The growth enhancement by irradiating plasma to seeds reduces fertilizer consumption and provides environmental adaptability. Plasma-activated fertilizer reduces the CO_2 emission for the production of nitrogen fertilizer.

The plant response regulation using plasma has attracted much attention.[2] Plasma irradiation to seeds enhances germination, growth, and harvest.[3-6] The seeds with high-temperature stress show degraded germination characteristics and rice quality. This is an example of the deterioration of food productivity due to global warming. We have demonstrated that plasma irradiation of heat-stressed seeds improves germination characteristics.[7] The plasma changes the gene expression related to abscisic acid (ABA) and α-amylase.[7] Further, plasma irradiation alters methylation in DNA promotors related to ABA and α-amylase. These results show that plasma irradiation to seeds allows us to regulate epigenetics, altering DNA methylation. It suggests that plasma is promising to provide plants with environmental adaptability. This method helps to recover from the decline in food productivity caused by global warming and reduces fertilizer consumption.

Key words: Plasma agriculture, Seed, Atmospheric pressure plasma, Planetary Boundary, SDGs.

References: 1. 10.1038/461472a; 2. 10.3390/pr8081002; 3. 10.1016/j.cap.2013.11.056;
4. 10.7567/APEX.9.016201; 5. 10.35848/1347-4065/ab7698; 6. 10.1002/ppap.202000181;
7. 10.1021/acsagscitech.0c00070.

* Correspondence: Kazunori Koga, koga@ed.kyushu-u.ac.jp

Invited lectures

COMBINING ADVANCED OXIDATION TECHNIQUES FOR DISINFECTION OF WATER FOR (CLOSED) IRRIGATION

Gregor Primc[1]*, Arijana Filipić[2], Miran Mozetič[1], Matevž Dular[3], Martin Petkovšek[3], Alja Štern[2], Bojana Žegura[2], Ion Gutierrez Aguirre[2], David Dobnik[2], Rok Zaplotnik[1]

[1] Department of Surface Engineering, Jozef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia
[2] National Institute of Biology, Večna pot 121, 1000 Ljubljana, Slovenia
[3] Faculty of Mechanical Engineering, University of Ljubljana, Aškerčeva 6, 1000 Ljubljana, Slovenia

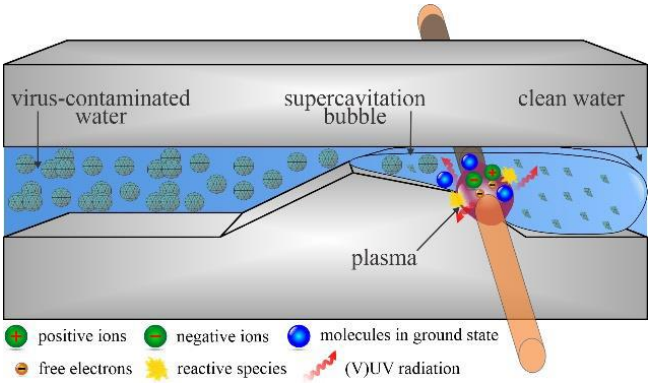
ABSTRACT

Water scarcity is a critical global challenge, with around 70% of the world's water used for agricultural irrigation.[1] As climate change and food shortages intensify, water scarcity issues are expected to worsen. Reducing agricultural water consumption, particularly through closed-loop irrigation systems, is essential. The FAO predicts that improved irrigation practices will limit the increase in agricultural water use in developing countries to just 14%.[1] However, closed-loop systems face challenges, including agrochemical residues, nutrient imbalances, and the spread of pathogens. In these systems, a single plant can release pathogens like bacteria and viruses (even through the root system) into the circulating water, affecting others. UV disinfection is a common solution but is sensitive to water turbidity, while membrane filtration requires frequent cleaning. To address pathogen spread, we researched combining hydrodynamic cavitation and gaseous plasma. We designed a device using the 'supercavitation regime', forming a stable water vapor bubble where plasma can be ignited, producing oxidizing species and UV radiation.[2] We tested two systems, small- and medium-scale, for virus inactivation. Experiments using MS2 bacteriophage, a surrogate for human viruses, achieved a 5-log inactivation rate.[3] The process proved to be non-toxic and effective in deactivating or destroying MS2 bacteriophage, offering an environmentally friendly disinfection method without chemicals.

Key words: gaseous plasma, hydrodynamic cavitation, viruses, disinfection.

References: 1. FAO (https://www.fao.org/water/en/, accessed 5th September 2024);
2. US patent (US11807555B2); 3. 10.1016/j.envint.2023.108285.

GRAPHICAL ABSTRACT[3]



* Correspondence: Gregor Primc, gregor.primc@ijs.si

OPTIMIZATION OF POLYMERIC MEMBRANE COMPOSITION AND REAL-TIME TRACKING OF MEMBRANE EVAPORATIVE DRYING FOR MASS PRODUCTION OF ELECTROCHEMICAL SENSORS

Sara Krivačić[1,2*], Ayian Speck[1], Petar Kassal[2], Eric Bakker[1]

[1] Department of Inorganic and Analytical Chemistry, University of Geneva, Quai Ernest-Ansermet 30, 1221 Genève, Switzerland
[2] University of Zagreb Faculty of Chemical Engineering and Technology, Trg Marka Marulića 19, HR-10000 Zagreb, Croatia

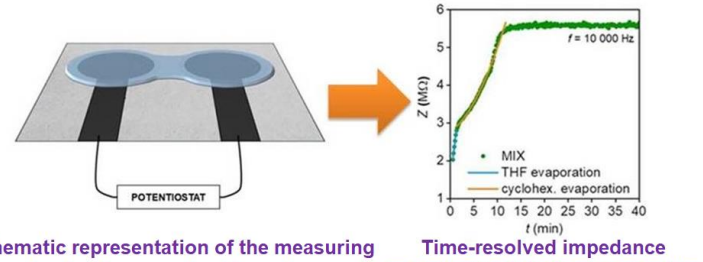
ABSTRACT

Solid-contact ion-selective electrodes are useful for quickly measuring charged substances in various samples. Most commercially available point-of-care electrochemical sensing systems are made using screen-printing. However, this method does not apply to the deposition of polymeric sensing membranes, due to the mismatch of the membrane physical characteristics to those intended for screen printing. We used an industrial automated dispensing machine for fast and controlled deposition of small volumes of ion-selective membrane (ISM). Unlike previously published methods, the dry solute membrane content was not altered. Rather, by optimizing the solvents for the ISM preparation, we significantly lowered the required number of membrane deposition steps. It was shown that dry membrane uniformity strongly depends on the solvent carrier system, with the best uniformity for the membrane prepared in a solvent mixture consisting of equal volumes of tetrahydrofuran and cyclohexanone. The volume of a single membrane deposit (spot) was estimated based on colorimetric absorbance[1] measurements to be 0.20 µL. We were able to achieve a good response to potassium ions with just two membrane spots, demonstrating the efficiency of our production technique. We also investigated the evaporative membrane drying for the first time using time-resolved impedance spectroscopy. This gave us valuable information about how the solvent composition affects the drying process. With this method, one can produce and dry the devices in less than 30 minutes, allowing the entire production and testing process to be completed within a few hours.

Key words: evaporative drying, plasticized poly(vinyl chloride), automated dispensing, electrochemical impedance spectroscopy, colorimetric absorbance.

References: 1. 10.1039/D0LC00028K.

GRAPHICAL ABSTRACT



Schematic representation of the measuring setup for time-resolved impedance spectroscopy

Time-resolved impedance measurement during evaporative membrane drying

* Correspondence: Sara Krivačić, skrivacic@fkit.unizg.hr

STRONTIUM-DOPED CALCIUM AND BARIUM MANGANITES AS CATALYSTS IN HETEROGENEOUS OXIDATION OF VOLATILE ORGANIC COMPOUNDS

Andreja Žužić[1]*, Filip Car[1], Jelena Macan[1], Vesna Tomašić[1], Andreja Gajović[2]

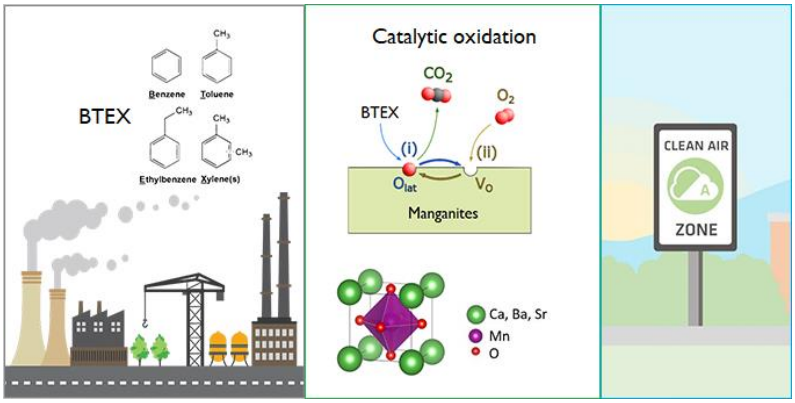
[1] University of Zagreb Faculty of Chemical Engineering and Technology,
Trg Marka Marulića 19, 10000 Zagreb, Croatia
[2] Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

ABSTRACT

As air pollution continues to be a growing concern, especially in large urban areas, developing efficient catalysts to remove volatile organic compounds (VOCs) like BTEX (benzene, toluene, ethylbenzene and *o*-xylene) has become increasingly important. Currently, expensive noble metal-based catalysts are used for the removal of VOCs, making it essential to find more affordable alternatives. This study highlights the potential application of the low-cost Sr-doped calcium (CSMO, $Ca_{1-x}Sr_xMnO_3$) and barium manganites (BSMO, $Ba_{1-x}Sr_xMnO_3$) as effective catalysts for air purification. The samples were synthesized using both autocombustion and co-precipitation methods, with varying levels of strontium doping (x = 0, 0.3, and 0.5). The effectiveness of these catalysts in removing BTEX compounds was tested at different temperatures ranging from 100 °C to 450 °C. Less stable components, such as toluene, ethylbenzene, and *o*-xylene, were completely removed by all catalysts at 350 °C, regardless of the synthesis method or Sr doping level. However, benzene, the most stable compound in the mixture, was more challenging to oxidize. The highest benzene conversion of 78 % was achieved with the CSMO sample prepared by co-precipitation and doped with 0.3 mol of Sr. In contrast, the best result was obtained for the BSMO sample prepared via the autocombustion method, where 86 % benzene conversion was observed with the same Sr doping level of 0.3. These results indicate that CSMO and BSMO catalysts are promising, cost-effective alternatives to noble metal-based catalysts for VOC removal.

Key words: autocombustion, BTEX oxidation, catalysts, coprecipitation, manganites.

GRAPHICAL ABSTRACT



* Correspondence: Andreja Žužić, azuzic@fkit.unizg.hr

DESIGN AND CHARACTERIZATION OF CELLULOSE-BASED OPTICAL PH SENSORS WITH POTENTIAL APPLICATIONS IN CHEMICAL WEARABLES AND SMART FOOD PACKAGING

Iva Žuvić[1], Gerhard J. Mohr[2], Ivana Murković Steinberg[1*]

[1] University of Zagreb Faculty of Chemical Engineering and Technology,
Trg Marka Marulića 19, HR-10000 Zagreb, Croatia
[2] Joanneum Research Forschungsgesellschaft mbH – Materials,
Franz-Pichler-Straße 30, Weiz A-8160, Austria Affiliation

ABSTRACT

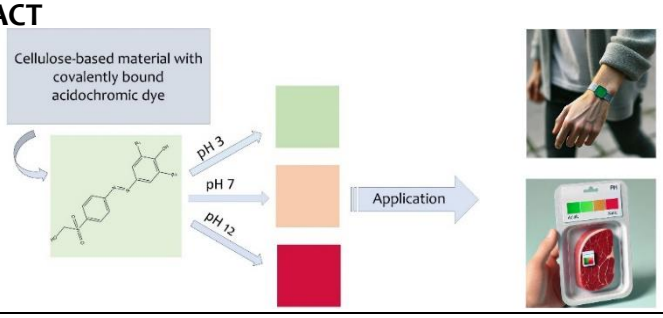
The goal of this research has been to design and characterize different forms of cellulose biopolymer materials for the purpose of reversible optical pH sensing. Natural and biocompatible materials containing hydroxyl functional groups such as cellulose, polyurethane hydrogel, and hydroxyalkyl methacrylate can be covalently functionalized with acidochromic vinylsulfonyl dyes using Michael addition.[1,2] Characterization included UV-Vis absorbance and reflectance spectroscopy and pH titrations. Subsequent data analysis revealed functional sensing properties, *e.g.* the acid-base equilibrium constants, expressed as apparent pK_{app} values, obtained by fitting titration data to the Boltzmann function. In general, these materials change color from yellow to red as the pH increases. However, by incorporating a blue pH-insensitive pigment, the pH-sensitive color change can be shifted from green to red, similar to a traffic light, making it easier to read with the naked eye.[1] Fine-tuning of the spectral and functional sensing properties is possible by using dyes with specific substituents in combination with the appropriate form of the cellulose material (cellophane transparent thin films, paper, cellulose nanocrystals and cellulose microparticles).[1,2] In conclusion, these materials are biocompatible, light, thin and flexible, making them ideal for incorporation into integrated chemical systems such as wearable chemical sensors. Since the pK_a values range from 5.5 to 7.8, potential applications include continuous monitoring of pH of skin, sweat, or wound fluid and as pH indicators in smart food packaging.

Acknowledgments: this work was partly supported by the Croatian Science Foundation under the project WearSense HRZZ IP-2022-10-2595

Key words: Optical sensors, pH indicators, Polymers, Cellulose, Acidochromic dyes.

References: 1. 10.1007/s00216-008-2428-7; 2. 10.1016/j.snb.2014.09.104; 3. 10.1007/BF00321245.

GRAPHICAL ABSTRACT



* Correspondence: Ivana Murković Steinberg, ivana.murkovic@fkit.unizg.hr

Poster presentations

IMPROVEMENT OF THE RHEOLOGICAL PROPERTIES OF DECELLULARIZED EXTRACELLULAR MATRIX HYDROGEL THROUGH ADDITION OF POLYSACCHARIDES

Teodora Prebeg*, Sara Čubek, Marta Pleše, Gordana Matijašić

University of Zagreb Faculty of Chemical Engineering and Technology,
Trg Marka Marulića 19, 10000 Zagreb, Croatia

ABSTRACT

The decellularized extracellular matrix (dECM) has demonstrated good biocompatibility in previous studies and, as a material obtained from natural tissue, contains a specific cell adhesion motif of the amino acids arginyl-glycyl-aspartic acid (RGD). Therefore, it is a good candidate as a biomaterial for tissue engineering purposes.[1] Although it provides a natural environment for cells, dECM-based hydrogels do not have good mechanical properties. Therefore, it is necessary to reinforce such hydrogels. There are various ways to do this, such as preparing hybrid hydrogels or the addition of another phase.[2] In this research porcine liver was decellularized and solubilized by digestion with pepsin. A hydrogel was prepared by adjusting the pH and temperature to physiological conditions. To improve the rheological properties, various polysaccharides were introduced to the dECM hydrogel: chitosan, alginate and gum arabic. Each mixture was prepared in the mass ratios 0/100, 25/75, 50/50, 75/27 and 100/0. The rheological properties and gelation kinetics were measured and evaluated. The results indicate that gum arabic does not promote gelation; instead it significantly retards the process and reduces viscosity when combined with dECM. Alginate showed similar behavior, while chitosan increased the gelation speed and viscosity in dECM blends.

Key words: Extracellular matrix, hydrogel, polysaccharides, rheological properties.

References: 1. 10.1177/20417314221101151; 2. 10.1002/adma.201902026.

* Correspondence: Teodora Prebeg, tprebeg@fkit.unizg.hr

CAM ASSAY AS A TOOL FOR EVALUATING THE BIOCOMPATIBILITY OF BORIC ACID/CHITOSAN/HYDROXYAPATITE SCAFFOLDS

Iva Bazina[1*], Luka Dornjak[1], Marta Protrka[2], Inga Urlić[2], Anamarija Rogina[1]

[1] University of Zagreb, Faculty of Chemical Engineering and Technology,
Trg Marka Marulića 19, 10000 Zagreb, Croatia
[2] Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia

ABSTRACT

Tissue engineering is an ever-evolving field, challenging the limits of regenerative medicine. Notable attention in this respect is directed toward biomaterials supporting tissue regeneration, among which chitosan-based scaffolds stand out as they are biocompatible, biodegradable, hydrophilic and non-toxic. Modified with boric acid and hydroxyapatite, these materials promise great potential for accelerating tissue healing and bone regeneration.[1,2] Boric acid is a source of boron, a bioactive agent known to modulate angiogenesis and neovascularization, crucial for effective tissue repair.[1] Meanwhile, hydroxyapatite exhibits osteoinductive behavior and can improve the mechanical properties of the material.[3] Any material used in biomedicine must be biocompatible, which is where the CAM assay applies. The chorioalantoic membrane (CAM) of the developing chicken embryo is a highly vascularized tissue, often used as a suitable substitute for in vivo options for angiogenesis, tumor growth, and metastasis studies, drug testing, and assessing biocompatibility. Composite boric acid/chitosan/hydroxyapatite scaffolds were prepared by covalent cross-linking with genipin.[2] The final product was a highly porous material with interlocking orifices that allow cell attachment and nutrient exchange. To demonstrate that the material has no harmful effects and would not be rejected by a living organism the scaffolds were implanted into CAMs, harvested after 7 days, embedded in paraffin, sectioned and stained with haematoxylin and eosin. Microscopic examination revealed abundant tissue integration and the presence of numerous blood cells within the scaffold pores. These results, indicating the angiogenic potential and biocompatibility of the scaffolds, make them a good candidate for subsequent research and possible future biomedical applications.

Key words: CAM, chitosan, scaffold, biocompatibility

References: 1. 10.1080/00914037.2019.1581202; 2. 10.3390/polym14214753;
3. 10.31803/tg-20230912143145.

* Correspondence: Iva Bazina, ibazina@fkit.unizg.hr

PHYSICALLY CROSSLINKED CHITOSAN HYDROGELS BY AUTOCLAVING

Yusuke Yamashita*, Yoshihiro Ohzuno, Masahiro Yoshida, Takayuki Takei

Graduate School of Science and Engineering, Kagoshima, Kagoshima University

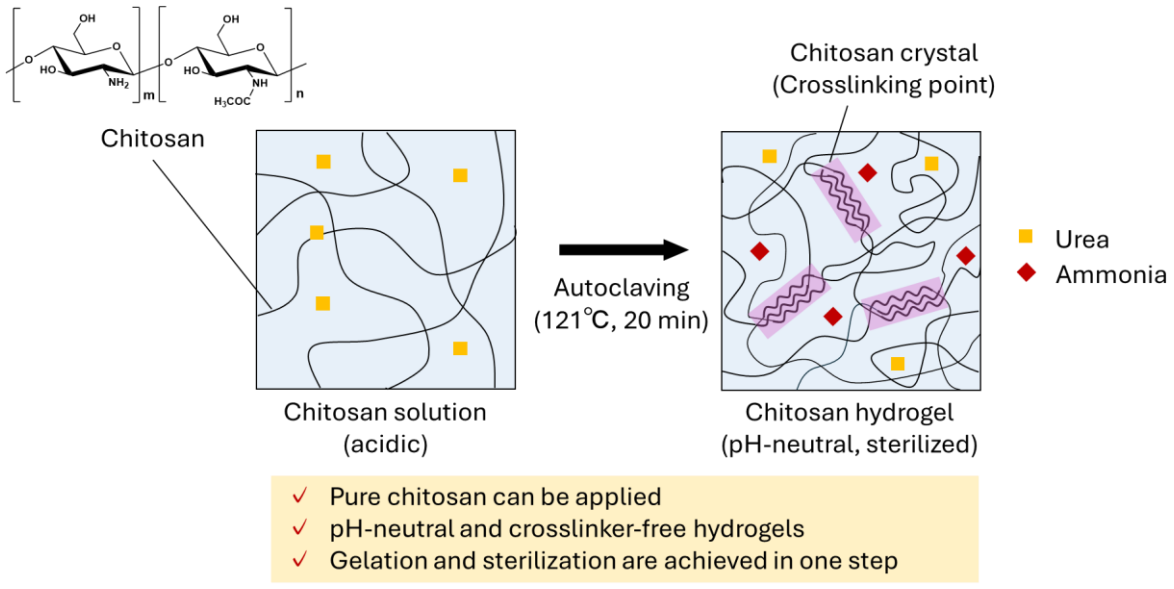
ABSTRACT

Chitosan is a polysaccharide with excellent biological properties such as biocompatibility, biodegradability, antibacterial properties, and wound healing promoting effect. Conventionally, chitosan hydrogels are acidic and contain toxic chemicals because chitosan dissolves only in acidic solvents and require toxic additives such as crosslinkers and polymerization agents to prepare chitosan hydrogels. These properties limit the use of chitosan hydrogels as medical materials. To solve these problems, various chitosan derivatives have been developed that dissolve in neutral water or gellable without cross-linking agents.[1,2] In this study, pure chitosan hydrogels were prepared by a simple process using urea hydrolysis by autoclaving (steam sterilization, 121°C, 20 min) without any crosslinkers[3]. When autoclaved, urea hydrolyzes in a chitosan aqueous solution, and ammonia is produced, which increases the pH of the solution, and chitosan becomes insoluble, leading to the formation of a chitosan hydrogel. The pH and osmotic concentration of chitosan hydrogels could be adjusted to be suitable for physiological conditions (pH: 7.4, and osmotic concentration: 285 mOsm/L) by changing the concentration of urea in chitosan solutions (chitosan: 2.5% (w/v), urea: 0.75–1.0% (w/v), pH: 5.5). The hydrogels had extremely low cytotoxicity without the washing process. The autoclaving technique for preparing low-toxic sterilized chitosan hydrogels in a single step is practical for medical applications.

Key words: chitosan, hydrogel, autoclaving.

References: 1. 10.1016/j.actbio.2011.10.005; 2. 10.3390/gels9040280; 3. 10.3390/macromol4020021.

GRAPHICAL ABSTRACT



* Correspondence: Yusuke Yamashita, k5290028@kadai.jp

ANTIBODY GENE INTEGRATION INTO CHICKEN PRIMORDIAL GERM CELLS USING TWO-STEP GENOME MANIPULATION TECHNOLOGY

Yuya Kaneko[1], Yoshinori Kawabe[1], Ken-ichi Nishijima[2], Masamichi Kamihira[1*]

[1] Department of Chemical Engineering, Faculty of Engineering,
Kyushu University, Kyushu University, Japan
[2] Graduate School of Bioagrocultural Sciences, Nagoya University, Nagoya, Japan

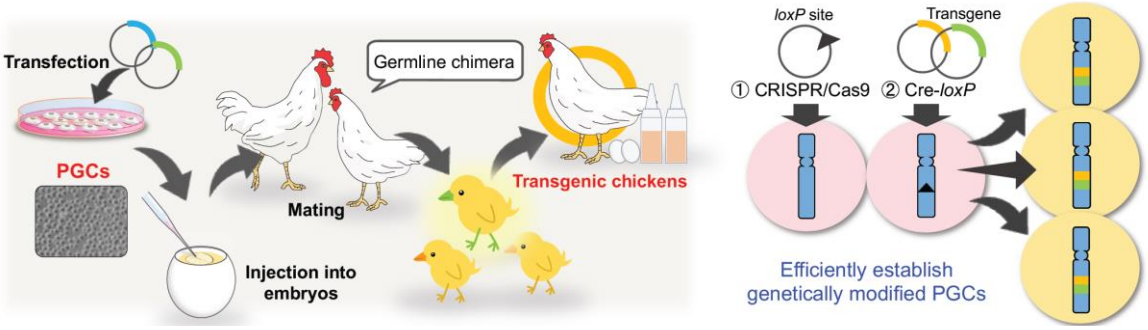
ABSTRACT

Demand for biopharmaceuticals has grown rapidly in recent years. However, limitations in production capacity and escalating manufacturing costs pose significant challenges, necessitating the development of more efficient production systems. We have proposed the use of chickens as bioreactors for the production platform of biopharmaceuticals.[1] Recombinant proteins have been successfully produced in the serum and eggs of transgenic chickens generated using a retroviral vector system.[1] While retroviral vectors can efficiently deliver transgenes into the cell genome, the uncontrollable integration site carries the risk of transgene suppression. We focused on chicken primordial germ cells (PGCs), which can differentiate into gametes such as sperm and oocytes, as a cell source. We have developed a two-step, site-specific transgene integration technology that combines CRISPR/Cas9 with Cre-loxP systems. Previously, genetically engineered PGCs with loxP sites at specific chromosomal locations were established by knocking in donor vectors using CRISPR/Cas9. In this study, we performed the targeted integration of two different antibody genes into founder PGCs using Cre-loxP. Retargeting vectors containing these antibody genes, along with a Cre recombinase expression vector, were introduced into the cells, followed by drug selection to obtain PGC clones. Genomic DNA from PGC clones was subjected to PCR analysis using primer pairs that only amplify if the Cre-loxP recombination occurs. Amplicons of the expected molecular size were confirmed in all clones, and the PGCs stably produced each antibody in the culture medium. This technology offers a promising approach for generating transgenic chickens that produce recombinant antibodies.

Key words: Transgenic chickens, PGCs, CRISPR/Cas9, Cre-loxP, Recombinant antibody.

References: 1. 10.1128/jvi.79.17.10864-10874.2005.

GRAPHICAL ABSTRACT



* Correspondence: Masamichi Kamihira, kamihira@chem-eng.kyushu-u.ac.jp

WHOLE-ORGAN ENGINEERED LIVER CONSTRUCT – A TRIAL TRANSPLANT

Lucija Štefan[1*], Mario Kokichi Uehara[1], Yasuhiro Ikegami[1], Yusuke Sakai[1], Yo-Ichi Yamashita[2], Shinichi Aishima[3], Hiroyuki Ijima[1]

[1] Kyushu University, Department of Chemical Engineering, Kyushu, Japan
[2] Japanese Red Cross Oita Hospital, Department of Surgery, Oita, Japan
[3] Kyushu University, Department of Scientific Pathology, Kyushu, Japan

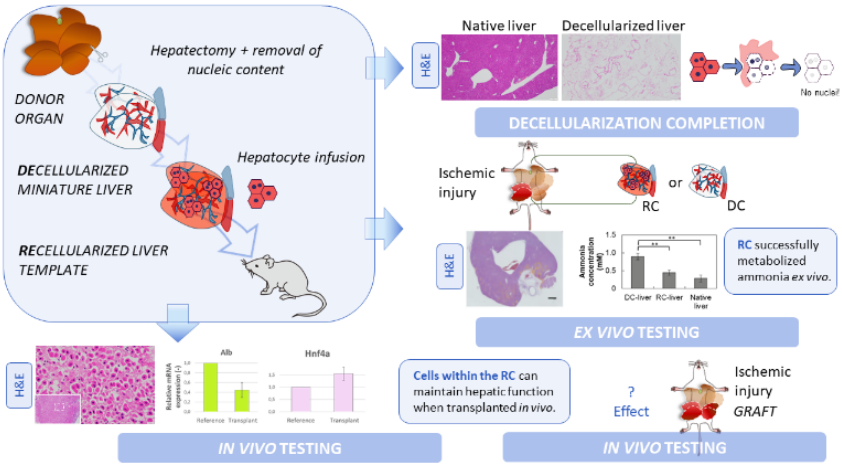
ABSTRACT

This research addresses the growing issue of organ donor shortage by utilizing the decellularization-recellularization technology for the construction of a miniature liver. By preserving the complex organ structure and native extracellular matrix composition, an excellent environment to promote cell functionality can be created. Key challenges include removing donor cellular components to prevent immune rejection, optimizing recellularization, ensuring oxygenation, preventing thrombosis and promoting long-term functionality.[1] In our study we focused on constructing a biocompatible liver based on the right lobe of mouse[2], to conserve the amount of valuable cells necessary to repopulate it. The completely decellularized liver was seeded with 1-2×10^7 primary rat hepatocytes corresponding to 1-2% of total liver mass in the graft and evaluated through blood extracorporeal systems in surgically induced liver injury models. Six hours post-reperfusion, the grafts successfully metabolized ammonia, demonstrating function comparable to a normal liver. Next, the viability of the cells within the graft was tested *in vivo*, demonstrating hepatic function (Alb, Hnf4α) can be maintained for up to one day with apoptosis levels (Casp3) close to normal. Finally, the grafts are planned to be tested in liver injury models and the results are discussed in this symposium.

Key words: liver tissue engineering, decellularization, primary hepatocytes.

References: 1. 10.3390/cells9020304; 2. 10.1016/j.jbiosc.2013.05.020.

GRAPHICAL ABSTRACT



* Correspondence: Lucija Štefan, stefan.lucija.771@s.kyushu-u.ac.jp

EXPERIMENTAL AND COMPUTATION STUDY OF THERMAL TRANSFORMATIONS OF STYRYL THIOPHENES IN ACIDIC MEDIA

Valerija Čolig[1]*, Vilma Lovrinčević[1], Dragana Vuk[1], Ines Despotović[2]

[1]Department of Organic Chemistry, University of Zagreb Faculty of Chemical Engineering and Technology, Trg Marka Marulića 19, 10000 Zagreb, Croatia
[2]Division of Physical Chemistry, Ruđer Bošković Institute, Biljenička cesta 54, 10000 Zagreb, Croatia

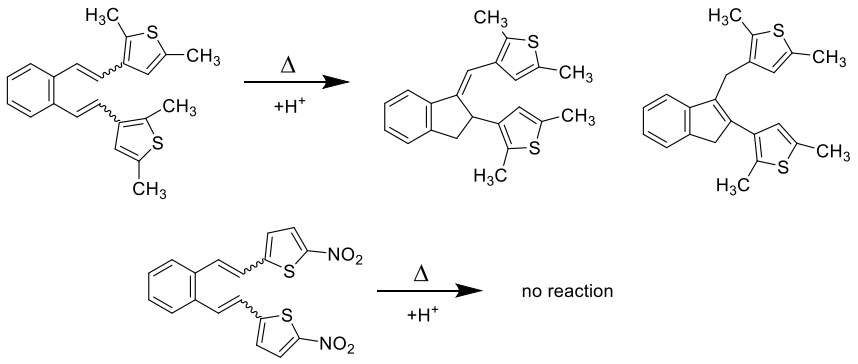
ABSTRACT

Computational methods may play a key role in modern chemistry, enabling detailed analysis and understanding of chemical reactions at the molecular level. Theoretical chemistry, which provides a mathematical description of chemical phenomena, serves as the foundation for computational chemistry. This branch of chemistry uses computational techniques and algorithms to investigate a variety of chemical problems, allowing for a deeper understanding of molecular processes and interactions.[1] In this study, the synthesis and thermal transformation in acidic media of styryl thiophene derivatives were presented and deep insight into the reaction mechanism was provided by quantum chemical calculations using the density functional theory (DFT) method.[2] Derivatives with different positions of substituents and heteroatoms were investigated, and all experimental results were in accordance with the computational study.

Key words: computational chemistry, density functional theory, thermal transformation, thiophene.

References: 1. 10.4172/2150-3494.1000127; 2. 10.1039/D3NJ02245E.

GRAPHICAL ABSTRACT



Reactivity of styryl thiophene derivatives

* Correspondence: Valerija Čolig, vcolig@fkit.unizg.hr

ASSESMENT OF THE ELECTRIC FIELD IN LOW-TEMPARATURE PLASMA FOR PLANT GROWTH ENHANCEMENT

Naoya Hidaka, Takamasa Okumura, Pankaj Attri, Kunihiro Kamataki, Naho Itagaki, Kazunori Koga*, Masaharu Shiratani

Kyushu University, Kyushu, Japan

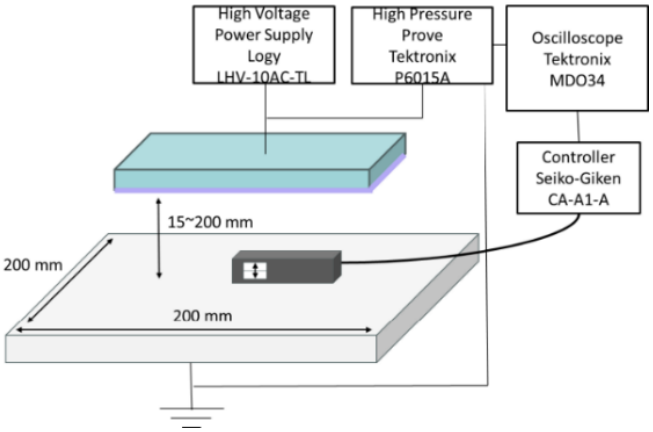
ABSTRACT

Plasma irradiation of seeds has gained significant attention as an innovative technology for enhancing plant growth.[1,2] Plasma generates various reactive species, photons, electric fields, and charged particles, which may lead to synergistic effects on plant development. We have found the growth enhance meant by seed treatments using a scalable dielectric barrier discharge (SDBD) plasma.[1,2] To elucidate the growth mechanism of the plasma induced growth enhancement. The quantitative measurement of plasmas is crucial. In this study, we measured the electric field generated by the SDBD using Pockels cells. The SDBD electrode has twenty ceramic-coated electrode rods. Powered rod and grounded rod are alternated to form like plate. High voltage pulse of 5.12 kV in peak-to-peak voltage and 12.7 kHz in reputation frequency was applied to the powered electrodes. A Pockels cell was positioned 10 mm from the center of the electrode to measure the electric field perpendicular to the surface. The results showed that the frequency of the applied voltage and the electric field were consistent. The high-frequency component likely resulted from the applied voltage. Previous studies indicate that a potential gradient above 15 kV/m impacts plant cells[3], causing about a 10% decrease in dry weight. In contrast, the electric field measured in our system was 6 kV/m, which could still affect plant cellular processes, though likely with less intensity. The interaction between this lower electric field and other plasma-generated species may influence the overall plant response, providing insight into the possible advantages of using plasma technology for plant growth enhancement.

Key words: Plasma agriculture, Pockels cell, Seed, DBD, Atmospheric pressure plasma.

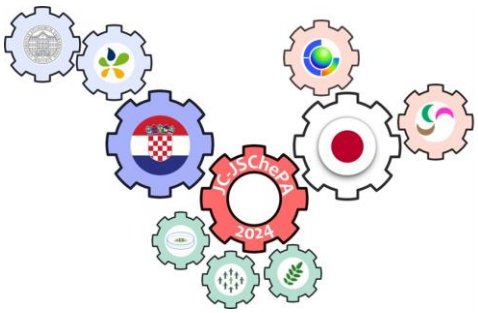
References: 1. 10.1016/j.cap.2013.11.056; 2. 10.7567/APEX.9.016201; 3. 10.1038/200490b0.

GRAPHICAL ABSTRACT



Schematic of electric field measurement generated by plasma.

* Correspondence: Kazunori Koga, koga@ed.kyushu-u.ac.jp



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