2ND NOVEL FLUIDIC TECHNOLOGIES AND APPLICATIONS WORKSHOP

WORKSHOP

9-10 April 2015

IZMIR/TURKEY

2ND NOVEL FLUIDIC TECHNOLOGIES AND APPLICATIONS WORKSHOP - ABSTRACT BOOK

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Dear Colleagues,

Researchers at the Department of Bioengineering at Ege University and Institute of Thermal Separation Processes at Technical University of Hamburg-Harburg brought their expertise together to carry out biotransformation in microfluidic devices and to establish long-term networking in microfluidics. The proposed INTEN-C project (113M050) is supported by BMBF and TUBITAK. One of the objectives of the project is to improve the collaboration between Turkish and German Universities. Therefore, the project is concentrating on reinforcing its science and technology potential by enhancing interactions between institutes, researchers and young scientists. It is supporting its existing human resources and new recruits by providing young scientists with solid and up to date knowledge in biocatalytic reactions in micro devices, sol-gel technology as an immobilization tool and mathematical modeling through exchange programmes, fellowships, training courses and workshops.

"**2nd Novel Fluidic Technologies and Applications Workshop**" which is held at Bioengineering Department, Ege University Izmir/TURKEY on **9-10 April 2015** is a result of these joint efforts. Within the past two decades, microfluidic technology has evolved from a highly advanced tool for engineers to a versatile field at the interface of physics, engineering, medicine, chemistry and biology. Over the past years, microfluidic approaches have been used in biotechnology, pharmaceuticals, life sciences, defense and public health for a variety of applications, including point-of-care diagnostics, high throughput screening of drug molecules, cell sorting, DNA sequencing on a chip and microchip capillary electrophoresis. Likewise, the trends in supercritical fluids have been extended to major life science applications such as encapsulation of drug molecules, enzymatic treatments and drying of scaffolds.

The scope of the workshop is to bring together scientists and young researchers to discuss breakthrough research in the field of fluidic technologies, specifically microfluidics and supercritical fluids focusing on life science applications and to enhance skills of young researchers thorough knowledge exchange. The second day of the workshop will be dedicated to young researchers with podium presentations in a competitive environment and the selected best three presentations will be rewarded by "Young Researcher Excelling in Novel Fluidics" award.

We look forward to the scientific exchange and the profile you bring to this meeting.

On behalf of the Organizing Committee,

Assoc. Prof. Dr. Ozlem YESIL-CELIKTAS

Workshop chair,

Ege University

Department of Bioengineering

2nd NOVEL FLUIDIC TECHNOLOGIES AND APPLICATIONS WORKSHOP (09-10 APRIL 2015)

	09.04.2015 (THURSDAY)
9:00-9:30	Registration
9:30-10:00	Opening session
10:00-10:45	Lab-on-a-chip for cancer cell biology: Controlled in vitro microenvironments (Assoc. Prof. Dr. Devrim PESEN OKVUR)
10:45-11.30	Novel fluidic technologies with emphases on life science applications (Assoc. Prof. Dr. Ozlem YESIL CELIKTAS)
11:30–12:15	Applications of FTIR spectroscopy for online process analytics and control (Dr. Anne BOOS)
12:15-13:30	Lunch
13:30-14:15	Design and fabrication of a microfluidic device for synthesis of biopolymeric nanoparticles (Mehmet Dogan ASIK)
14:15-15:00	High throughput protein microarrays determining humoral immune responses (Ass. Prof. Dr. Sultan GULCE IZ)
15:00-15:45	Application of porous organic and inorganic sol-gels for immobilization of enzymes in new reaction and separation systems (Rene HEILS)
15:45-16:15	Coffee break
16:15-17:00	Machining-based fabrication of microfluidic devices for biotechnology (Assoc. Prof. Dr. Barbaros CETIN)
17:30-19:30	Cocktail

2nd NOVEL FLUIDIC TECHNOLOGIES AND APPLICATIONS WORKSHOP (09-10 APRIL 2015)

	10.04.2015 (FRIDAY)	
10:30-11:00	Seda Nur Topkaya İlbey Karakurt	(Ege University, Faculty of Pharmacy) (Bilkent University, Mechanical Engineering Department)
11:00-11:30	Coffee break	
11:30-12:30	Elif Özdemir Kaynak Oğuz Kayıllıoğlu Aslıhan Kazan Reza Rassoli	(COMU, Bioengineering Department) (Koç University, Department of Physics (Ege University, Bioengineering Department) (Bilkent University, Mechanical Engineering Department)
12:30-13:45	Lunch	
13:45-15:30	Hamdullah Yanık Cansu Yavuz Deniz Kankale Ece Yıldız Öztürk Gizem Batı Dilan Karabulut Erdem Çağatay	(IYTE, Department of Molecular Biology and Genetics) (Ege University, Bioengineering Department) (IYTE, Department of Bioengineering) (Ege University, Bioengineering Department) (IYTE, Department of Bioengineering) (Ege University, Bioengineering Department) (TOBB Uni. of Eco.&Tech., Mech. Eng. Dept.)
15:30-16:00	Coffee break	
16:00-17:00	İrem Cemre Türü Emre Tarım Cahit Müderrisoğlu Bilal Demir	(Ege University, Materials Science and Eng. Department) (IYTE, Department of Material Science and Technology) (Ege University, Bioengineering Department) (Ege University, Biochemistry Department)
17:00-17:30	Award ceremony and closing remarks	

Preface

In the past two decades, fluidic technologies such as supercritical fluids and microfluidics transformed life science applications by employing engineering principles and methods. These fluidic technologies are currently undergoing a paradigm shift owing to revolutionary advances in nanomedicine, biomedical and nanotechnologies and beginning to converge in unexpected ways.

As we are organizing the **2nd Novel Fluidic Technologies and Applications Workshop**, supercritical fluids and microfluidics are on the edge of a more advanced stage. More documents are published on these topics, journals are dedicated to supercritical fluids and microfluidics and moreover the number of products using microfluidics is on the rise. It is an exciting field in which to work, with simultaneous advances being made on many fronts. Given the dynamic nature of the field of study, the materials presented in this abstract book are intended to bring the reader to a thorough understanding of the current state of the art regarding life science applications of supercritical fluids and microfluidics.

Furthermore, the section tagged as “Podium presentations” contains studies carried out by young researchers which will be presented in a competitive environment and evaluated by a committee based on the following criteria; (i) elicitation of the scientific problem / request questioning if a logical hypothesis/statement of problem is presented, background information is relevant and summarized well along with the clarity of connections to previous literature and broader issues are clear, (ii) objective of the study, (iii) originality providing evidence that the study exceeds state of the art, (iv) contribution to science and (v) presentation. The best three studies will be rewarded by “**Young Researcher Excelling in Novel Fluidics**” award.

Hopefully, this abstract book will provide the readers with the knowledge necessary to create an understanding in the field of novel fluidic technologies.

Assoc. Prof. Dr. Ozlem YESIL-CELIKTAS

Invited Speakers

Lab-on-a-chip for cancer cell biology: controlled *in vitro* microenvironments

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Keywords: Cancer cell biology, lab-on-a-chip, PDMS, UV lithography

Introduction: The leading cause of death for cancer patients is metastasis. Metastasis defines both the process of spreading of cancer cells from the primary tumor and the resulting secondary tumors. During metastasis of carcinoma (cancer of epithelial tissue), tumor cells degrade the underlying basement membrane and degrade into the connective tissue, migrate towards blood vessels, intravasate, extravasate and seed secondary sites in distant organs. As cancer cells metastasize, they interact with various extracellular molecules and stromal cells. Current *in vitro* cell culture systems do not reflect the organization and complexity of the *in vivo* microenvironment. 3D in vitro culture systems are now proving to be a necessary step linking *in vitro* studies, *in vivo* animal models and clinical trials.

Lab-on-a-chip (LOC), which has started from microelectromechanic systems applications produced by nano- and micro-fabrication methods in the semiconductor industry, is now being applied to chemistry, biology and medicine. The market share of LOC was 2.6 billion dollars in 2010 and is expected to reach 5.6 billion dollars in 2015. The economic significance of LOC is based on the many advantages it provides: Small liquid volumes (pL), precise spatial & temporal control, ability to successfully mimic the physiological context, high-throughput analysis, low fabrication costs, portability and safety.

From LOC, the applications have evolved into cell-on-a-chip, organ-on-a-chip and are aiming for human body-on-a-chip [1].

Controlled *in vitro* microenvironments (CivMs) are realizations of LOC technology at the cell and organ levels [2, 3, 4]. UV lithography can be used to generate PDMS-based CivMs. Mimicking the tumor microenvironment faithfully offers tremendous advantages to gain insight into the cell biology of cancer and to move closer to solutions.

Here, results of current research projects in the Controlled *in vitro* Microenvironments Laboratory will be presented.

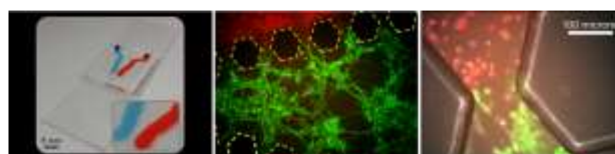

Fig. 1. Interactions of breast cancer cells and macrophages can be investigated in 3D CivMs.

Discussion & Conclusions: Cell-on-a-chip/ organ-on-a-chip are the next frontiers of LOC. Controlled *in vitro* Microenvironments (CivMs) can offer solutions to important questions in cancer cell biology shaping new paths to diagnostics and therapy.

Acknowledgements:

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Novel fluidic technologies with emphases on life science applications

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Keywords: Novel fluidic technologies, supercritical fluids, microfluidics, life science applications

Introduction: In the past two decades, fluidic technologies such as supercritical fluids and microfluidics have seen a phenomenal growth with increasing applications from basic sciences to most engineering disciplines. These fluidic technologies are undergoing dramatic changes with the emergence of the field of nanotechnology. The most obvious impact is the emergence of the life science applications of supercritical fluids such as drug encapsulation techniques allowing fabrication of nanoparticles with narrow particle size distributions, processing of scaffolds and usage of supercritical CO_2 as a reaction media. The developments in microfluidics coupled with nanotechnology are paving the way for growing number of applications for human welfare.

Discussion & Conclusions: Various life science applications of supercritical fluids have been carried out. A rosemary extract exhibiting anti-proliferative activity against various human cancer cell lines was encapsulated by polycaprolactone using solvent evaporation method and gas anti-solvent process at 300 bar, 40 °C using supercritical CO_2 at a flow rate of 20 g/min. Rosemary extract encapsulated using gas antisolvent process exhibited a narrow particle size distribution, a lower mean particle size and higher encapsulation efficiency (254.5 nm, 82.8 %) compared to the traditional fabrication method (617.5 nm, 62.2 %) [1]. A chitosan based scaffold was prepared by fabrication of hydrogel followed with supercritical CO_2 drying. Under elicited optimum conditions, a porosity of 87.03% was achieved which enhanced cell attachment, thereby providing an efficient platform for tissue engineering applications [2]. Hydrolytic enzymes such as amylase [3] and protease [4] were treated and retreated with supercritical CO_2 where activities were enhanced (**Fig. 1**). Currently, enzymes targeting hydrolyses of glyosidic bonds are investigated in microfluidic devices (**Fig. 2**) in order to obtain aglycone structures which have high value applications in pharmaceutical industry.

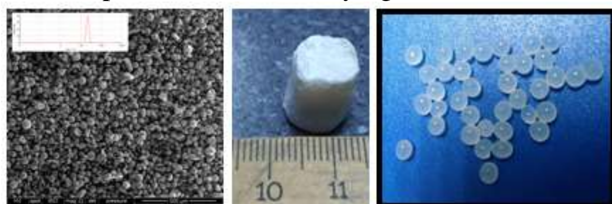

Fig. 1. Gas antisolvent encapsulated nanoparticle, chitosan scaffold, alginate particles (from left to right).

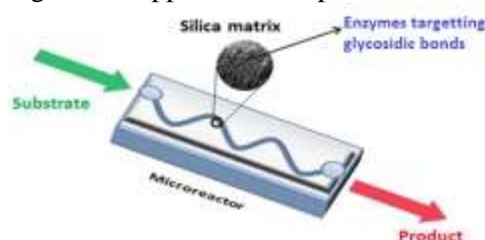

Fig. 2. Enzyme immobilized microfluidic chip used for hydrolysis of saponins.

Based on patenting trends in enzyme related applications [5], immobilization procedures carried out at room temperatures, incorporation of novel hybrid materials, the integration of supercritical fluids and microfluidics, employing ionic liquids as wall-less microreactors, designing low cost, high performance microfluidic devices pose challenges in various life science applications.

Acknowledgements:

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Process analytical technology for online process control

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Keywords: Biocatalysis, FTIR spectroscopy, process analytics, process control

Introduction: The monitoring of the progression of processes in chemical or biotechnological production via sample-drawing (offline) methods is costly in terms of labour and time. Therefore, the ability to analyze the components of such a reaction online is advantageous. If the monitoring is done in real-time, it is possible to react to the analysis and control the process. Infrared spectroscopy provides qualitative information on molecular structure and allows quantitative determination of concentrations. Thus it is a valuable tool to monitor reactions online and in real-time [1]. In combination with an ATR probe and chemometric modeling [2], product or substrate concentrations can be determined even in multiphase environments [3, 4].

Introduction: In the mid infrared range (4000 – 400 cm^{-1}) molecules are in their fundamental state of vibration. Absorption bands in this range are more intense and less broad in comparison to the near-infrared range (12000 – 4000 cm^{-1}). Thus, an assignment of characteristic functional groups to single bands is possible. Due to the relatively high absorbance of samples in the mid-infrared, a layer thickness of only a few micrometers is required for spectroscopy in transmission geometry. This limitation can be overcome either by utilizing Attenuated Total Reflectance (ATR) or by miniaturizing the equipment using microsystem technology.

Biotransformations on the Microscale – Reaction Screening: The strong absorbance of organic molecules in the mid IR is a challenge for measurements in transmission but also a chance as it allows the miniaturization of the equipment and space-resolved experiments in IR-transparent devices (i. e. made of silicon or CaF_2). Different approaches have been pursued to improve screening and reaction monitoring applications.

State of the art IR-transparent microtiter plates are suitable only for the measurement of dried films. In our institute, a new concept for microtiter plates is in development together with the institute of microsystem technology that allows the measurement of solutions, circumventing problems arising from capillary forces and evaporation. The microtiter plates are compatible with a commercial IR plate reader (HTS-XT, *Bruker*) and pipetting robots. Within a single well of the plate, the monitoring of (biocatalytic) reactions is possible. An approach for space-resolved reaction monitoring are IR-transparent microreactors [5]. Similar to our microtiter plates, those are compatible with a commercial microplate reader. When continuously pumping the reaction components through the microreactor, the measurement position then corresponds to the reaction time. This can be used for the monitoring of a reaction and the screening of process conditions. The small volume of the reactor reduces the amount of chemicals needed and thereby costs and waste. The monitoring of a biocatalytic ester hydrolysis has been successfully performed in an IR-transparent microreactor [6].

Industrially Relevant Biotransformations – Process Development and Analytics: FTIR spectroscopy in transmission geometry is limited to small path lengths and single-phase systems. Measurements in turbid or strongly absorbing solutions are not possible. Attenuated Total Reflectance (ATR) probes can resolve these challenges. ATR probes comprise a high refractive index crystal. At the boundary of this crystal, total reflectance of an infrared beam occurs, which

causes an evanescent wave penetrating a few micrometers inside the lower refractive index material in contact with the probe. The evanescent wave interacts with the sample and the beam in the ATR probe is attenuated. The ATR probe enables FTIR spectroscopy in strongly absorbing and multiphase environments, and can therefore be utilized to monitor solvent-free reactions in bubble column reactors (**Fig. 1**). The ATR-FTIR spectra are analyzed using a chemometric model to reveal the concentrations of the compounds. During the progress of a reaction, magnitudes like conversion or even molecular weights in case of a polycondensation can be monitored [7]. This information can be utilized for process control. In principle, a single probe is sufficient to control a process. By adding more probes at different locations in the reactor, space-resolved information can be acquired (**Fig. 1**).

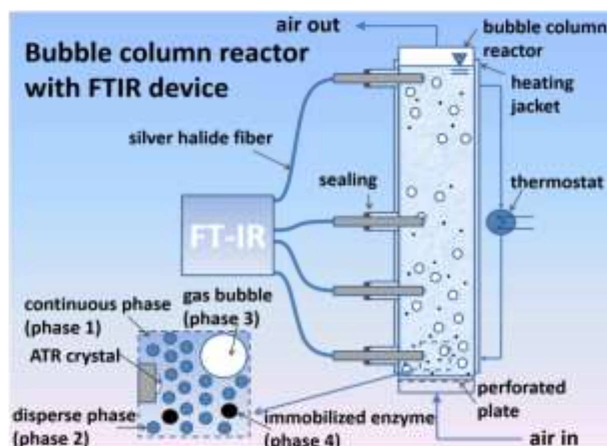

Fig. 1. Setup of an ATR-FTIR device for spectroscopy inside a bubble column reactor; adapted from Ref. [3].

Current investigations in the microdevice field concentrates on further improving the devices itself as well as broaden their range of applications, i. e. adding an inlet for a gaseous sample to the microreactor. Current limitations in commercial microplate readers are low intensities of the IR radiation sources and large beam radii leading to insufficient spatial resolution. The emergence of quantum cascade lasers emitting in the infrared may have the potential to solve those problems.

FTIR spectroscopy with ATR probes is currently applied to various systems, e. g. glycerol esters typically used in the cosmetics industry. Further studies will focus on broadening its applicability on biomaterials.

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Design and fabrication of a microfluidic device for synthesis of biopolymeric nanoparticles

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Keywords: Nanoparticles, targetted drug delivery

Introduction: The use of microfluidics for biomedical applications has a number of clear advantages. Microfluidic devices have been proposed and tested in many diagnostic applications. Diagnostic devices based around microfluidic chips exploit the high throughput and specificity that can be achieved when the size of systems is reduced. Innovative designs allow for multiple tests to be performed on a single chip, often paired with optical detection methodologies to create fast reliable diagnostic tools. In addition, the intrinsic low cost of microfluidic diagnostic devices makes them attractive for environments where classical test are not appropriate, such as military applications and developing world medicine [1].
The other significant and emerging area for the application of microfluidics in biomedical problems is in the preparation of novel drug delivery systems, specifically micro- and nano-particles. Microfluidic systems allow for exquisite control flow parameters, temperature, mixing and other physical parameters important in the synthesis and purification of micro- and nano-particles. In addition there are some processes that are only possible in microfluidic chips due to their unique properties [2].
Increasingly people are interested in drug delivery with micro- and nano-particles. This is mostly due to the advantages properties of very small particles, such as improved tissue penetration. Encapulation of active molecules has been used extensively in agriculture and the cosmetics industries for many years now, and increasing the pharmaceutical industry is looking to exploit these technologies. Metal nanoparticles have found application in cancer treatment [3] and biopolymer nanoparticles are under investigation due to their intrinsic biocompatibility. The surface functionalization of micro- and nano-particles is being investigated to improve drug delivery specificity.
Micro-particles have been being synthesized for many years, typically in batch or large scale continuous processes. However the most popular processes for their synthesis have not been transferable to biomedical application. In addition nanoparticles are often much harder to synthesis via traditional methods. An interesting type of micro- and nano-particle that is very promising for biomedical application is made from biopolymers, such as: chitosan, alginate, casein, gelatin, fibroin etc [4]. These materials are particularly challenging for large scale synthesis as the physical conditions need to be tightly controlled. Microfluidic systems are therefore very effective at solving these problems, and biopolymer nanoparticles, loaded with active molecules have been demonstrated [5].
The other great challenge in the preparation of biopolymer micro- and nano-particles formulations is purifying the particle prior to formulation. Typically the concentration of particles is very low and there are a number of unwanted, albeit harmless, contaminant. Microfluidic technologies also offer solutions towards purification, removing the need to filter or centrifuge the material for purification. By exploiting lamina flow and the charge or polarizability of micro- and nanoparticles they can be effectively concentrated and purified [6].
Discussion & Conclusions: The use of microfluidics to prepare micro- and nano-particles from biopolymers for biomedical applications is a new and exciting field. We expect that research into this area will generate exciting technical developments as well as real life medical products.

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High throughput protein microarrays for determining humoral immune responses

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Keywords: Protein microarrays, humoral immune responses

Introduction: The very high-accumulated data in genomics and proteomics made possible to utilize high throughput analysis. After the wide range applications of cDNA microarrays, protein microarrays are also gained importance. Protein microarrays can be simple defined as immobilisation of proteins onto a solid surface to study the interactions of protein-protein; nucleic acid; small molecule or antibody in a sensitive and reproducible microenvironment [1].
To determine humoral immune responses by protein microarrays, the rationale is to print whole proteome of the infectious agent and probe the clinical sera with known serology. After interpretation of the array data, it is possible to detect immune reactive antigens among the proteins on the protein microarray chips. These antigens are promising candidates for subunit vaccines and diagnostic proteins [2]. The main steps of high throughput protein microarray fabrication are given on **Fig.1**.

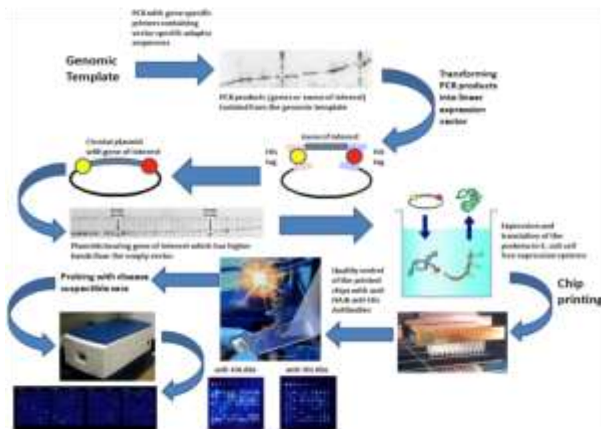

Fig. 1. The overview of high throughput protein microarray fabrication

As a case study, serological cross-reactivity between *Plasmodium falciparum*, *P. vivax* and *Toxoplasma gondii* will be discussed during the presentation [3].
Discussion & Conclusions: Protein microarray technology like cDNA microarrays allows us to screen hundreds or thousands of proteins simultaneously. Using protein microarrays, entire microorganism proteomes can be screened to identify promising vaccine and diagnostic antigens while data interpretation is combined with novel bioinformatics tools.

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Application of porous organic and inorganic sol-gels for immobilization of enzymes in new reaction and separation systems

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Keywords: Enzyme immobilization, sol-gel materials, microreactors, integrated processes

Introduction: The entrapment of enzymes in highly porous sol-gel materials is a widely applied immobilization technique. In the first place, the immobilization in a polymer-matrix increases the stability as well as the reusability of the enzyme for application in biosynthesis. In addition, the nature of the sol-gel process allows easy loading of immobilized enzyme into microreactors or coating of structured packings in reactive distillation columns. By adjusting the sol-gel parameters the chemical and structural properties of the matrix can be altered in a way that they improve the stability of the enzyme in the respective reaction system [1]. This work describes the development of sol-gel matrices for the immobilization of selected enzymes in two different process examples, in microfluidic systems and in reactive distillation columns.

Enzymes in microfluidic systems: In the first part, the applicability of enzymes in microfluidic systems for future use with saponins is studied. Saponins are glycosidic substances from which valuable compounds can be obtained by selective enzymatic hydrolysis. The reaction can be catalyzed with the enzyme β-glucosidase.

Fig. 1. Microfluidic system.

Compared to stirred tank reactors, microreactors offer larger surface to volume ratios and well-defined reaction times. Due to the small size of the microchannel system, heat and mass transfer as well as energy efficiency are improved.

In addition, the miniaturized reactors require only small volumes of resources leading to less usage of enzyme. For immobilization of catalysts into the microreactor, silica-based systems are well described and established as carrier for enzymes in microfluidic systems [2]. The liquid precursor solution can easily be filled into the small channels of the microreactor and subsequently solidify as an aqueous gel within these structures.

Compared to the often used silica gels, the application of organic alginate gels offers several benefits as enzyme carrier material. While silica-based carriers show shrinkage in aqueous system which leads to bypass flows in µ-reactors, alginate gels rather take up water and indicate a swelling behavior. In addition, the alcohol formation in the sol-gel reaction was reported to lower the enzyme activity [3]. The alginate formulation are completely aqueous based and therefore correspond to a rather mild immobilization media. In this work, the feasibility of alginate gels for the immobilization of β-glucosidase is investigated and the performance of enzymes immobilized in alginate gels is compared with silica based systems.

Enzymes in reactive distillation: In the second process example, a silica-based coating was developed for commercial structured packings in order to immobilize lipase B from Candida Antarctica (CALB) in reactive distillation columns [4]. In that way, the increased surface area of the packing can be used both for a better vapor-liquid mass transfer and as contact area between substrates and biocatalyst. The composition of the biocatalytic coating was adjusted to the favoured conditions of the enzyme. Lipase CALB showed for example an increased activity in more hydrophobic coatings consisting of alkoxysilanes.

The biocatalytic coatings were successfully test-

ed in a batch reactive distillation for the kinetic resolution of 2-pentanol. The integrated setup allowed in-situ removal of the product which drove the conversion beyond the equilibrium limitation.

Fig. 2. Structured packing with silica-based coating containing lipase CALB.

In addition, the feasibility of an in-situ coating process for structured packings with biocatalytic coatings is presented. This method allows renewing the biocatalyst in reactive distillation setup without disassembly of the internals. In spite of the instability of the biocatalyst, this in-situ coating method enables to prolong the operation time of the column and avoids the exchange of cost-intensive catalytic column internals. **Conclusions:** This work demonstrates that tailor-made sol gels can be used to immobilize enzymes in new reaction systems (microreactors) as well as integrated processes (reactive distillation) und in that way expand the operation window for the technical biocatalysis.

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Machining-based fabrication of microfluidic devices for biotechnology

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Keywords: Microfluidics, bio-particle manipulation

Introduction: Microfluidics and lab-on-a-chip technology offers unique advantages for the next generation devices for biotechnology applications. When the fabrication of the microfluidic devices is concerned, there are basically two common approaches: direct substrate manufacturing (photolithography, etching, laser ablation etc.) and mold-based techniques (hot embossing, injection molding or soft-lithography). Photolithography has good ability to manufacture very small and complicated microchannel structures, but it usually involves multi-step processes which take considerable time and specific chemical requirements especially for etching steps in high tech facilities such as a clean-room environment. One alternative method to fabricate the microfluidic device is to use mechanical micromachining (i.e. CNC-machining) either for direct substrate manufacturing or for the fabrication of the mold.

Mold-based techniques require a mold (sometimes mold is referred as the mask) to be fabricated. Although the fabrication of the mold may need lithography-based, relatively complicated fabrication process; once the mold is fabricated, the mold may well be used for several times. After the completion of the mold, the rest of the fabrication procedure is simple and highly reproducible (i.e. low-cost replication), which makes mold-based techniques very suitable for mass production. A common material used in the fabrication of the microchannels is the Polydimethylsiloxane (PDMS) due to its low cost, low toxity and transparency. Bonding PDMS with glass can be achieved using a straightforward surface treatment process with oxygen plasma.

For direct substrate manufacturing, the limits of the process is constrained by the size of the milling fabrication of the mold, the process is limited by the *xyz*-accuracy of the tool-positioner of a CNC-machine since the negative of the microfluidic structure is fabricated as the mold. With today's technology, by using magnetic bearings for their positioning systems, the *xyz*-accuracy of a conventional CNC-machines are around 5 micrometer. Therefore, a mold can be fabricated using mechanical machining within couple of hours without any need for clean-room equipment within the desirable accuracy limits for micro-fluidic devices. Moreover, CNC-machining can generate 3D structures without any difficulty.

In this talk, different aspects of machining-based microfluidics devices will be discussed. Moreover, some recent microfluidic devices which have potential to be used in biotechnology based applications and were fabricated by machining within Bilkent University Microfluidics & Lab-on-a-chip Research Group will be demonstrated.

Podyum Presentations

for

“Young Researchers Excelling in Novel Fluidics” Award

Disposable electrochemical biosensors for detection of DNA hybridization

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Keywords: DNA hybridization, electrochemical DNA biosensors, point of care diagnostics

Introduction: Since the first biosensors were demonstrated by Clark and Lyons in 1962 [1], the idea behind biosensors has been improved in many areas related to clinical diagnostics. As much as new sensing systems are being developed today, a great deal of effort is spent for the integration of biosensor system within an efficient detection strategy to deal with for real sample analysis. In this respect, electrochemical DNA biosensors offer a promising route for the detection of polymorphisms/mutations, alterations of genes and potential drug-DNA interactions via hybridization because of their short assay time, miniaturization, portability and low-cost [2, 3]. The principle of electrochemical DNA biosensors is based on the conversion of hybridization into the analytical signals via a transducer. Herein, the immobilization strategies of DNA onto the surface of the different disposable electrodes and experimental parameters for optimization of the biosensors for an effective DNA hybridization will be presented. The strategies for biomarker, mutation and polymorphism detections will be exampled. Future goals to create Point of Care Diagnostics (POCs) will also be discussed.

Materials & Methods: The height of the guanine oxidation peaks of DNA were used as analytical signals**.** The signals of probe (single stranded DNA), hybrid (double stranded DNA) and control oligonucleotides were measured with Differential Pulse Voltammetry (DPV) by using 3 electrode systems (graphite electrodes and screen printed electrodes as the working electrodes, a reference electrode and a platinum wire as the auxiliary electrode).

Results & Discussion: In order to provide best hybridization, experimental parameters as probe and target concentrations, hybridization-immobilization periods and surface activation conditions were optimized. Hybridization was detected by the decrease of the probe signals after interaction with its complementary target due to all guanines in the probe were partly closed to oxidation after the hybridization. By using electrochemical techniques, we successfully achieved to detect hybridization for various important biomarkers such as DNA hypermethylation in prostate cancer and single point mutation in myeloproliferative disorders.

Conclusions: We need effective, simple and easy to handle assays for point-of-care tests for the rapid and cheap detection of various diseases. In the case of clinical specimens, such as blood, urine, saliva and tissue, the challenge of sample preparation and detection of biomarkers within these samples can be reduced by benefiting from nanotechnology via integrated-miniaturized sensing systems. With respect to high sensitivity of electrochemical techniques, we can create POCs by using modified electrode systems.

Acknowledgements:

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Continuous flow microfluidic PCR reactor

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Keywords: Microfluidics, polymerase chain reaction

Introduction: Polymerase-chain-reaction (PCR) is a thermal cycling process (repeated heating and cooling of PCR solution) for amplifying DNA. PCR devices have many biomedical applications. One of the most important aspects for the success of PCR is to control the temperature of the solution precisely at the desired temperature levels required for PCR in a cyclic manner. Microfluidics offers great advantages over conventional techniques in PCR; Faster heating and cooling rates due to reduced thermal mass, reduced sample volume, disposability, portability [1, 2, 3]. In this study a computational model to study and simulate such a microfluidic device having a spiral microchannel, which was previously presented in recent research [4], is developed and experimentally verified.

Computational Model: The computational model that was developed using COMSOL Multiphysics software. This tool allowed us the combine different physical phenomena with ease. In order to solve the temperature locally along the microchannel, the continuity, momentum and energy equations within the microchannel were solved for using the Pipe-flow module which simplifies all the equations to their 1 dimensional form taking only the tangential components into account. In order to obtain the heat generation values from the electrodes due to the Joule heating was determined using the Electrical shell module. To determine the temperature of the PDMS chip, the conduction equation was solved. The use of the Pipe-flow module and the Electrical shell module simplify the model and decrease the computational power which in turn decrease the computation time [5].

Fabrication: The microfluidic chip was fabricated in 5 step:

(1) A mold was fabricated via CNC machining
(2) The mold was used to create the micro-channel structure by using PDMS
(3) The shadow mask used for electrode deposition was manufactured via Electro Discharge Machining.
(4) The shadow mask was used to deposit chrome on to a silicon wafer.
(5) The PDMS microchannel structure and the silicon wafer are strongly bonded together via a straightforward plasma treatment.

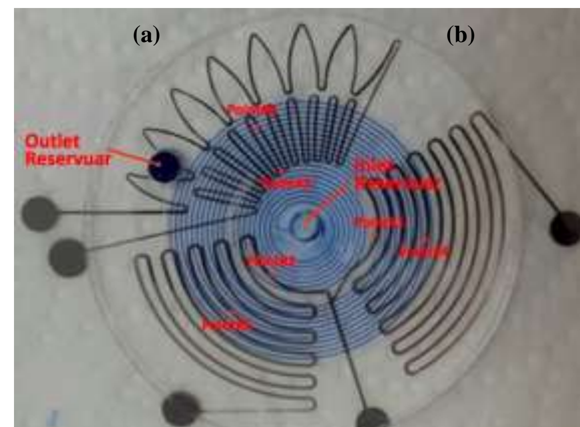

Fig 1. (a) Shadow mask**, (b)** Mold of the chip, **(c)** microfluidic chip.

Experiments: Following the fabrication of the chip, the experiments were conducted. The water solution was degassed prior to the experiments. The fluid flow was generated using syringe pump with the desired volumetric flow rate. Six thermocouples were located on the chip to monitor the temperature (two for each temperature zone with varying distances to the center of the microchannel). The temperature values were recorded via data acquisition device and PC. DC power supplies were used to supply the required voltage for the electrodes.

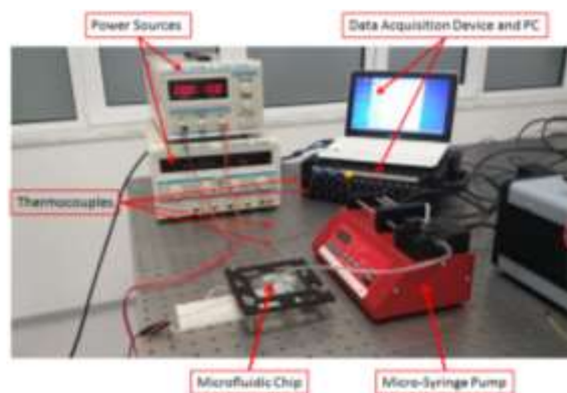

Fig. 2. Experimental set-up.

Results: PCR has a predefined thermal cycle that must be met in order to obtain good results from the process. Different chip parameters (channel width, electrode geometries, channel length, etc.) were studied with the computational model. The chip parameters were optimized to obtain the best the thermal cycle (i.e. temperature zones with minimum temperature gradients). **Fig. 3-(a)** shows the computational results for the optimum design. In order to verify the computational model, experiments were conducted. **Fig. 3-(b)** shows the experimental data that was obtained the thermocouples. As seen from the figure, the experimental results are within the desired temperature ranges as predicted by the computational model. Moreover, the voltage values are in good agreement with the computational model. The voltage values determined from the computational model was 2.0V, 5.1V and 9.3V, and during the experiments the voltage values were found as 2.2V, 4.9V and 9.3V for different temperature zones.

The total process time for a 100µL sample was found to be approximately 40 minutes. For this preliminary study, water was used as the buffer solution. The use of the PCR mixture as the buffer solution both in the computational model and the experiments will be our future research direction.

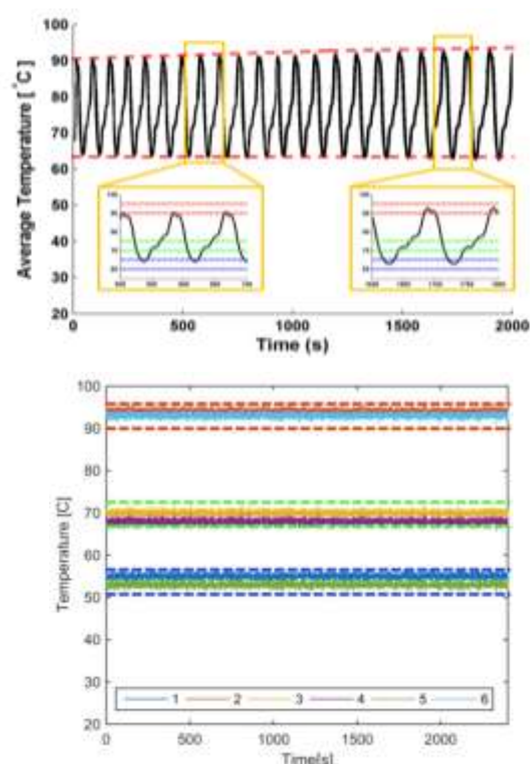

Fig. 3. Temperature values of the microfluidic chip: **(a)** computational, **(b)** experimental.

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Fabrication of bone tissue engineering scaffolds with supercritical CO_2 processing

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Keywords: Supercritical CO_2 drying, bone tissue engineering, polymeric scaffold, porosity, osteoblast

Introduction: Supercritical Fluid Technology (SFT) has already confirmed to be feasible for many pharmaceutical applications, represents a recent approach for the enhanced processing of many materials and is appearing as a promising trajectory for the preparation of injectable particles and three-dimensional structures suitable to be used in tissue engineering. Supercritical Fluids (SCFs) especially supercritical carbon dioxide (SC-CO_2) have distinctive properties as alternative to organic solvents for use as added substances in polymer processing due to it is relatively cheap, non-toxic, non-flammable and non-ozone depleting [1, 3].
SC-CO_2 foaming process for fabricating one of the three components of tissue engineering, scaffold, is selected among other different methodologies, including electro-spinning, solvent casting, particulate leaching, melt molding, solid free form fabrication, gas foaming and freeze drying with having a number of unique properties that could be utilized for polymer synthesis in these media by force of liquid-like densities and gas like viscosities and diffusivities, ideal for penetrating into porous structures [2, 3, 4].
Methods: Scaffolds were fabricated by two techniques as schematically presented in **Fig. 1**. Effects of process parameters were investigated and optimum conditions were determined as 250 bar, 45°C and 120 min yielding the best porosity and the stable morphological structure based on the characterization by mercury porosimetry and scanning electron microscopy. Scaffolds were also tested for cell holding capabilities with the Saos-2 cell line and cell-scaffold constructs were analyzed for their osteoblastic differentiation using polymerase chain reaction (PCR) [3].

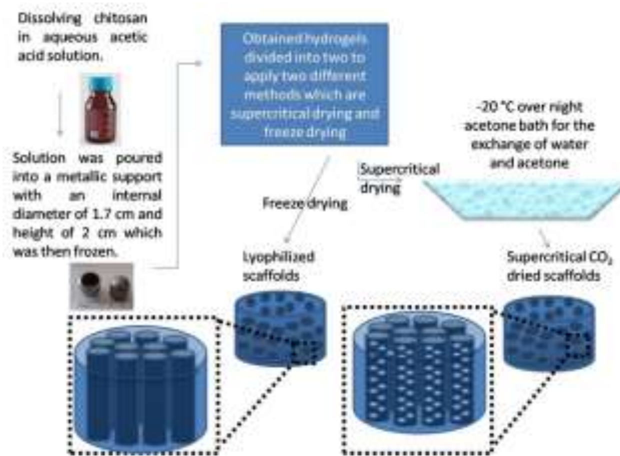

Fig. 1. Flow chart for the preparation of the scaffolds by lyophilization and SC-CO_2 drying methods.

Results & Discussion: Results showed that SC-CO2 treated scaffolds revealed the same surface area (87.03) as the lyophilized one (88.68) but with a reduction of 95.83% in process duration. Furthermore, SC-CO2 dried scaffold was observed to be a suitable support for cell attachment, thereby providing an ideal basis for transport of nutrients and cell adhesion (**Fig. 2**).

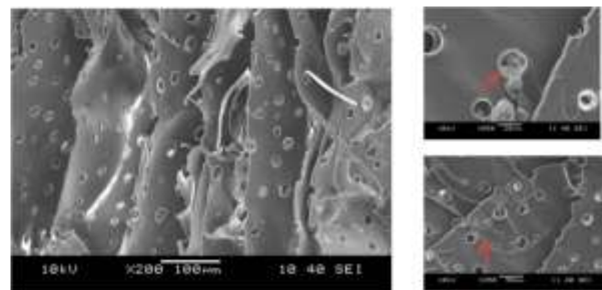

Fig. 2. SEM images of Saos-2 cells attached to nano and micropores of SC-CO_2 dried scaffolds after 3 weeks.

Conclusions: From a green chemistry perspective, production of highly pure and solvent-free structures is possible by using SCF technology. Consequently, SCF technology proves to be a more efficient and rapid alternative for tissue engineering and regenerative medicine applications.

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Laminar flow-induced dissolution of hydrodynamically trapped benzyl benzoate micro droplets within immiscible environment

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Keywords: Benzyl benzoate, dissolution, hydrodynamic trapping, microfluidics, surfactant

Introduction: For the past 30 years, numerous noncontact methods have been demonstrated for trapping micro/nano-particles using forces generated by optical, electrical, acoustic, and acoustic potential fields [1, 2, 3]. Despite the versatility of these methods, new approaches are still needed for confinement and observation of objects with arbitrary shapes for studies in protein folding, single polymer dynamics, or single cell mechanics. Recently, hydrodynamic trapping has been introduced as a powerful tool for trapping and manipulation of microbeads, DNA molecules, and cells [4, 5, 6]. Here, we extend this previous work to the hydrodynamic trapping and manipulation of oil microdroplets. First, we demonstrate a novel method for trapping and manipulating microdroplets in liquid media using a hydrodynamic trap. This method enables confinement and long-term observation of biological objects such as cells and macromolecules. Subsequently, we show that despite the presence of an immiscible liquid environment, substantial dissolution is observed in hydrodynamically trapped benzyl benzoate microdroplets. We explain this by the presence of the planar extensional flow that enhances the solubility of oil in glycerol-water. Thirdly, we present the effect of surfactant concentration and flow rate on dissolution.

Materials & Methods: Hydrodynamic trapping is based on generating a stagnation point at the intersection of two opposing laminar streams. An automated feedback control system enables trapping of single particles at the semi-stable stagnation point. Feedback control loop uses the relative flow rates through the outlet channels as the feedback parameter for stabilizing the position of a target particle at the microchannel junction.
The automated feedback control mechanism also enables particle manipulation along the direction of the outlet channels.

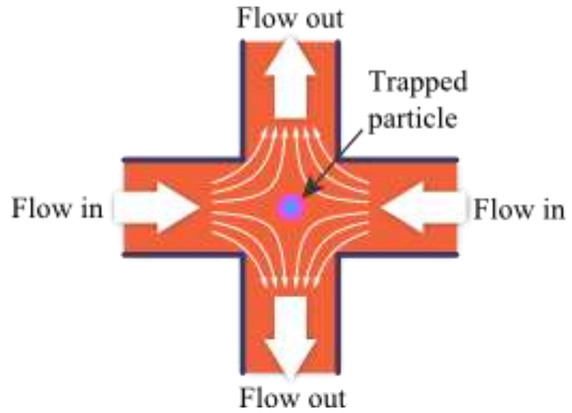

Fig. 1. Trapping region in the microfluidic chip.

Hydrodynamic trapping is performed in conventional polydimethylsiloxane (PDMS) microfluidic chips fabricated by multilayer soft lithography. A 100 µm-thick fluidic layer is positioned between a glass substrate and a control layer. A thin elastomeric membrane (30-100 µm thickness) separating the control and the fluidic layer is deflected down onto the fluidic layer by applying pressure to the control layer, thereby acting as a pneumatic valve, enabling flow rate control through the fluidic layer. Controlling relative flow rates through the outlet channels in the fluidic layer enables us to control the stagnation point position and to trap single oil microdroplets at the junction within the fluidic layer. In **Fig. 2** we show a layout of the microfluidic chip for hydrodynamic trapping.

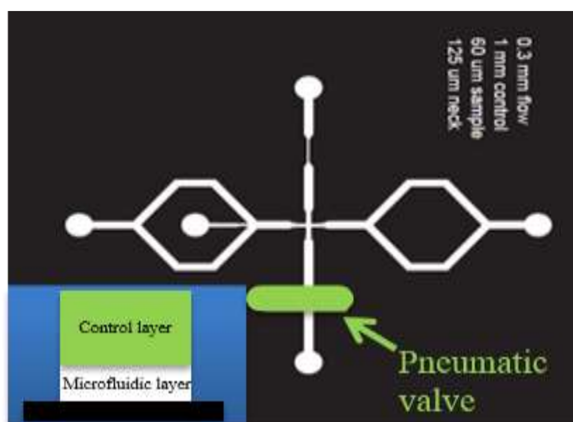

Fig. 2. Layout and cross section of multilayer microfluidic chip.

To prevent benzyl benzoate microdroplets sticking onto microchannel walls, we add a surfactant, Docusate Sodium Salt (DSS, Sigma Aldrich) at different concentrations.

Results & Discussion: Fig. 3 shows the effect of flow rate on dissolution for glycerol-water solution with 10mM surfactant. Here, the plots with red, green, purple shows microdroplet diameter as a function of time at three different flow rates, 20, 30 and 40 µl/hr, respectively. Microdroplet diameters are measured by image analysis. Under no flow conditions there is no change in droplet diameter; however, with fluid flow, we approximately observe a 60% change in droplet diameter within 55 minutes.

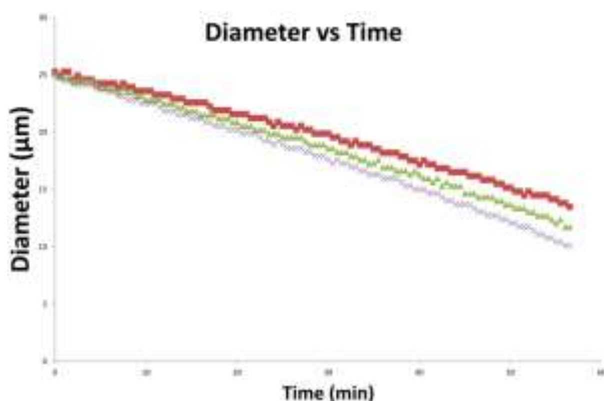

Fig. 4. The change in microdroplet diameter as a function of time under three different flow rates.

We also studied the effect of surfactant concentration on dissolution. We observed that increasing the concentration of DSS greatly increases the dissolution of microdroplets. **Fig. 4** shows the dissolution graph of a microdroplet (microdroplet diameter as a function of time) in glycerol-water solution with 50mM DSS at 40ul/hr flow rate for 22 minutes. By comparing the dissolution data at 40 µl/hr presented in **Fig.3.** (10 mM DSS) and in **Fig. 4** (50 mM DSS), we observe dissolution rate increased significantly at a higher surfactant concentration. A 25 µm-diameter droplet dissolves into a 10-µm droplet in 55 and 22 minutes at 10 and 50 mM DSS, respectively.

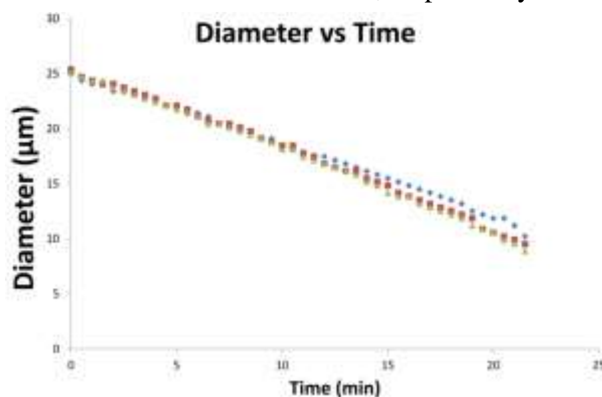

Fig. 3. Dissolution of microdroplets under different flow rates for higher surfactant concentration.

Conclusions:

In this work we present a novel method for trapping and manipulating microdroplets using fluid flow. We also studied dissolution of benzyl benzoate microdroplets in an immiscible fluid and demonstrate the effect of flow rate and surfactant concentration on microdroplet dissolution. Laminar flow-induced dissolution of microdroplets can be used to test existing theories for dissolution of immiscible fluids and extend them in the presence of laminar flow [7].

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Activity and stability enhancement of protease under supercritical CO_2 conditions

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Keywords: Enzyme, immobilization, optimization, protease, supercritical CO_2

Introduction: Enzymes have the important advantages of high chemo-, stereo and regio-selectivity in organic synthesis, generating the main class of synthetically relevant biological catalysts [1]. Today, nonaqueous medium for enzymatic applications is a well-accepted procedure which presents opportunities for the fundamental studies in regards to functions and structures of enzymes [2]. The use of supercritical fluids (SCF) seems to be a promising strategy to satisfy the requirements of green chemistry concept in industrial processes [3]. Compared with conventional reaction media, supercritical fluids (SCFs) including CO_2 may have several advantages, which are relatively mild reaction conditions and high rates of molecular diffusion and heat transfer, the absence of gas–liquid mass transfer limitations and the possibility of molecular interactions with the dissolved reacting species [4, 5].

One of the important disadvantages of enzymes is their short lifetime. Enzymes are sensitive molecules and lose their activity under some conditions such as high temperature, pH changes and organic solvents. Immobilized enzymes have various advantages over free enzymes including reuse, easy handing, enhanced stability and recovery from the reaction medium [6, 7].

The aim of this study was to enhance the activity and stability of protease from *Bacillus* sp. under SC-CO_2 conditions and determine the reusage of the enzyme after consecutive enzymatic reactions with immobilized form.

Materials & Methods: The activity and stability of protease were evaluated according to the effects of operational variables, temperature (28–80 ◦C), pressure (60–300 bar), CO_2 flow (2–10 g/min) and process duration (60–180 min). In order to determine the reusability of adsorbed enzyme on sponges, the activity was assayed several times for the hydrolysis of casein and when a lower activity value than the initial activity of the untreated enzyme was recorded, the immobilized samples were retreated with SC-CO_2. Consecutive reactions were carried out till the activity can not be increased with SC-CO_2 retreatment in comparison to the initial activity of the untreated enzyme. In addition, the kinetic parameters, pH and temperature stability of SC-CO_2 treated and untreated protease as a model enzyme are presented in this study.

Potential mechanisms for pressure stimulated activation and stabilization were investigated by NMR, SEM, FTIR, SDS-PAGE and XPS analyses.

Results & Discussion: Optimum conditions were determined as 300 bar, 54 °C, 6 $gmin^{-1}$ CO_2 flow and 120 min of process duration yielding 54.4% (417.50 µmol/ml/min) higher activity than the untreated enzyme.

After pH and temperature stabilization optimum conditions were pH 9 and 37 °C for the enzymatic reaction, whereas the kinetic parameter values, Vmax and Km were calculated using Michaelis-Menten model.

Conclusions: While most current applications of high pressure are for inactivating deleterious enzymes, in this study the potential to increase activity and stability was examined. The present study indicated that SC-CO_2 treatment of protease has been effective for the activity enhancement. As a result, applications of SC-CO_2 medium for enzyme catalyzed processes are going to become important and sustainable with environmentally friendly and economical synthetic protocols.

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Assessment of different element orders for boundary element formulation of particulate flow in microchannels

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Keywords: Microfluidics, boundary element method, particle motion

Introduction: The investigation of particle motion in a Stokes flow is a well-suited for microfluidic applications. Motion of particles with different shapes and properties has many applications in many fields of engineering and science such as bioengineering, chemical engineering, micro vascular fluid dynamics nuclear engineering and etc. [1]. One approach to model the particle trajectory within the microchannel is the stress tensor approach. In this approach, the field variables are solved with the presence of the finite-sized particle. Dvinsky and Popel [2] used a numerical method for investigating the motion of a rigid circular cylinder between plane parallel boundaries. For the particles with spherical shape, Wagner [3] used extended FEM for small spherical particles. Boundary element method (BEM) is a numerical tool that is well applied to many problems in engineering. BEM has boundary only discretization, which results in less computational effort in meshing and remeshing. In BEM formulations, continuity and compatibility conditions for the governing equations are satisfied exactly (not approximately) within the solution domain [4]. Unlike, FEM or FVM, geometric order of discretization is critical, since the line integrals are approximated which effects of the overall accuracy. It is typical in a microfluidic application that a circular and/or elliptic particle flow in a channel with straight walls. The discretization of the channel wall can be performed by line segments; however, for circular and elliptic particles the discretization can be performed with circular segments rather than line segments.

In this study, two different geometry order and two different function order elements are studied and compared. The discretization is performed with line and circular segments for linear and circular geometric order, respectively. For the functional order, both constant and linear functions are implemented. For the constant function, the value of the variables over each element is constant. For the linear function the variation of the variables is linear.

Formulation: The Stoke's equation (also called creeping flow) that is for low Reynolds flows and well-suited for micro scale applications can be written as [4]:

$$-\nabla P + \mu \nabla^2 u = 0 \qquad (1)$$

where P is the modified pressure that can be expressed as:

$$P = p - \rho g.X \qquad (2)$$

Where X is the position vector in Cartesian coordinates. μ represents the viscosity of the fluid and u is the velocity vector of the fluid.

$$C_{ij}(A)u_j(A) = \int_C G_{ij}(A,P)t_j(P)dS - \int_C H_{ij}(A,P)u_j(P)dS \qquad (3)$$

u_j and t_j are the components of velocity and traction vector respectively. C_{ij} is a constant that depends on the position of the node. The equation above in the matrix form is:

$$H.u = G.t \qquad (4)$$

For the linear function we can write:

$$t = t^{(1)}\emptyset_1 + t^{(2)}\emptyset_2 \qquad (5)$$

$$u = u^{(1)}\emptyset_1 + u^{(2)}\emptyset_2 \qquad (6)$$

$$\emptyset_1 = \frac{1}{2}(1-x) \qquad \emptyset_2 = \frac{1}{2}(1+x) \qquad -1 \le x \le 1 \qquad (7)$$

t and u are the value of the variables over each element. $t^{(1)}$ and $u^{(1)}$ are the values at the first point of each element and $t^{(2)}$ and $u^{(2)}$ are at the second point.

Verification: To verify our results, we compared the results with a benchmark problem in which the drag force on a stationary cylinder located in a channel is analyzed [5]. The Reynolds number is taken as 2×10^{-4}. The last row is the analytical solution.

Table 1. Values for the dimensionless drag force.

# elements on the particle	4	8	16	32
LC	12.831	15.188	15.935	16.137
LL	11.995	14.799	15.843	16.115
CC	16.152	16.207	16.209	16.208
Faxen results[5]	16.207	16.207	16.207	16.207

LC: linear geometry, constant function
LL: linear geometry, linear function
CC: circular geometry, constant function

As expected, as the number of the elements on the particle increases, the dimensionless drag force get closer to the analytical solution. The results circular geometry element is superior to other orders. Even with 36 elements, the result is within 0.5% of the Faxen result. Another important observation is that increasing the functional order from constant to linear does not improve the result. Therefore, it can be concluded that the improvement in the discretization is more critical than improvement in the functional order. Another important observation is the performance. Circular element geometry has the same computational performance with the linear geometry. We used an intel core i5-3230M CPU 2.60 GHz with 8.00 GB RAM memory. On the other hand, the computational performance of the linear function is less than the constant function.

Particle Motion: After verifying the results with the benchmark problem, the particle trajectory of a circular and an elliptical particle in a straight channel is simulated. The length and width of the channel is 500 and 100 µm respectively. The inlet velocity is assigned as uniform and 300 µm/s. The density and viscosity are taken 1000 kg/m^3 and 0.001 Pa·s, respectively. On the channel wall, no-slip velocity, and the outlet of the channel zero traction boundary condition is assigned. The circular particles has a diameter of 10 µm which resembles a cell. The elliptic particle has a size of long-axis diameter of 10 µm and short-axis diameter of 6 µm which resembles a bacteria. As seen form the figures, the particle motion can be predicted quite well. For the particle motion, 16 elements were assigned on the particle and the simulations are performed for a time period 1s.

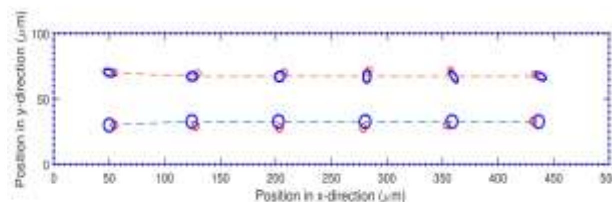

Fig. 1. Circular and elliptic particle motion in a microchannel.

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Construction of 2D protein structures by using microcontact printing

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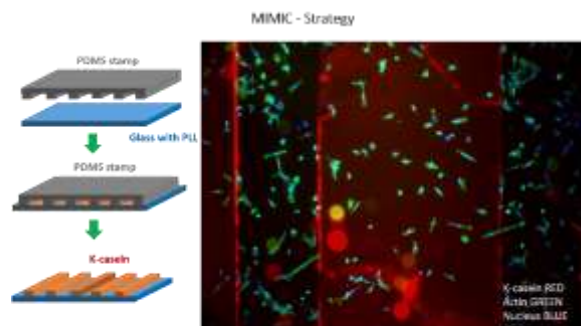
Keywords: Cell adhesion, cell patterning, microprinting, mimicking

Introduction: Cell polarity can be identify as the manifestation of the morphological and functional asymmetry of the cell compartmentalization relative to a polar axis [1]. Adhesion of the cells regulate the turnover of tissue during the developmental progress [2]. Result of this cell polarity, cell-cell, cell-ECM are effected. Cell adhere to the ECM with help of superfamily of integrins [3].

Materials & Methods: First, a silicon master is fabricated, which contains the micropattern of interest. After fabrication, the master can be used multiple times to make stamps. Second, a polydimethylsiloxane (PDMS) stamp is fabricated. Unlike fabrication of the master, this step can be performed without specialized equipment. The PDMS stamp is inked with extracellular matrix proteins. Proteins are printed on a substrate.



Result & Discussion: By manipulating micropattern shapes, cells were shown to precisely adapt their cytoskeleton architecture to the geometry of their microenvironment. Remodelling of actin and microtubule networks participates in the adaptation of the entire cell polarity with respect to external constraints. These modifications further impact cell migration, growth and differentiation.

Acknowledgements:

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Sterilization of microchips for biomedical applications

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Keywords: Microfluidic chip, sterilization, supercritical CO_2, PDMS, PMMA

Introduction: Microfluidic technologies have shown great potential for microscale biochemical systems for analysis and synthesis [1, 2]. Microfluidic devices are provided for biological assays to perform analysis with efficient throughput and use of samples or reagents in small fluid amounts (µL, nL, pL) and analysis of single cells, cell-based assays and long-term cell culture [3, 4, 5]. Although sterility is required in most of the life science applications, contamination risk in microchip technologies is a significant limitation to realize these applications in aseptic conditions. In medical applications, the standard sterilization methods include steam, ultraviolet (UV), ethylene oxide and hydrogen peroxide sterilization which has drawbacks in certain applications. The aim of this study was to develop sterilization protocols for microchips fabricated from PMMA and PDMS materials and to analyze characteristics of these materials subsequent to sterilization applications. **Materials & Methods:** Techniques such as heat, UV, ethylene oxide, hydrogen peroxide and alternatively supercritical CO_2 were applied for sterilization of microchips (**Fig. 1**). Afterwards, the sterilized microchips were incubated in Tryptic Soy Broth and Thioglycollate Broth at 27 and 37ºC for 7 days. Thus, the application was performed under steril conditions in order to assess the microbiological loads.

Fig.1. Microchips fabricated with PMMA and PDMS.

Furthermore, the sterilized microchips were analyzed using Scanning Electron Microscope (SEM), Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR) to determine microchannel sterility and possible chemical deformations in the material. **Results & Discussion:** The surface morphology of the autoclaved microchips was negatively altered, whereas UV application has not caused adverse effects. Chemical sterilization applications which might cause toxicity in biomedical applications were regarded as the weaknesses of the protocols. Although complete sterility was achieved in the applications, they were not suitable for heat sensitive and porous materials. Therefore, high-pressure CO_2 could be an option as a new sterilization protocol due to non-toxic, non-flammable and inert property of CO_2 along with a low critical temperature. The optimum parameters for supercritical CO_2 sterilization of PMMA microchips were elicited as 120 bar, 40 ºC and 60 min which provided complete sterility. Studies regarding sterilization of PDMS microchips are going on as well.
Conclusions: Supercritical CO_2 can be proposed as a new sterilization technique particularly in life science applications as risks of toxicity and chemical residues have been eliminated.

Acknowledgements:
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Pre-seeding method on PDMS surface that ensures cell viability

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Keywords: 2D microenvironment, cancer, microbubble, soft lithography

Introduction: Soft lithography provides many advantages to cell biology research due to its biocompatibility and patterning range from micrometers to nanometer scale [1]. Targeted drug delivery is a significant concern in the cancer treatment. However, mimicking in vivo structure, especially interactions of molecules with cells under flow is difficult. In addition, bare PDMS does not favor cell adhesion. Microbubbles are made up of lipids and air; therefore they ascend. To provide interaction of cells and microbubble, cells need to be at the upper surface of PDMS microchips. However, cells do not attach normally to the PDMS surface. Here, we fabricated 2D system which will allow us to test drug loaded and targeted microbubbles.

Materials & Methods: By using soft lithography, master molds were formed; PDMS micro-channels were fabricated and permanently bonded to slide with UV/ozone [2]. With the aim of ensuring cell attachment, cells were cultured on PDMS; PDMS+PLL and PDMS+PLL+FN. Cells are loaded on microchannels and kept 6 hours inverted for adhesion of cells on upper PDMS surface. After that microfluidic device was rinsed with media to remove unbound cells, filled with media and incubated glass side down for at least 10 days.

Fig. 1. Light microscopy images of cells cultured on PDMS; PDMS+PLL and PDMS+PLL+FN.

Results & Discussion: To achieve microbubble and cell interaction in the PDMS channels, we seeded cells on the PDMS side of microfluidic channels which were initially incubated upside down. The best results is observed when the PDMS surface is first coated with PLL (1:10) and then FN protein which is one of the most abundant glycoproteins in the extracellular matrix. We performed experiments with two different cell lines, MDA-MB-231 (breast cancer cell line) and MCF10A (normal breast epithelial cell line). Both cell lines adhered to PDMS surface by 6 hours of initial incubation and were viable for at least 10 days.

Conclusions: We develop a new pre-seeding method on PDMS surface that ensures cell viability for at least 10 days. Future studies will investigate interactions of drug loaded and targeted microbubbles with breast cancer and normal mammary epithelial cells inside microfluidic channels.

Acknowledgements:
The financial support for this study was provided by TUBITAK- The scientific and Technological Research Council of Turkey, Project Number: 213M668.

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Difusion phenomena in microfluidic 3D cancer models

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Keywords: Breast cancer, carnosic acid, microfluidic 3D models, diffusion, Fick's law

Introduction: According to World Health Organization (WHO), cancer is a leading cause of death worldwide [1]. Consequently, important resources are directed towards bettering treatments and outcomes. Cancer is difficult to treat due to its heterogeneity, plasticity and frequent drug resistance. New treatment strategies should strive for personalized approaches [2]. Traditional animal cancer models and *in vitro* cancer models are limited in their ability to recapitulate human structures and functions, thus hindering the identification of appropriate drug targets and therapeutic strategies. The development and application of microfluidic 3D cancer models have the potential to overcome some of the limitations inherent to traditional 2D in vitro models. Novel 3D culture systems can provide improved biological models and functionality while reducing required volumes and cost. In particular, microscale 3D in vitro models represent a potential alternative to improve both functionality and throughput of traditional 3D systems (**Fig. 1**) [3].

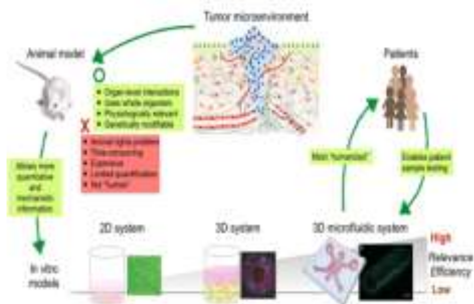

Fig. 1. Comparison of cancer cell research models.

One of its major advantages over standard drug screening assays is a possibility of long-term observation of the cytotoxic effect and evaluation of the activity of repeated doses of the drug [4]. Studies indicate that purified components from rosemary such as carnosic acid and its extract display significant growth inhibitory activity on a variety of cancers, especially human breast cancers [5]. Carnosic acid alone or in combination with the anticancer drugs may offer a good strategy for the treatment of a variety of human cancers that are resistant to chemotherapy [6].

The aim of this study is to demonstrate the effect of carnosic acid towards human breast cancer cells and investigate diffusion phenomena in butterfly-shaped microchip. We employed a 2-D diffusion equation model based on the Fick's Law to describe the diffusion process of the carnosic acid within the microchip by adapting the model terms to each compartment of a 3-compartment butterfly-shaped microchip (**Fig. 2**).

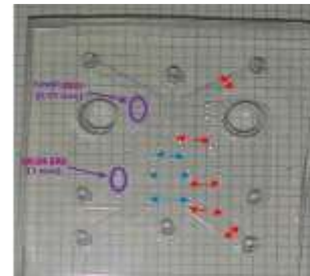

Fig. 2. Butterfly-shaped microchip and dimension of chambers.

Materials & Methods: MCF-7 and MDA-MB231 breast cancer cells are used. Either MCF-7 or MDA-MB 231 cells are cultured in one chamber of the microfluidic device whereas, healthy human skin fibroblast (HS-2) cells are cultured in the other chamber of this device. All cells are immobilized in matrigel and fed with DMEM (1:1) to chambers. Carnosic acid (in DMEM) was fed in central chamber in order to diffuse to cancer and healthy cells.

Results & Discussion:
IC_{50} values of carnosic acid in 2D culture for MDA-MB 231 was 15 μg/mL, whereas the values were 20 μg/mL and 30 μg/mL for MCF-7 and HS2 cells, respectively. In this regard, carnosic acid is selective especially for estrogen independent breast cancer cells.

Diffusion coefficient for the carnosic acid in DMEM and matrigel mixture which was to be exploited in the diffusion model was determined experimentally by making use of a two-chambered diffusion system [7]. Since the cells were trapped in their compartments, the model included a cellular uptake term for the related (left- and right-side) compartments whereas the diffusion process was the only event considered to occur in the middle compartment. The microchip used had a non-regular shape making the initial and boundary conditions difficult to incorporate into the model. Therefore, we developed and used an image processing approach on MATLAB in which we first converted a well-describing image of the chip into a pixel data matrix and specified the initial and boundary conditions by filtering the data matrix.

Conclusions: With the advances in 3D culture techniques and mathematical modeling, promising 3D microfluidic cancer models will serve as the bridge between 2D monolayer culture and cancer xenografts to accelerate the translation of novel therapeutics to the clinic.

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3D controlled *in vitro* microenvironments to test drug loaded and targeted microbubbles

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Keywords: 3D microenvironment, cancer, microbubble, soft lithography

Introduction: Soft lithography provides many advantages to cell biology research due to its biocompatibility and patterning range from micrometers to nanometer scale [1]. Targeted drug delivery is a significant concern in the cancer treatment. However, mimicking *in vivo* organization, especially flow within blood vessel and tissue is difficult. Furthermore, cancer and normal cells should be simultaneously tested. Here, we attempt to develop 3D co-culture system which contains breast cancer cells and normal epithelial breast cells in a lab-on-a-chip under flow conditions.

Materials & Methods: Master molds are formed by soft lithography, using SU-8 2075. PDMS samples are cleaned and permanently bonded to slide with UV/ozone. In order to achieve proper loading, channel hydrophobicity is ensured by holding at 80ºC for 24 hrs. [2]. Hamilton syringe needle is introduced to microchip parallel to slide surface. Then, matrigel is loaded into microchannels at +4 ºC, and incubated at +37 ºC for 30 min left for polymerization. Finally needle is pulled out and flow is started.

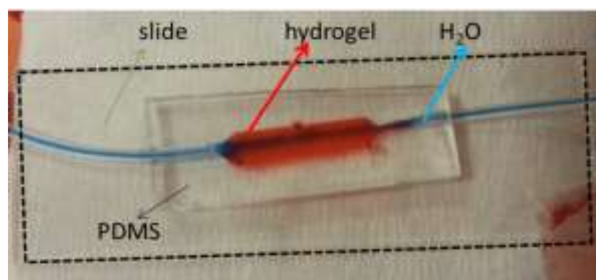

Fig. 1. Proof of concept with hydrogel and blue fluid.

Conclusions: Preliminary results are promising for creating a 3D controlled in vitro microenvironment for testing specificity and efficiency of drugs.

These 3D controlled *in vitro* microenvironments will be used to test drug loaded and targeted microbubbles.

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The financial support for this study was provided by TUBITAK- The scientific and Technological Research Council of Turkey, Project Number: 213M668.

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A miniaturized device for hydrolysis of ginseng RB1

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Keywords: β-glucosidase, biocatalytic conversion, ginseng RB1, microfluidics, sol-gel

Introduction: Silica sol-gel derived materials are very suitable for enzyme immobilization with their porous structure and high surface area [1]. Microreactor systems are consistently gaining relevance recently with their excellent performance in comparison to conventional reactors [2]. The objective of this study is to synthesize a sol-gel silica based biomaterial for preparation of β-glucosidase immobilized monolith microreactor system as a miniaturized device for catalysis of ginseng RB1 molecule into its aglycone form and compare acid catalysis, batch enzymatic hydrolysis and enzymatic hydrolysis in microreactor.

Materials & Methods: Tetraethylorthosilicate (TEOS) derived silica monoliths were prepared with two step sol-gel method with some modifications of previous work [3]. The TEOS sols were mixed with buffered enzyme solution and polyethoxysilane (PEO) and 3-aminopropyltriethoxysilane at ratio of 1:1 (v/v). For preparation of monolithic microreactors based on macro porous TEOS gel, TEOS sol and buffered enzyme were loaded into the single sinuous channel microreactor with scale down approach following aging 24 h at +4°C. enzyme kinetics has been evaluated with pnpg substrate [4]. The dimensions of the channel was 500 µm width, 500 µm depth and 40 cm long and fabricated with injection molding technique by using polydimethylsiloxane (PDMS). Biocatalysis of pnpg within microreactors was performed with continuous flow with 15 min retention time.

Activity of enzyme was determined by pumping 300 µl substrate solution continuously into the monolithic channel including 0.004 mg of enzyme for 45 minutes. Simultaneously, the same amount of enzyme in solution and 300 µl of substrate were incubated for 45 min in order to compare free and immobilized enzyme activity. Biocatalysis of ginseng RB1 achieved by pumping ginseng RB1 substrate (1 mg/ml) into microreactors including 0.02 mg of enzyme with continuous flow at 1 µl/min flow rate for 5 hours. Batch enzymatic reaction was carried on with incubation of equal amount of enzyme and substrate in tampon solution for 5 hours. For acid hydrolysis, ginseng RB1 (0.2 mg/ml) prepared and hydrolysed with HCl 10% at ratio of 1:10 under reflux for 4 hours. In this study conversion yields of hydrolysis of RB1 in β-glucosidase immobilized into sol-gel derived silica monoliths, free enzymatic hydrolysis and acid hydrolysis were compared.

Characterization of silica monoliths was achieved with Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR) analysis.

Results & Discussion: In this study, relative activity of enzyme immobilized in TEOS and EGMS derived monoliths 68% and 60% respectively. Vmax values of free and immobilized enzyme in TEOS derived monolith were determined as 0,018 ± 0,000495 mmol mg^{-1} min^{-1} and 0,0062 ± 0,000849 mmol mg^{-1} min^{-1}. Km values was determined 29,8 ± 1,152584 mM and 29,65 ± 3,747666 mM, respectively. Activity of immobilized enzyme in microreactor and enzyme in solution were compared. As a result, maximum conversion yield were 25% and 19% for TEOS-PEO and EGMS-PEO derived monolithic microreactors, respectively while 72% of pnpg was converted to glucose with enzyme in solution. Aglycone form of RB1 only obtained by acidic hydrolysis with conversion yield of 15.5. Aglycone form could not obtained with enzymatic hydrolysis but another form of molecule was obtained with conversion yield of %37.88 after 5 hours with 0.02 mg enzyme in TEOS-PEO derived

monolithic microreactor, but no conversion product was obtained with free enzyme.

Conclusion: The results showed that both TEOS and EGMS derived silica monoliths are suitable materials for immobilization of β-glucosidase. Catalysis of pnpg in microreactor was also performed and activities of free and immobilized enzymes were compared. While activity of enzyme could not be increased with microsystem for pnpg substrate, promising result obtained for RB1 substrate showed that it is possible to obtain aglycone form of saponins with further optimization of parameters in microsystem. These results indicated that microreactor used in this study is a promising biodevice for future works based on biocatalytic reactions. Further studies will be carried out for biocatalysis of substrates with complex molecular structures.

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An integrated microfluidic device for particle wash and particle separation

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Keywords: Microfluidics, acoustophoresis, dielectrophoresis, particle wash and separation

Introduction: Microfluidics is the combination of micro/nano fabrication techniques together with knowledge of fluid behaviour at the microscopic level to pursue powerful techniques in controlling, manipulating and measuring chemical, physical and biological processes at micro/nano scale. For chemical, biological and biomedical analyses in microfluidic systems, there are some fundamental operations such as separation, focusing, filtering, concentration, trapping, sorting, detection, counting, washing, lysis of bio-particles, and PCR-like reactions. The combination of these operations led to the complete analysis system or LOC system for a certain application. Manipulation of the bio-particles is the key ingredient for the aforementioned operations. Acoustophoresis (ACP) and dielectrophoresis (DEP) are two label-free particle manipulation techniques which utilizes the intrinsic acoustic and dielectric properties of the particles. ACP and DEP has been utilized for particle manipulation by many research groups [1]. In this study, ACP and DEP were utilized in an integrated manner to combine two different operations on a single chip in sequential manner, namely particle wash (buffer exchange) and particle separation.

Materials & Methods: Fig. 1 shows the schematic of the device presented in this work. For ACP piezoelectric materials (PZT) and for DEP two metal electrodes are utilized. For the fabrication of the microfluidic device out of polydimethylsiloxane (PDMS), a mould was designed and fabricated using mechanical machining.

The principle of ACP is generating stationary acoustic waves and forces on the microparticles with PZT. The forces depend on compressibility, density and size of the particles [1]. With a proper frequency the particles can be manipulated to the centreline of the microchannel. The principle of DEP is the manipulation the articles within a non-uniform electric field with the interaction of the dipole moment of the particles with the electric field gradient [2].

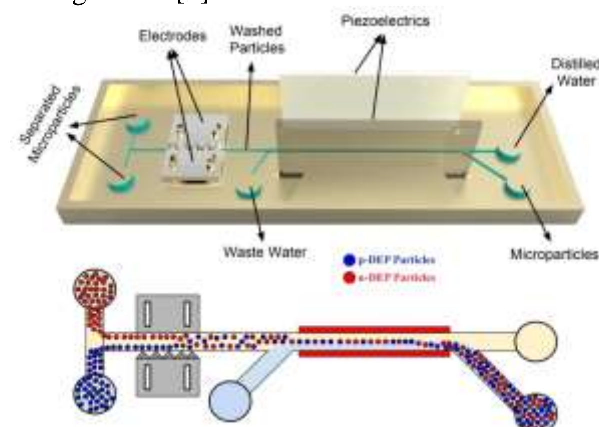

Fig. 1. (a) Schematic representation of the device design. (b) The working principle of the device presented.

In this study, the particle wash (buffer medium exchange) was utilized using ACP and particle separation was utilized by DEP. The particles which are originally in a high conductivity buffer solution were loaded into the device through a side inlet, and were pushed towards the centerline where a low conductivity buffer solution flowed with the help of ACP. Following particle wash, the particles flow through the separation section in which two electrodes were located for DEP manipulation. DEP force is produced in the transverse direction to the flow via applying voltage on asymmetric electrodes. The flow is loaded in the microchannel by using a laboratory syringe pump.

Results & Discussion: First, the computational fluid dynamics (CFD) analysis of the chip is carried out in COMSOL (**Fig. 2**). It shows that the distilled water (blue line) and water with particles

(red line) do not mix each other due to laminar flow. The experiments are performed using poly-

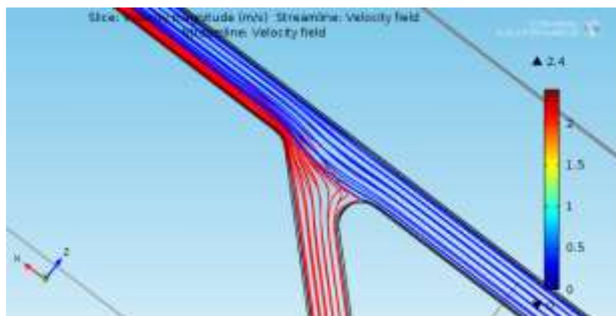

Fig. 2. The computational fluid dynamics (CFD) analysis of the chip is carried out in COMSOL.

styrene particles. More detailed computational model regarding this design can be found elsewhere [3]. Then, the fabricated device is characterized experimentally. Prior to the experiments, the liquid level of the side outlet was adjusted such that the particles flowing through the side inlet flew out the side outlet when the PZT were off. Then the PZT were switched on. To check the repeatability of the experiments, PZT's were switched on and off repeatedly, and consistent particle behaviors were observed. Following the particle wash experiments, electric field was switched on and off to observe the DEP based manipulation. Due to the dielectric properties of the polystyrene particles, only n-DEP response could be observed. **Fig. 3** shows the experimental results of the particle motion for PZT and electric field on and off. To verify the particle wash, the electrical conductivity of the incoming and exiting buffer solutions were measured. The desired particle motion was achieved with 39.8 V_{pp}, 2.223 MHz, for PZT and 16 V_{pp} and 3 MHz for DEP electrodes.

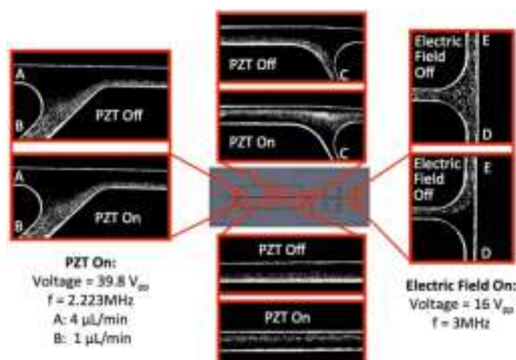

Fig. 3. An image showing the presented device during operation.

For the particle wash experiments electrical measurements of the buffer solutions were measured. The electrical conductivity of the buffer solutions at the inlet was performed using conductivity meter. Since minute amount of buffer solution was collected at the outlet reservoir, the electrical resistivity of the buffer solutions at the exit was measured by loading the collected buffer into a separate microchannel.

Table 1. Electrical conductivity measurements for particle wash.

	Inlet-A	Inlet-B	Outlet D&E	Outlet D&E (Complete mixing)
σ [S/cm]	730	1790	---	942
Resistivity [MΩ]	2.64	0.99	2.45	2.30

Table 1 shows the electrical measurements during the experiments. As seen from the table, the particles which are originally at a higher conductivity buffer was transferred to a buffer solution with a lower electrical conductivity.

Conclusions: This paper presents an integrated microfluidics device, which can wash and separate particles. Future work will involve testing the device for high throughput and operate the proposed devices with the bio-particles.

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Fabrication of 3-D cryogels for flow-through separation of EPSs from algal broth

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Keywords: Algae, *B. braunii,* cryogel, EPS, PHEMA

Introduction: Cryogels are gel matrices that are formed in frozen solutions of monomeric or polymeric precursors. Cryogels have interconnected macropores or supermacropores with a pore size range 10–100 mm. These interconnected supermacropores of cryogel structure permit the free passage of microparticles, nanoparticles or bioparticles without blockage of the gel matrice [1, 2]. Cryogels are very good alternative to remove or purify substances from biological matrices with many advantages including large pores, short diffusion path, low pressure drop and very short residence time. [3, 4]. *Botryococcus braunii* is an algae of particular interest due to its behavior of accumulating extracellular hydrocarbons, where the three races of this colony-forming algae have developed different metabolic strategies to produce the oil [5]. Apart from that, it contains exopolysaccharides which can be converted to value added bio-products.

Materials & Methods: In this study (**Fig. 1**), preparing a cryogel column was aimed for efficient separation of exopolysaccharides (EPSs) from *B. braunii* algae non destructively. For this purpose, PHEMA based cryogel matrix containing a hydrophobic group was prepared as monolithic form by polymerization 2-hydroxyethyl methacrylate (HEMA) via cryogelation technique. Then, the surface was modified by coupling Concanavalin A. Before the algal broth, a synthetic exopolysaccharide solution comprised of arabinose, galactose and glucose was prepared and pumped through the cryogel to test the efficacy. After that, the gel was washed with a buffer solution in order to release the captured exopolysaccharides and quantification was carried out using the spectrophotometric phenol-sulphuric acid analysis. The same method was repeated with algal broth.

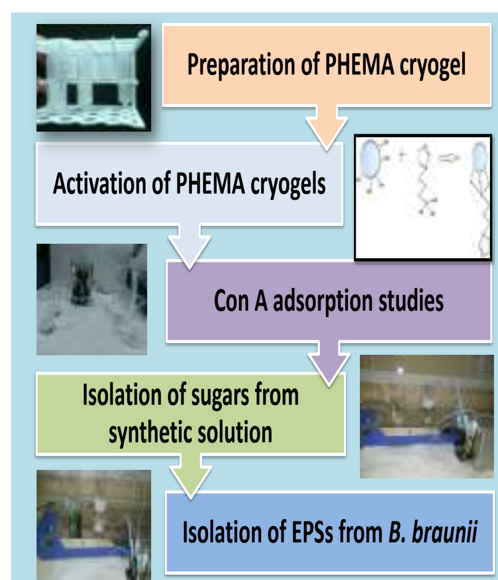

Fig. 1. Schematic representation of the study.

Results & Discussion: EPSs were isolated via cryogel columns with a maximum adsorption capacity of 3.313 mg EPS/g cryogel corresponding to adsorption yield of 86%. In a study, Con A binding with the mannose attached PHEMA cryogel from Con A aqueous solution was 5.2 mg/g. In another study, adsorption capacities of blood proteins were obtained as 1.0 mg/g for fibrinogen and 1.7 mg/g for albumin. Our result is quite competitive in regards to cryogel studies for separation purposes reported in the literature [6, 7]. In order to increase EPS adsorption capacity, optimization studies in the cryogel preparation steps are still ongoing.

Conclusions: It can be seen that isolating hydrophilic materials effectively by modifying surface of a monolithic cryogel column using a hydrophilic molecule is possible. Also algal EPSs should be isolated non-destructively, for this reason, viability tests will be carried out.

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Methods that positions cell-laden or cell-free matrices at defined positions from each other inside a single microfluidic channel

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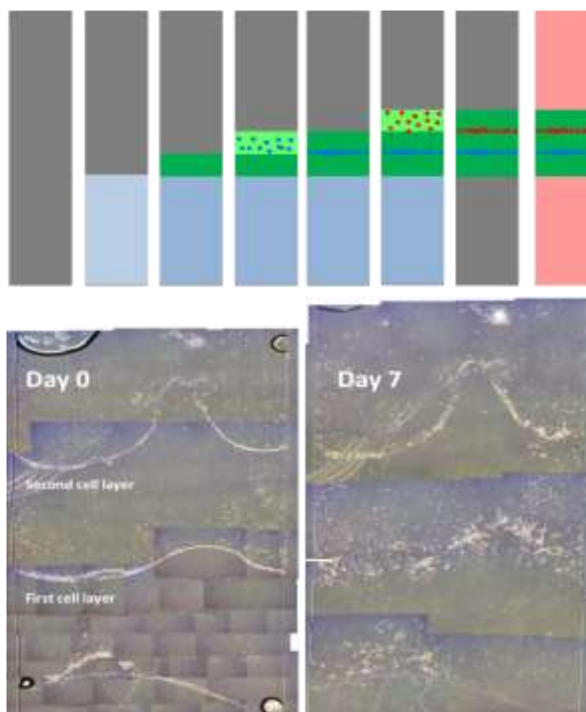
Keywords: Lab on a chip, microfluidics, cell culture

Introduction: Microfluidic refers to the science and technology of fluid system that process or manipulating small amount of liquids at nanoliter to femtoliter scale [1]. Microfluidic technology is multidisciplinary field of engineering, physics, chemistry, biochemistry, nanotechnology and biotechnology. Culturing cells in microfluidic devices have been used to study for many biological process and responses such as comparing with macro scale cell cultures and large scale bioreactors in testing conditions [2]. The aim of this project was to develop a method that positions groups of cells at certain distances from each other in a microfluidic channel.

Materials & Methods: First, SU-8 master mold is created by using UV lithography method then polydimethylsiloxane (PDMS) mold is created by using soft lithography method. Cell-free and cell-laden hydrogels were loaded into the microfluidic channel and organized by centrifugation.

Results & Discussion: Gelatin or agarose were first loaded as sacrificial layers at one end of the microfluidic channel. Cell-free matrigel was loaded and centrifuged to create an even surface. Finally two rounds of loading and centrifugation successfully generated two layers of cells. The sacrificial layer was removed and both ends of the microfluidic channel was filled with culture medium. Cells were viable and proliferated for 15 days.

Conclusions: In this work, we developed an easy centrifugation based method that positions groups of cells at certain distances from each other inside a microfluidic channel.



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Immobilisation and characterisation of β-glucuronidase in silica sol-gel matrix

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Keywords: Sol-gel fabrication, enzyme immobilisation, microreactor, β-glucuronidase, saponine

Introduction: Enzymes have great application potential for the environmentally friendly production of high value-added compounds. However, many enzymatic processes have not been commercialized, because of problems in either stability and reusability of the enzymes or cost and efficiency of the reactions. Thus, there have been demands for innovation in enzymatic process engineering for better product quality with lower cost [1]. Sol-gels are a new class of materials that have been found to be suitable for the immobilization of enzymes and other biological molecules. Compared to the other immobilization matrices, sol-gel matrices have many advantages such as entrapment of large amount of enzymes, thermal and chemical stability, simplicity of preparation without covalent modification and flexibility of controlling pore size and geometry [2]. Hence in this study, β-glucuronidase enzyme catalysing the hydrolysis reaction of the glycyrrhizic acid saponine ($C_{42}H_{62}O_{16}$) was entrapped in the sol-gel matrices in order to create a larger surface area which will enhance the reachability of the substrate to the active sites of the immobilized enzyme thus liberating the aglycone part suitable for pharmaceutical applications. For that purpose, activity of entrapped enzyme was determined along with chracterization of the fabricated gel structures.

Materials & Methods: In order to obtain enzyme doped sol-gel monolithic matrices, firstly sols were prepared by mixing tetraethylorthosilicate (TEOS), ultrapure water and HCl (0.1 M) at room temperature for 2.5 hours. Secondly buffered enzyme and buffered polyethylene oxide (PEO, MW: 100,000) were prepared separately and mixed at 1:4 (v/v) ratio. Finally the resulting solution cocktail was mixed with prepared TEOS-sol at 2:1 (v/v) ratio [3]. Enzyme activity inside the gels (**Fig. 1**) were determined with 4-Nitrophenyl β-D-glucuronide by employing Sigma β-glucuronidase enzyme protocol and compared with that of free enzyme.

Results: β-glucuronidase activity was expressed as unit/ml where one unit was defined as the amount of enzyme releasing 1 μmol *p*-Nitrophenol in one minute. Activity of immobilised enzyme within porous gel structure was determined as 17,378 U/ml whereas free enzyme was 58,592 U/ml. Consequently the immobilised enzyme exhibited 30% residual activity.

Fig. 1. Enzyme doped TEOS-derived sol-gel matrices fabricated inside shringes.

Discussion: As the residual activity of the enzyme is comparatively low, further optimisation studies will be implemented in order to avoid the activity loss occuring after immobilisation process.

Conclusions: After determination of the conditions for immobilisation of enzyme into TEOS-PEO derived gels and characterisation of the gel structures as well as examination of entrapped enzyme activity, the studies will continue inside microreactors.

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Bio-investigation of formulated natural products *via* microfluidic cell culture

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Keywords: Cell culture, formulated natural products, microfluidic bio-investigation

Introduction: Many polyphenolic and flavonoid compounds which have used to be derived from plants are known for the cornerstone of the medicinal nutrients such as phytomedicines and applied in health maintenance and disease management since the dawn the beginning of history. Most of these phytomedicinal compounds, especially phenolics, are poorly-adsorbed in the body by posing a challenge in clinical applications [1]. In order to solve this problem, many strategies have been developed to enhance the bioavailability of phytomedicines from plant extracts such as encapsulation in liposomes, micelles and polymeric particles [2]. Phospholipids as the major compound of liposomal membrane can generate complexes with natural active ingredients and constitutes "Phytosomes" which was emerged as a new technology in 1989 [3].
Microfluidic technology can be used to supply and transfer media, buffers, and even air while the waste products by cellular activities are drained in a way resembling the human circulatory system [4].
Compared with traditional culture tools microfluidic platforms provide; much greater control over the cell microenvironment, reagents and drugs, a rapid optimization of media composition using relatively small numbers of cells, in vivo like environment, high-throughput cell based experimentation, cost-effective platform, decreased contamination risk and fast response time [5].
In this work, natural products in vesicular systems were first tested in traditional cell culture. After the toxicity, proliferative effect against oxidative stress and wound healing (scratch assay) studies, the developed natural products will be tested on microfluidic cell culture platform.

Discussion and Conclusion: The purpose of the present work was to prepare various nano-formulations of different bioactive compounds and to investigate their biological effects as a supportive agent for wound healing and their antioxidant activity. Because of the increase in the developed nanomaterials paved the way of looking for new rapid and automatical methods. Within this aspect, microfluidic cell culture has been a basic tool for all cell-based applications including toxicological studies, drug discovery studies, cell and tissue engineering efforts.
In our studies optimized formulations of both gold nanoparticle (AuNP) loaded phytosomes and nanoemulsions (containing lipoic acid capped AuNPs, *Nigella sativa* seed oil and *Calendula officinalis* extract) were applied to Vero (Monkey kidney like fibroblasts) cells and showed greater activity for antioxidant and wound healing.

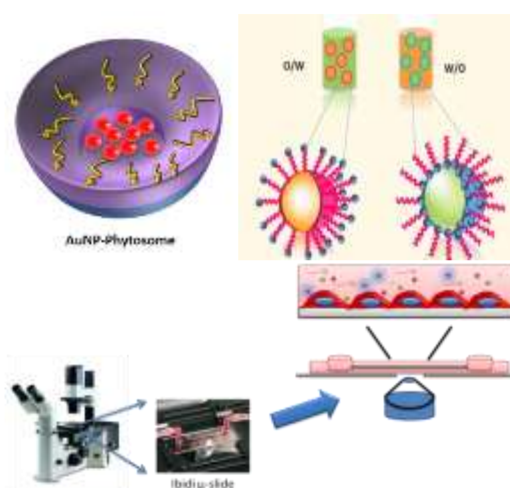

Fig.1. The formulated natural products (Phytosomes and nanoemulsion) and bio-testing platform for microfluidic cell culture.

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Poster Presentations

Design and fabrication of microfluidic device that allows investigation of distance dependent interactions of two different cell types

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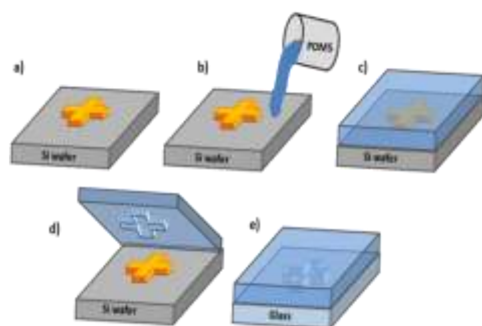
Keywords: Lab on a chip, microfluidics, cell culture

The main studies of in this project, the mold and a microfluidic device are achieved by using SU-8 photoresist and PDMS polymer.

Introduction: The study of fluidics at the microscale for applications is defined as microfluidic. Those applications are cell culture, drug delivery and sample handling [1]. As it is written above, microfluidics specialized for the applications at the microscale; so that these devices are manufactured with features in micrometer scale that can process fluids with volumes in nanolitres.

Materials & Methods: Once a desired thickness is chosen, a grade of SU-8 must be chosen according to the desired thickness and poured onto the wafer through a spin coater. The wafer is soft baked at 65°C, then at 95°C. It is exposed to UV radiation underneath a laser-printed mask transparency. Afterwards, the wafers are post exposure baked at 65°C, then at 95°C. Next, the covered wafer is developed in SU-8 developer, rinsed, and dried. PDMS molding fabrication process for creating polydimethylsiloxane (PDMS) polymer microfluidic chips from the masters and PDMS polymer. PDMS pre-polymer in a 10 to 1 ratio of base to curing agent is mixed and poured onto the masters. Next, PDMS mold and glass is bonded.



Result & Discussion: SU-8 photoresist was poured onto the wafer through a spin coater. The wafer was soft baked firstly at 65°C, then at 95°C, and then cooled down. it was exposed. Afterwards, the wafer was post exposure baked at 65°C, then at 95°C. Next, the substrate with the photoresist was developed in SU-8 developer, rinsed, and dried. All parts were performed in clean room. After that, PDMS and glass were bonded each other's and microfluidic device was fabricated successfully.

Acknowledgements:
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