ESF Exploratory Workshop on

MANIPULATION OF BIOMATERIALS SURFACE BY PLASMA PROCESSING

Iasi (Romania), 26-30 May 2010

Convened by:
Prof. Nicoleta Dumitrascu
Faculty of Physics, Alexandru Ioan Cuza University of Iasi, Romania

SCIENTIFIC REPORT

Co-sponsored by

National Authority for Scientific Research of Romania
Alexandru Ioan Cuza University of Iasi
1. Executive summary

General logistics

The workshop was hosted by Alexandru Ioan Cuza University of Iasi, Romania. Participants arrived on 26 May 2010 and the departure was fixed for 30 May 2010. The effective lectures period was from 27 to 29 May 2010. The lectures were held in Ferdinand room of Alexandru Ioan Cuza University. Wireless Internet access was available in the conference room during entire workshop period. General logistics and local organization details were assured by the staff of Plasma Physics Laboratory, Alexandru Ioan Cuza University of Iasi.

All participants, excepting the local ones, stayed in Hotel Unirea. A bus transfer schedule from Hotel Unirea to the University and return was assured daily. Flights to Iasi were booked for all foreign participants via a travel operator recognized by our University and transfer from Iasi International Airport to the hotel was assured. Lunches were served in a restaurant nearby our University and dinners were served in different locations of our city. All these costs were supported from the workshop budget for most of participants, with the following exceptions: Dr. Greg Byrne (University College Dublin, Ireland) and Phd Student Tinneke Jacobs (Ghent University, Belgium). They agreed to participate on their own laboratory costs.

Financial support from two additional sponsors has made possible the successful organization of our workshop:

- National Authority for Scientific Research of Romania (ANCS), with a grant that served for bags, printing & photocopying, general event signalization and publication of scientific contributions from participants in one volume edited by Prof. Nicoleta Dumitrascu and Dr. Ionut Topala (editorial work in progress);
- Alexandru Ioan Cuza University of Iasi (UAIC), which provided access to the meeting room, equipment and Wireless Internet access for 3 days.

For this scientific event a web page was created and it is accessible to the general public at the following address: http://www.plasma.uaic.ro/ESF_MBSPP2010/.

Participants

Our meeting had a wide European participation, as country of origin and scientific field of the 26 participants. After announcing the list the founded projects, we sent letter of invitation to all participants included in the workshop proposal. Replacements were necessary for 4 speakers, due to busy agenda in the period of time 26-30 May 2010 (option of the majority of participants). Access in the conference room was restricted only to invited participants and the two ESF representatives. Ample discussions and debates were launched after each presentation and during the coffee breaks. The broad range of participant’s specialization (e.g. plasma physics, materials physics and chemistry, medicine) allowed clear identification of actual problems in plasma biomaterials processing. New strategies and approaches for further development in the field were discussed.

Two ESF rapporteurs, i. e., A. Margioris (School of Medicine, Heraklion University of Crete, Greece) and M. Tichy (Charles University in Prague, Czech Republic) participated at the workshop. An overview about the European Science Foundation was delivered at the opening ceremony.

Last-minute Changes

Due to medical reasons Dr. Dimitrios Mataras (University of Patras, Greece) canceled his participation to our workshop two weeks before the meeting starting day. The cancellation costs for his thicket are supported from the workshop budget. He was replaced by his collaborator, Dr. Eleftherios Amanatides. All local costs (hotel, meals, transfer) were supported from the workshop budget, only the airplane ticket was supported from they own laboratory sources.

Objectives
The workshop was focused on the following objectives:

1. To bring together researchers with high expertise in physics, chemistry, medicine and materials science to build a particular network of contacts having a common ground: what are the beneficial effects of plasma processing on the biomaterials surface and how do we get to improve its use for medical applications.

2. To make a comparative analysis of plasma technologies at low and atmospheric pressure, in order to standardize (homogenize) certain experimental arrangements for medical purposes. Characterization and optimization of plasma processing conditions were discussed, looking for the main effects of plasma on the properties of surface materials.

3. To prove the efficiency of plasma processing in certain medical applications: synthesis of polymeric membranes/films for cell growth, deposition of biofunctional coatings using plasma polymerization, sterilization and decontamination of biomaterials surface, and immobilization of biomolecules onto the polymeric supports.

4. To compare the effects of plasma processing with other techniques (chemical and biochemical) used for surface functionalization and immobilization of biomolecules.

5. To debate and develop new strategies in surface modification of biomaterials by plasma processing and establish future collaborative activities.

Main Conclusions

This workshop conclusion can be easily drawn in the form a SWOT analysis.

As strength points we can note:
- at the meeting participated research groups from different fields (physics, chemistry, biology), all working with plasmas in relation with medical proposes;
- all the research groups have complementary expertises in plasma diagnosis, surface chemistry and biological tests;
- the presented results show that all groups benefit of large research infrastructure;
- we noticed a clear and fundamental interest of each group to cooperate on a large scale on the subject;
- the discussions were very constructive and inspired, with original comments and often suggestions for future research of speakers.

As weak points we can mention:
- cooperation with medical doctors and biologists is still week; the main approach of the subjects is from the physics and chemistry point of view;
- present economical crisis has an impact on medium and long term cooperation in the field;
- in general regulation on the bioethical subjects is rather complicated and makes research difficult;

As opportunities we identified:
- a large demand to have better medical services and medical assistance, which needs new techniques for drug delivery, more accurate diagnostic methods etc.;
- the existence of a rather large community with strong expertise and competence in field of physics, chemistry and biology;
- for the Romanian side is a special situation, we have the opportunity to apply for structural funds for PhD and postdoc programs;
- existence of European Research programs (FP7, ESF etc.) which allow applications for common projects.

As threats we can look after:
- an extent of the present economical crisis;
- continuation of very fragmented activity of different groups without cooperation.
Scientific contributions received from participants are assembled in a volume entitled (provisional title) *Biomaterials and Plasma Processing*, edited by the organizers and published by Alexandru Ioan Cuza University Editorial house.

2. Scientific content of the event

Biomaterials are used as implants and fibers in surgery or artificial material restoring a function of living tissues, as biosensors in medical devices or as simple bags for transport of the blood being involved in all stages of a disease, from diagnostic to therapy and healing. In order to respond at these applications the biomaterials must have mechanical and physico-chemical bulk properties that complete well the restrictive conditions of the medical applications, even over those asked in the human. Unfortunately, their surface biocompatibility is often poor, altering the physiological environment through adverse reactions, infection, inflammation, cancer effects and thrombosis formation. More, their low surface energy does not concur to an easy and stable connection with the biological molecules.

The invited speakers emphasized the importance of these surface properties, such as mechanical stress, scratch and wear resistance, hardness, chemical corrosion resistance, wettability, adhesivity and biocompatibility and that plasma technology represents a unique, precise and selective method for modification of surfaces from micrometer to nanometer length scales for use in biological environment. The main part of the lectures was focused on the effects of plasma processing on the surface characteristics and three lectures were dedicated to chemical methods which may be used to prepare substrates for medical applications. *All of lectures included basic research, various methods to prove the results and specific tests sustaining the pointed/final medical scope.*

In the following we summarize the main results communicated by our invited speakers which can be considered as efficient and practical solutions in improving the quality of materials for use in human:

1. creating and incorporating/grafting of functional groups onto the polymeric surfaces such as liposomes and heparin (E. Amanatides), enzymes (N. Dumitrascu) etc., also incorporation of acid groups in the organic matrix (P. Kingshott), for the subsequent immobilization of various bioactive species;
2. grafting onto plasma functionalized membranes by an air-to-air-process for hollow fibers which are whole blood compatible and remove bacterial endotoxines (C. Oehr);
3. deposition of Ag, Ti and modified Ti, also nanocomposites onto various substrates/polymeric films and metallization of yarns with antibacterial efficacy (D. Hegemann) and development of active wound dressing which combats infection (R. Forch);
4. deposition of hybrid highly ordered binary and ternary nanopatterned surfaces using mixtures of plasma polymers, metals, oxides and colloids, with multiple specific functionalization for biological applications (P. Kingshott);
5. spray deposition of polyelectrolyte multilayers used as photostimulated antibacterial activity (V. Ball);
6. synthesis of polymeric membranes (V. Rouessac) and deposition of biofunctional coatings with specified transport properties (A. Hollander);
7. chemical method based on core-shell nano/micro particles by soapless emulsion polymerization to create supramolecular structures (B. Simionescu);
8. treatments of various substrates by stimulating the surface functionality or to remove residuals (J. M. Martinez) and control of plasma parameters (Gh. Popa);
9. explanations about the mecanisms of decontamination processes by plasma based on a combined effect of UV (fast process) and plasma etching (slow) (F. Rossi);
10. use the plasma effects to improve the ELISA test assay (F. Epaillard);
11. plasma surface activation biocompatible and biodegradable polymers (polycaprolactone) (C. Leys);
12. atmospheric pressure plasma processing for biomedical applications (G. Byrne);
13. plasma deposition technology at atmospheric pressure for modifying the surface chemistry both on 2-D and 3-D structures (J. Bradley);
14. Method of investigation of carbohydrate-protein interaction using the quartz crystal microbalance (M. Barboiu).

The above results have been obtained in various type of plasma reactors, used as energetic medium for treatments and chemical reactions, including polymerization, followed by deposition of films with specific physico-chemical and biological properties, in the depth from µm to nm and over a large area. The chemical methods presented demonstrate that an interdisciplinary collaboration between the groups can lead to better solutions in improvement of implant’s medical requirements.

Characterization, optimization and standardization of these experimental set-ups for plasma processing must be correlated with analysis of its effects and also with the methods of plasma diagnosis, such as emission spectrometry, tunable diode-laser absorption spectrometry, laser induced fluorescence, electrical probes and electrostatic analyzers, mass spectrometry and ICCD imaging.

The management and the control of these 4 steps (plasma diagnosis, plasma effects, medical requirements and biological testing) presume very complex aspects and demand interdisciplinary researches, including competences in the field of physics, chemistry and biochemistry, medicine and materials science.

Since the parameter pressure was one major concern regarding the plasma reactors applied for biomaterials processing, in the following lines we summarize the type of plasma reactors presented by the invited speakers:

- **Radio-frequency plasma at low pressure** (both inductively and capacitively coupled) used for: PET films surface modification and a-C:H deposition (E. Amanatides), allylamine deposition (J. Bradley), carbon nanofibers and carbon nanowalls synthesis (G. Dinescu), reduction of cell and bacterial adhesion (R. Forch), incorporation for Ag into functional groups and crosslinking (D. Hegemann), control of surface chemistry for the immobilization of proteins (A. Hollander), synthesis of protein/cell resistant surfaces (P. Kingshott), plasma treatment of rubber (J. Martin-Martinez), plasma treatment for surfaces used for fibroblast growth and plasma treatment for blood cleaning and cell adhesion (C. Oehr), plasma chemistry as a tool for improving ELISA protocols (F. Poncin-Epaillard), sterilisation and decontamination of medical devices sensible at high T, efficient for removal of microorganisms, pyrogenic agents, fungi, protein residues (F. Rossi);
- **Magnetron sputtering** combined with **glow discharge plasma polymerization at low pressure** used to obtain functional plasma polymers cytocompatible and stable in aqueous environments, or metallization of yarns with antibacterial efficacy (D. Hegemann);
- **Low frequency and low pressure plasma reactors**, used to obtain microporous and permselective membranes (V. Rouessac);
- **Dielectric barrier discharge at medium and atmospheric pressure**, applied for: polymer surface treatment and plasma polymerization for biomolecules immobilization (N. Dumitrascu), plasma deposition of polymethylmethacrylate and surface activation of polycaprolactone (C. Leys);
- **Atmospheric pressure plasma sources in jet configuration**, applied for: polymer surface treatment and plasma polymerisation (J. Bradley, G. Byrne), processing internal parts of narrow tubes and organic or carbon residuals removal from surfaces (G. Dinescu), plasma treatment of rubber (J. Martin-Martinez), surface modifications on biodegradable polymeric substrates (J. Mano).
After all presentations and summarizing the ideas issued during discussion, the following conclusions can be pointed in relation with the scientific content of the workshop:

- importance of the plasma processing as a dry, efficient and eco-friendly technology, offering an attractive alternative to add new physico chemical and biological functionalities to biomaterials, such as intelligent morphology with micro and nano-scale control, long-term hydrophilicity, mechanical, electrical and antibacterial properties as well as high purity, low toxicity, bio-compound immobilization ability, film forming ability, high selectivity etc.;
- collaborative activities are necessary for a standardization of certain plasma devices and comparison of the results/ effects induced onto the surface; combination of experimental arrangements at low and atmospheric pressure;
- control of the main processes in the gas phase using various techniques; relation between kinetics in volume and chemical quasi-equilibrium at interface;
- importance/ attention to the stability of coatings in the biological environment, including the human (temperature, pH, enzymes etc.);
- better correlation between the surface free energy and bacterial and cell adhesion, in particular importance of the streaming potential;
- optimization of the in vivo tests and strategy for better collaboration with biologists and physicians.

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

After 3 days of lectures and discussions the following points can be noted:
- we had a benefic and necessary exchange of knowledge between speakers with different scientific background; challenges and strategies in this field were establishment;
- it was established the common experimental facilities for complementary measurements and reciprocal help; moreover a strong collaboration between plasma physicists, chemists, biologists and medical doctors is necessary to improve the physico chemical properties and the biological response of biomaterials;
- it is necessary to implement new plasma techniques for drugs (antibiotics, anticoagulants, antialergic etc.) immobilization onto the polymeric supports (drug delivery systems), and to collaborative actions must be encouraged for optimization and standardization of these techniques;
- it is necessary to improve the exchange of researchers, visiting professor and other opportunities: exchange of students, co-directed PhD thesis.

During the meeting identification of more problems than solutions became obvious. It was clear that plasma technology is an efficient and expected solution in developing new biomaterials which accomplish better the medical requireements. However it is impossible to find a unique plasma technology for all medical applications of biomaterials. We need a clear identification of research objectives and directions in the field, so that scientific effort is sharp and well defined. We must identify clear situations were plasma can help or even “is going to save life” and we must consolidate a strong network that is focused on these subjects.

One of the common questions during the debates was what type of plasma source is necessary for biomaterials processing? Atmospheric pressure plasma sources represent easiest solution, nevertheless chemically well defined surfaces cannot be obtained by atmospheric pressure plasma processing. In this way was arisen the need to study the pressure parameter in plasma technology applied for biomaterials processing and the necessity for accurate plasma diagnostics during biomaterials processing.

Looking for further research directions in the field some subjects were identified like the plasma processing of porous materials, plasma for antibacterial adhesion, plasma surface modification to promote cell attachment, plasma diagnostics in relation with biomaterials.
surface modification, plasma processing of materials used in specific clinical diagnostic kits, in order to improve these tests and to make them accessible to large community.

The necessity of further collaborative actions was the main conclusion of this workshop. In Europe promising results are obtained in many groups, still the scientific effort in the field is dissipated. During this workshop most of participants presented their own results on plasma and biomaterials; nevertheless a strong collaboration is demanded between plasma scientists, biologists and medical doctors. Homogenization and standardization of biological response in case of new biomaterials and specific assays for biomaterials performance testing must be released to researches. Furthermore an open dialogue must be launched with medical doctors: what are the problems in biomaterials field from the medical point of view. In this way physicists and chemists will know better the needs of medicine and they will find ways to overcome these problems. It was clear that further meetings between plasma scientists and medical community at large scale will be fruitful for all of us. In this respect an ESF Research Conference is a solution to bring together medical doctors with all the other scientists that work on biomaterials.

Due to the interdisciplinary approach of the field Plasma – Materials – Biology, specific education of young researchers is needed in order to work in this domain, so we must correlate the educational programmes to support this domain. Creation of a network for education and research in Plasma Science represents a starting point to reach this goal (at national level some examples are UK Nonthermal Plasma Network, Plasma Germany, Réseau Plasmas Froids in France). This can be constructed as a PhD students training network but also as a common platform of knowledge and infrastructure in plasma technology applied for biomaterials processing. Application for ESF events may represent a solution for networking in the domain of Plasma and Biomaterials.
4. Final programme

Thursday, 27 May 2010

08.30-10.00 OPENING CEREMONY
Vasile ISAN (Rector Alexandru Ioan Cuza University of Iasi) Dumitru LUCA (Dean of the Faculty of Physics)
Presentation of the European Science Foundation (ESF)
Andrew MARGIORIS (ESF Standing Committee for the European Medical Research Councils (EMRC))
Milan TICHY (ESF Standing Committee for Physical and Engineering Sciences (PESC))
Nicoleta DUMITRASCU (Convenor, Alexandru Ioan Cuza University of Iasi, Faculty of Physics, Iasi, Romania)

10.00-18.30 SESSION: PLASMA PROCESSING FOR BIOMEDICAL APPLICATIONS
(Chairperson: Gheorghe POPA)
10.00-10.30 “Plasma chemical modification of 2 and 3-D structures in cell-surface interaction studies”
James W. BRADLEY (University of Liverpool, United Kingdom)
10.30-10.45 Discussion
10.45-11.00 Coffee / Tea Break
11.00-11.30 “Decontamination and sterilization of medical devices using low pressure plasma”
Francois ROSSI (The Institute for Health and Consumer Protection JRC-IHCP, Ispra, Italy)
11.00-11.15 Discussion
11.45-12.15 “Cold expanding plasma sources for processing of biomaterials surfaces”
Gheorghe DINESCU (National Institute for Laser, Plasma and Radiation Physics, Bucharest, Romania)
12.15-12.30 Discussion
12.30-14.00 Lunch
14.00-14.30 “Using plasma polymers and colloid crystals to create adhesive and non-adhesive patterned surfaces”
Peter KINGSHOTT (Aarhus University, Aarhus, Denmark)
14.30-14.45 Discussion
14.45-15.15 “Nanostructured plasma coatings for medical applications”
Dirk HEGEMANN (EMPA Research Institute, St. Gallen, Switzerland)
15.15-15.30 Discussion
15.30-15.45 Coffee / Tea Break
15.45-16.15 “Plasma diagnosis for biomaterials surface modification”
Gheorghe POPA (Alexandru Ioan Cuza University, Iasi, Romania)
16.15-16.30 Discussion
16.30-17.00 “Biocompatible surfaces by plasma treatment: the relation between the conformational changes of proteins on plasma treated surfaces”
Mehmet MUTLU (Hacettepe University, Ankara, Turkey)
17.00-17.15 Discussion
17.15-17.45 Thematic conclusions
17.45-18.30 Visit of Plasma Physics laboratories
20.00 Dinner
Friday, 28 May 2010

09.00-19.00 SESSION: EFFECTS OF PLASMA ON THE PROPERTIES OF MATERIALS USED IN MEDICAL APPLICATIONS (Chairperson: Gheorghe DINESCU)

09.00-09.30 "Plasma polymers as substrate for biomolecules immobilization"
Nicoleta DUMITRASCU (Alexandru Ioan Cuza University, Iasi, Romania)

09.30-09.45 Discussion

09.45-10.15 "Medium and atmospheric pressure plasma treatment of polymers used in biomedical applications"
Christophe LEYS (Ghent University, Ghent, Belgium)

10.15-10.30 Discussion

10.30-10.45 Coffee / Tea Break

10.45-11.15 "Plasma treatment for blood cleaning and cell adhesion"
Christian OEHR (Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Stuttgart, Germany)

11.15-11.30 Discussion

11.30-12.00 "Surface chemistry for the immobilization of proteins"
Andreas HOLLANDER (Fraunhofer Institute for Applied Polymer Research IAP, Potsdam, Germany)

12.00-12.15 Discussion

12.15-14.00 Lunch

14.00-14.30 "Improvement of the wettability, adhesivity and biocompatibility of polymers by plasma treatments"
Eleftherios AMANATIDES (University of Patras, Patras, Greece)

14.30-14.45 Discussion

14.45-15.15 "Antiadhesive Ti-implant surfaces by plasma assisted surface modification"
Renate FÖRCH (Max Planck Institute for Polymer Research, Mainz, Germany)

15.15-15.30 Discussion

15.30-15.45 Coffee / Tea Break

15.45-16.15 "Reactive layer-by-layer coating by means of spray deposition"
Vincent BALL (University of Strasbourg, Strasbourg, France)

16.15-16.30 Discussion

16.30-17.00 "Use of plasmas to enhance the surface properties of rubber materials"
Jose Miguel MARTIN-MARTINEZ (University of Alicante, Alicante, Spain)

17.00-17.15 Discussion

17.15-17.45 "Membranes and plasma processing – from synthesis to applications"
Vincent ROUESSAC (European Membrane Institute, Montpellier, France)

17.45-18.00 Discussion

18.00-18.30 Thematic conclusions

20.00 Workshop Dinner
**Saturday, 29 May 2010**

09.30-16.00  **SESSION: PERSPECTIVES OF PLASMA PROCESSING IN BIOMATERIALS SCIENCE** (Chairperson: Nicoleta DUMITRASCU)

09.30-10.00  “Functional Polymers, Macromolecular and Supramolecular Architectures Designed for Biomaterials”
Bogdan SIMIONESCU (Petru Poni Institute of Macromolecular Chemistry, Iasi, Romania)

10.30-10.15  Discussion

10.15-10.45  “Plasma chemistry as a tool for designing new ELISA protocols”
Fabienne PONCIN-EPAILLARD (University of Maine, Le Mans, France)

10.45-11.00  Discussion

11.00-11.15  Coffee / Tea Break

11.15-11.45  “New techniques for biofunctional structures analysis: Quartz crystal microbalance biosensors-complex roadmaps toward sensitive devices”
Mihai-Dumitru BARBOIU (European Membrane Institute, Montpellier, France)

11.45-12.00  Discussion

12.00-13.30  Lunch

13.30-14.00  “Plasma-based surface modifications on biodegradable polymeric substrates for tissue engineering applications”
João F. MANO (University of Minho, Braga, Portugal)

14.00-14.15  Discussion

14.15-14.45  “Protein and cell interactions with atmospheric plasma jet modified surfaces”
Greg BYRNE (University College Dublin, Dublin, Ireland)

14.45-15.00  Discussion

15.00-17.00  Workshop conclusions and networking perspectives

18.00  City Tour followed by Dinner

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**5. Final list of participants**

**Convenor:**
1. Nicoleta DUMITRASCU (Faculty of Physics, Alexandru Ioan Cuza University of Iasi, Romania) nicoleta.dumitrascu@uaic.ro

**ESF Representatives:**
2. Andrew MARGIORIS, Standing Committee for the European Medical Research Councils (School of Medicine, University of Crete, Heraklion, Greece) andym@med.uoc.gr

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**Participants:**
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7. James W. BRADLEY (Department of Electrical Engineering and Electronics, University of Liverpool, United Kingdom) J.W.Bradley@liverpool.ac.uk

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16. Jose Miguel MARTIN-MARTINEZ (Department of Inorganic Chemistry, University of Alicante, Spain) JM.martin@ua.es

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18. Christian OEHR (Interfacial Engineering and Materials Science, Fraunhofer Institute for Interfacial Engineering IGB and Biotechnology, Stuttgart, Germany) christian.oehr@igb.fraunhofer.de

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20. Gheorghe POPA (Faculty of Physics, Alexandru Ioan Cuza University of Iasi, Romania) ghpopa@uaic.ro

21. François ROSSI (Biomedical Materials and Systems, The Institute for Health and Consumer Protection (IHCP) Ispra, Italy) francois.rossi@jrc.it

22. Vincent ROUESSAC (Institut Européen des Membranes, ENSCM/ UMII CNRS-UMR 5635, Montpellier, France) vincent.rouessac@iemm.univ-montp2.fr

23. Bogdan SIMIONESCU (Laboratory of Physical Characterisation of Polymers, Petru Poni Institute of Macromolecular Chemistry, Iasi, Romania) bcsimion@cmpp.ro

24. Tinneke JACOBS (Ghent University, Belgium), tinneke.jacobs@ugent.be

25. Mauricio SCHIEDA (Institute of Materials Research, Geesthacht, Germany) mauricio.schieda@gkss.de
6. Statistical information on participants

*Age structure:* below 30 (3 participants), between 30 to 50 (12 participants), past 50 (11 participants)

*Gender repartition:* out of a total of 26 participants, 4 (15%) were women and 22 (85%) were men.

*Countries of work:* the participants work in 14 different countries. Number of participants from each country is listed in the following table:

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<th>Number of participants</th>
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