EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

Exploratory Workshop Scheme

Standing Committee for Physical and Engineering Sciences (PESC)

ESF Exploratory Workshop on

MICROREACTOR TECHNOLOGY FOR HEALTHCARE APPLICATIONS

Fiesa (Slovenia), 26. Sep. – 28 Sep. 2010

Convened by:
Prof. Dr. Mihael Junkar and Assist. Prof. Dr. Joško Valentinčič

Faculty of Mechanical Engineering, University of Ljubljana, Slovenia

SCIENTIFIC REPORT

Executive summary

Microreactor technology for healthcare application is inherently interdisciplinary scientific research field as disciplines such as chemical technology, biotechnology, biomedicine, biocybernetics and mechanical engineering are essential for modelling, design, fabrication and application of microreactors.

The community of micromanufacturing technologies is essential for innovation on the field of applications. The community is growing constantly, thus it is substantial to discuss the conditions and assets for sustainable knowledge-intensive production, including construction, development and validation of new paradigms responding to emerging industrial needs. The subject of development of new engineering concepts exploiting the convergence of technologies (e.g. nano, micro, bio) for the next generation of high added value products and services was discussed in view of future collaborative research activities.

The Exploratory Workshop took place from September 26st to September 28th, 2010 in Fiesa, Slovenia and it was attended by fifteen (15) scientists from six ESF countries, one participant was from private sector. In provisional participant list approximately one third women scientists were expected. Due to personal reasons one female scientist was substituted with equally competent participant coming from the same institution. In the end one fifth of all participants were women scientists. Talks were organized in three thematic sessions. In each session four talks were presented. Each lasted for half an hour with ample time for discussion available after every talk. After presentations lively discussions were facilitated by most of the participants which resulted in prolonging the daily program of the workshop.

The venue on Slovenian coast provided an excellent environment for academics to interact and exchange ideas with its relaxing environment. The workshop's administration was run by the staff members from Laboratory of Alternative Technologies, Faculty of Mechanical Engineering, University of Ljubljana with previous experience in management of such events. The organizers provided a comfortable environment that allowed participants to focus on the tasks at hand. Surroundings of the hotel where the workshop took place permitted additional informal interaction due to its hiking paths along the coast line. Additional time for discussion on future collaborative activities was also available on evening walks to and from the restaurant where dinner took place.

The workshop itself focused on applied microtechnologies for biomedicine, life sciences, biotechnology, chemical technology, electrical engineering and mechanical engineering. It was devided into three main sessions:

- Design of Microreactors;
- Micro-Manufacturing Technologies;
- Microreactors in Biomedical and Biochemical Applications.

Most of the participating researches had no previously established collaboration schemes so one of the main goals of the workshop was to give the opportunity to learn about the ongoing research in other participants group, and discovering the common interests for future collaborations. This is especially important in this vastly interdisciplinary research field of microreactor technologies where for instance a biomedical scientist gets an insight into state of the art of micro-fabrication possibilities and vice versa.

Following is the list of main research topics identified among the participants which are promising for future collaboration through successful application for EU FP7 Research Programmes:

- usage of liquid interfacial media for microfluidic system boundaries instead of hard surfaces;
- design, fabrication, testing and usage of 3D microreactors with emphasis on cell and tissue biomedical engineering with special interest on nanoparticle-cell/tissue interaction (assessment of nanoparticle hazardousness). An application forseen also in this scope of 3D microreactors is to use them for drug delivery systems and cancer treatment research (electrochemotherapy). In this context the development of multiple use platform is a challenging task to be addressed by project proposal(s) (autoclavable platforms);
- development of microreactors for on-line chemical or/and bio analysis, namely the use of small-scale devices, from microwell plates to microreactors, speed up the development of bioconversion and fermentation processes allowing top research with reduced costs.

A major part of last morning session of the workshop was dedicated to discussion on follow-up activities, such as networking and collaboration opportunities within ESF and EU FP7 Research Programmes. Actually the whole MITHRA workshop idea emerged after a failed EU FP7 project application with emphasis on establishing a competence consortium to form programs allowing student training and exchange. In this context one of the actions planned for the near future is to rewrite the project proposal with consideration of the original project evaluation comments. An additional benefit for preparing a better proposal were also the comments of the ESF representative regarding the down sides of our ESF Exploratory Workshop application which can be applied to improve the envisioned EU FP7 project proposal.

The first output of the workshop was the installation of the on-line application (Dropbox) accessible for use for all workshop participants. Here we already collected ideas which would serve as a base ground for future collaboration actions. The response from most of participants was quick and constructive – an abstract of ideas and visions of future collaboration projects have already been gathered.

Feedback from the participants was generally very positive, and good momentum was established, on which we are actively working now. The overall conclusion of the workshop was that the participants have enough competences and cover wide enough range of expertise to be successful in applying for EU research funds. The inclusion of additional experts in clinical technologies would also greatly contribute to form a complete circle of expertise needed in the field of microreactor technologies for healthcare applications.

Scientific content of the event

It is thought that worldwide more than 10^9 analyses are now performed every day —with a double-digit growth rate. There is an increase in the demand for analytical chemical, bio-chemical, and bio-medicine information, because quality control has become a worldwide topic, agreed upon in many international trade contracts. For this reason, microreactors and microdevices for analytical and biosensors applications that provide rapid analysis, high sensitivity, and require small sample quantities can have great potential use and the major fields are environmental studies, quality control of chemically produced compounds and biomedical analysis, especially for medical diagnostics.

Microreactor technology for healthcare application is inherently interdisciplinary scientific research field as disciplines such as chemical technology, biotechnology, biomedicine, biocybernetics and mechanical engineering are essential for modelling, design, fabrication and application of microreactors.

The meeting opened with a welcome speech by the workshop convenor prof. Mihael Junkar explaining the origins of the formation of the scientific consortium gathered on the workshop. He explained that the whole idea of organizing the workshop was derived from unsuccessful FP7 project proposal. The issue of only non-formal acquaintance prior to project proposal submission was put forward. The members of the consortium had only a vague or no insight of partners research activities. By knowing the partner research activities and capabilities through personal meeting enabled through ESF exploratory workshop should and will enrich and improve the planned proposals for EU research funds. So the main workshop aim, to seek Europe-wide multidisciplinary collaborations between different excellent research groups to advance knowledge-based research and to reinforce the S&T potential by facilitating the communication between research entities, was alleged.

After the welcome speech the ESF representative prof. Salim Belouettar presented the main objectives of the ESF organization. He also enlisted some open EU research funds calls which would be suitable for the present consortium of participants to take into consideration when planning the future collaborative actions. An additional benefit for preparing a better proposal in the future were also the comments regarding the down sides of our ESF Exploratory Workshop application which can be applied to improve the envisioned EU FP7 project proposal. A main drawback of the formed consortium is that it should be more pan-European, involving more European countries. We should also have a higher input of participants from health sector, looking at experts in clinical technologies and clinical experts in specific areas that would increase the chances for successful project application.

The first scientific presentation was given by prof. Martin Richter titled "Microreactors with integrated micropumps for applications like cell cultivation". It was stressed out that for development of drugs or cosmetics, there is a general trend to replace animal experiments by cell tissue, which are manufactured by tissue engineering. Two drawbacks were mentioned: breeding cells in petri dishes is a manual process with a small throughput, and those cells don't have the same properties compared to cells inside the body (e.g. they are not polarised). His research group realized a flow through bioreactor for cell growth monitored by on-line impedance biosensor. As a result, the colon cells showed a similar growth structure as within the living body and the polarisation of the cells was improved.

In the following many ideas were shared by the author of the presentation and the participants regarding the overlapping of their research activities. Three main outlines, backed up by the competences of other participants research interests, that could be used as a nucleus of the future collaboration activities emerged: development of a holistic 3D bioreactor for growing cell tissue which could sustain sterilisation and would be cost effective; development of a microreactor for on-line chemical or/and bio-analysis available for multiple use; development of a specialized micro-reactor dedicated to drug delivery and cancer treatment.

Second presentation was given by Stephen Wilson titled "Nanoparticle interactions in silicone and perfluorinated oils". The relevance of this research field adheres to the subject of microreactor technology due to fact that the quantity and variety of applications for synthetic nanoparticle technology is growing rapidly in all of the major commercial and industrial sectors. The problem of determination of nanoparticle toxicology (skin, ingestion, inhalation), its environmental impact and recyclement are the main research outlines. Critical dimensions of nanoparticles, hazardous to health, were identified to be between 100-300 nm.

In the context of microreactor technologies for healthcare applications following challenges were identified in the discussion afterwards. As no systematic approach has been done to examine nanoparticle toxicology, micro bio-reactor approach could be one of the steps towards methodological bio-interaction quantification. Another promising application derived from the lecture was to use liquid 'walls' for cell entrapment and thus use it for nanoparticle interaction research.

Pedro Fernandes gave a talk titled "Key issues in scaling down fermentation and bioconversion systems". Main part of the talk was concentrated around the main advantages of using microreactors to speed up the bioconversion and fermentation processes, possibility of process parallelization and automation. Namely the findings gathered at microlitre (or lower) scale can often be translated to larger scales using very small sample volumes allowing top research with reduced costs. The issue of bioprocess integration to miniature scale was also thoroughly addressed.

The debate following the talk developed into defining the need to develop a cheap flowthrough microreactor allowing the on-line control of process parameters, pH identified as one of the most influential one. The issue of adherence of microbiological organisms to microtiter walls and microorganisms growth monitoring devices were also identified as one of the areas that could be successfully exploited in view of broadening R&D capacities by the participant consortium.

A presentation titled "Micromachined structures for microfluidics" was presented by Slavko Amon. His research group is highly competent in silicon based micro/nano technology having deep insight knowledge of state of the art silicon processing. Applications such as microchannel chips, micropumps, microvalves, microheaters and microsensors were discussed.

As the surface micro/nano texturing is very important in cell growth bio-reactors immediate responses and proposals for research collaboration emerged among the participants researching cells and tissues in bio-reactor setup. Being able to produce nanometric features opens a numerous applications among the participant research groups identifying such a competence as one of the key partners in envisioned project proposal.

A presentation of Luis Fonseca covered topics related to "Microreactors and other microdevices for analytical and biosensor applications". The presentation was focused on showing the potential of microreactors and microdevices in analytical and biosensors applications, providing rapid analysis, high sensitivity at small sample quantities. Main application fields identified are quality control of chemically produced compounds, biomedical analysis, especially for medical diagnostics and environmental studies.

In the discussion development of an enzyme-base analytical microreactor was put forward. Main challenges to overcome are production cost reduction, simplicity of microreactor handling, applying inexpensive method for enzyme immobilization and microreactor disposability. In the same context a development of portable microfluidic platform for individual cell and biological compounds (e.g. single strand DNA molecules) manipulation applied for pathogen microorganisms detection was discussed. These integrated bioanalytical portable microdevices can be operated in-situ by untrained personnel, and can be associated with applications regarding environment issues (pollution, food and water quality) and biomedical applications (e.g. bioterrorism).

Silvia Marson presented a talk titled "Manufacturing methods for 3D microfluidic systems". Disposability and high volume production of lab-on-a-chip applications used as miniaturized biological, chemical and medical devices was exposed as one of their key requirements. Due to the increase in demand for more complex microfluidic devices, true 3D structures, also known as 'out-of-plane' or 'vertical' architectures, offer the advantages of optimising the use of space to integrate more functionality or increase throughput.

A case study was presented for blood cells - plasma separation in 3D microfluidic device. Building block approach was used for separator fabrication. The discussion arose regarding the possibilities of producing a true 3D micro-, bio-reactors for participants specific applications. As closed 3D micro-geometry is a top notch research topic it should be emphasised in call proposal submission.

The second talk from Stephen Wilson titled "Integration of PZT thick-film materials into MEMS using batch-scale production techniques" presented the possibilities and realizations of thick films piezoelectric ceramic micro-electromechanical devices such as micro-pumps, micro-valves, and applications used for energy scavenging. The problem of processing incompatibility was put forward as the main barrier that hinders more widely availability of thick-film usage despite commercial desirability.

The discussion that followed went into the direction of applying the piezoelectric ceramics in micro-reactor applications since micro-pumps and micro-valves are one of the most important components of this microfluidic system. A main issue to be solved is the cost-effectiveness of the production route based on ultra-precision ceramic machining and full-wafer bonding.

A talk titled "DFM approach to custom made micromixers" was presented by Joško Valentinčič. It addressed the importance of simulations in order to design the microfluidic device in the concurrent engineering paradigm to be "right the first time". The whole process of design was presented on the base of a case study of a micro-mixer application. Namely, mixing under laminar flow regime, which is inherent to microfluidic applications, is a non-trivial task. The experience from macro-world have little use in designing a functional microstructure.

The participants agreed that the presented systematic approach, implementing the fabrication technology early on in the design phase is of key importance in order to produce the desired micro-component effectively, functionally and cost-effectively.

Xunli Zhang, from the University of Southampton, presented a talk titled "Microfluidics and applications in bioengineering". Numerous applications in research were presented such as stem cell analysis utilizing micro sensors and actuators, the research of blood flow in micro-meter dimensions in order to study blood vessel embolisation in the context of an alternative tumour treatment method, flow chemistry setups and biomaterials bio-reactor devices (e.g. development of 3D structures to enable growth of bone tissue).

As the content of the talk emphasized the importance of developing complex microdevices to enable cells and tissue growth in order to study pharmaceutical drug effects and for instance nanoparticles interactions (toxicology), many participants were very interested in new collaborative research activities on this subjects. One of the research challenges identified was a 3D physical realization of a capillary system scan. The participants affiliated with micro/nano-manufacturing technologies were also very interested in joining forces in researching this challenging task.

The opening talk of the second day was held by Igor Plazl with a presentation titled "Implementation of microstructured devices in biotransformation processes". Main subject of the talk was the miniaturization of biotechnological processes (e.g. various biotransformations in one or two-phase systems) and the implications of continuous downstream processes in the system of microchanells, which allow an integrated process set-up. Many case studies of tested reactions and downstream processes performed in the continuously operated pressure-driven microstructured devices were presented (e.g. laccase-catalyzed oxidation of a chosen phenolic compound in an aqueous phase with dissolved biocatalyst) indicating a clear intensification of the process. All of the performed reactions were also modelled with reaction-diffusion dynamics.

Present consortium of participants acknowledge the presented competences on the subject of biotransformation processes in microreactors as a valuable contribution to round up the competences of the whole consortium. Modelling of reaction kinetics is of vital importance for theoretically understanding the transformation process and to make possible its optimization through theoretical analysis.

The next presentation was given by Giuliano Bissacco titled "Micro and precision manufacturing". It was concentrated around manufacturing processes which remove the material from the workpiece by a finite unit volumetric fashion. Encompassed processes were micro milling, micro grinding, diamond turning and energy assisted material removal processes represented by laser micro machining, micro electrical discharge machining, electrochemical machining and some material additive processes like laser sintering, metal and ceramic ink jet printing and localized electroforming. As each single process presents inherent limitations in terms of machinable geometries, feature/part size, achievable accuracy, productivity, etc. For low costs mass production of micro components replication techniques have to be used (e.g. micro injection moulding).

The organisation of the available micro manufacturing technologies into optimal process chain is the main challenge in tackling the production costs. The presented micro manufacturing processes present an alternative to silicon based machining which was predominantly used in past decades for microstructure devices fabrication. As most of the

participants were not acquainted with vast possibilities that this modern processes present, implicating them in rapid prototyping for testing of new microreactor concepts is of great importance.

Finale talk, titled "Cell electroporation detection and separation", was given by Alenka Maček-Lebar. Firstly the phenomenon of electroporation was explained which represents increased cells' membrane permeability due to exposure to high voltage electric pulses. If exerted electric field is of adequate strength and duration, the membrane returns to its normal state (reversible electroporation). Electroporation is mainly applied for introduction of small and large molecules into cell interior, finding application in biochemistry, molecular biology and medicine (e.g. cancer treatment with electro-chemotherapy). For research reasons it is of upmost importance to know which cells were electroporated and which weren't. As cells' properties change significantly after electroporation (e.g. membrane permeability, cells' geometrical and electrical properties) this makes possible to separate them by means of dielectrophoresis.

The research institution of the lecturer has excellent cell technology equipment. Due to their experience with cancer treatment research, participants were interested in starting collaboration project(s) and transfer the research platform from petri dishes to micro-bioreactors, implementing cell growth functionality with monitoring and control sensors. Cancer treatment is always an important research subject and the consortium of participants see a good opportunity to enhance the newly established collaboration connections with a project proposal on this topic.

The workshop was concluded by a round table, where all participants put forward the possible goals of the future collaboration(s). Ideas were converging to the common goal and one week after the workshop, Martin Richter proposed two topics on which the participants can work together in the future, namely:

- Bioreactor for tissue membranes;
- Microreactor with online IR spectroscopy

The content of the proposed ideas is confidential and thus not given here.

Assessment of the results, contribution to the future direction of the field, outcome

Microreactor technology for healthcare application is inherently interdisciplinary scientific research field as disciplines such as chemical technology, biotechnology, biomedicine, biocybernetics and mechanical engineering are essential for modelling, design, fabrication and application of microreactors. Through presentations and corresponding scientific discussions following conclusions and overlapping base points were identified in a view of future collaborative research activities:

- usage of liquid interfacial media for microfluidic system boundaries instead of hard surfaces. This concept is considered as a novel approach to microreactor design which enables contactless interface for envisioned cell bioreactor development. Namely in conventional micro bioreactors, cells tend to get attached to the solid surfaces and have to be detached prior to the experimental usage;
- design, fabrication, testing and usage of 3D micro-bioreactors with emphasis on designing a sustainable cell and tissue bioreactor environment (biomedical engineering), implementing the functionality of growth of the implied organism (cell/tissue factories) as well as functionalities of on-line monitoring and perturbating the micro-biosystem. The development of such micro-device finds its applications in pharmacy (drug delivery research system, cosmetic industry) and in the cancer treatment studies (e.g. electro-chemotherapy treatment);
- in the context of developing a 3D micro-bioreactor; it should be made for multiple use i.e. the components should sustain autoclavation (pressure, temperature resistant components). To achieve that, different materials should be tested; e.g. the silicone membrane, a critical part of the micropump, could be replaced with steel membrane;
- a big challenge is to fabricate 3D microdevices that mimic functional natural structures (nano surface texturing enabling better cell/tissue growth, cell/tissue repellent surfaces, physical real 3D modelling of the capillary system to study embolisation, micro 3D 'sponge' structures for bone tissue growth);
- the outcome of previous bulletins would be a multipurpose micro bio/chemical reactor allowing systematic and highly controllable studying of biosystems (e.g. bio/non-bio interactions of nanoparticles on living cells/tissues). In a specialized version i.e. targeting diagnostic applications it should satisfy the following requirements: point-of-care applicability, very low-cost, quick to perform, minimal manipulation required by a non-expert;
- development of multiple use microreactor(s) applied in chemical technology, which is exerted to much harsher environment (pressure, temperature, aggressive chemicals) than bioreactors is also a vivid scientific challenge, namely the use of small-scale devices, from microwell plates to microreactors, speed up the development of bioconversion and fermentation processes allowing top research with reduced costs. It should implement on-line chemical- or/and bio-analysis;
- in the last decades the reduction of CO_2 became an important environmental issue. Research the use of microreactor (bio)catalyzed technology for this purpose is a relatively new research field.

The research objectives identified are based on the minutes taken at the meeting based on scientific presentations and the subsequent scientific discussion. The discussion continues with a help of a commercial on-line application Dropbox, which allows the participants to access all the workshop documentation (minutes, book of abstracts, project ideas, relevant

EU research funding calls documentation) and contribute new ideas and thoughts about already established funding proposal actions and identifying the future collaboration actions. The response from most of participants was quick and constructive – an abstract of ideas and visions of future collaboration projects have already been gathered.

Currently a proposal for FP7-REGPOT-2010 research potential call is being prepared within the formed consortium of participants. As the workshop organization brought the participants closer together, positive outcome from project proposal is expected, especially due to analysis of the failed proposal application by the consortium submitted previous year.

Final programme

Sunday 26 September 2010

Afternoon	*Arrival (Hotel Barbara Fiesa)*
19.00	*Conference dinner, get-together, social event, informal*

Monday 27 September 2010

09.10-09.20	**Welcome by Convenor** **M. Junkar** (Faculty of Mechanical Engineering, Ljubljana, Slovenia)
09.20-09.40	**Presentation of the European Science Foundation (ESF)** **S. Belouettar**, ESF Standing Committee for Physical and Engineering Sciences - PESC
09.40-12.00	**Morning Session: Design of Microreactors**
09.40-10.10	**Presentation 1 "Microreactors with integrated micropumps for applications like cell cultivation and nanoencapsulated drugs"** **M. Richter** (Fraunhofer Institute for Reliability and Microintegration IZM, Munich, Germany)
10.10-10.40	**Presentation 2 "Nanoparticle interactions at liquid-liquid interfaces"** **S. A. Wilson** (Microsystems and Nanotechnology Centre, Cranfield, UK)
10.40-11.00	*Coffee / Tea Break*
11.00-11.30	**Presentation 3 "Key issues in scaling down fermentation and bioconversion system"** **P. Fernandes** (IBB-Institute for Biotechnology and Bioengineering, Lisbon, Portugal)
11.30-12.00	**Presentation 4 "Micromachined structures for microfluidics"** **S. Amon** (Faculty of Electrical Engineering, Ljubljana, Slovenia)
12.00-13.00	**Discussion**
13.00-14.00	*Lunch*
14.00-17.00	**Afternoon Session: Micro-Manufacturing Technologies**
14.00-14.30	**Presentation 1 "Microreactors and other microdevices for analytical and biosensor applications"** **L. P. Fonseca** (Institute for Biotechnology and Bioengineering, Lisbon, Portugal)
14.30-15.00	**Presentation 2 "Manufacturing methods for 3D microfluidic systems"** **S. Marson** (Precision Engineering Centre, Cranfield University, UK)
15.00-15.30	*Coffee / Tea Break*
15.30-16.00	**Presentation 3 "Integration of functional materials into microsystems for batch-scale production"** **S. A. Wilson** (Microsystems and Nanotechnology Centre, Cranfield, UK
16.00-16.30	**Presentation 4 "DFM approach to custom made micromixer"** **J. Valentinčič** (Faculty of Mechanical Engineering, Ljubljana, Slovenia)
16.30-17.00	**Presentation 5 "Microfluidics and applications in bioengineering"** **X. Zhang** (School of Engineering Sciences, Southampton, UK)

17.00-18.30	**Discussion**
19.00	*Dinner*

Tuesday 28 September 2010

09.00-12.00	**Morning Session: Microreactors in Biomedical and Biochemical Applications**
09.00-09.30	**Presentation 1 "Implementation of microstuctured devices in biotransformation processes"** **I. Plazl** (Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia)
09.30-10.00	**Presentation 1 "Micro and Precision Manufacturing"** **G. Bissacco** (Technical University of Denmark, Department of Mechanical Engineering, Lyngby, Denmark)
10.00-10.30	*Coffee / Tea Break*
10.30-11.00	**Presentation 4 "Separation of electroporated and non-electroporated cells by means of dielectrophoresis"** **A. Maček-Lebar** (Faculty of Electrical Engineering, Ljubljana, Slovenia)
11.00-13.00	**Round table on follow-up activities/networking/collaboration**
13.00-14.00	*Lunch*
	End of Workshop and departure

Final list of participants

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Statistical information on participants

The participants came from the following countries:

Slovenia	:	5	(33%)
Portugal	:	2	(13%)
United Kingdom	:	3	(20%)
Romania	:	2	(13%)
Denmark	:	1	(7%)
Germany	:	2	(13%)

There were participants from 6 European countries. Giuliano Bisacco, previously affiliated to University of Padua, Italy at the time of documents submitted, transferred to Technical University of Denmark, so all corresponding data (travel costs, affiliation) are changed.

Age bracket:

21 - 30	:	1	(7%)
31 – 40	:	5	(33%)
41 - 50	:	5	(33%)
51 -	:	4	(27%)

Male/Female repartition:

Female participants	:	3	(20%)
Male participants	:	12	(80%)

Provisionally it was planned that there would be one more female participant but was substituted with her male colleague due to personal reasons.

The scientific background of the participants is vastly interdisciplinary and is presented in the following table.

Micromanufacturing technologies	:	6	(40%)
Microsystems engineering	:	3	(20%)
Electrical engineering	:	2	(13%)
Chemical engineering	:	2	(13%)
Biotechnology and Bioengineering	:	2	(13%)

One of the participants was from private sector, all the others were from research organisations.