EUROPEAN *CHITIN* SOCIETY *N*EWSLETTER

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EDITORIAL

Dear Society members,
I am pleased to send you the copy of the new issue of the European Chitin Society bulletin. I must say that it is an honor for me to become Secretary of EUCHIS and editor of the Chitin NewsLetter. I will do my best to carry out the duties as well as possible.
I would like to thank Prof. George Roberts for his service as Secretary and editor. He did excellent work and that is why he was elected as Honorary Secretary of the Society. His help was always priceless.

This year we could attend the 10th International Conference of European Chitin Society that was held on May 20-24th in St Petersburg (Russia) under the auspices of the Russian Chitin Society; you will find my short report later in this issue.
During the conference the winner of the Braconnot Prize was announced. This year it was Dr Ander Abarrategi Lopez who worked on application of chitosan as BMP-2 carrier in bone tissue engineering. Please find the abstract of his thesis in this issue.
There were also three poster awards. Jury took under account the scientific level and presentation of the subject. Abstracts of the awarded posters are also presented.
Finally, the report of the Treasurer of the Society is also included.

This year we had also the 9th Asia-Pacific Chitin & Chitosan Symposium, which was held in Nha Trang, Vietnam on 3-6th August and XVI Seminar of Polish Chitin Society will be held in Warsaw on 21st-23rd September, but impressions of these events will be published in the next issue of Chitin Newsletter.

Malgorzata M. Jaworska
Secretary

Dear members of EUCHIS family,

We are coming to the end of the 2011 with again lots of successful events in the Chitin World, the year which we have also celebrated the 100th year of the discovery of chitin by Braconnot. We had a very successful EUCHIS meeting, both socially and scientifically held in Russia-St. Petersburg. Many papers are published emphasizing the growing importance of chitin and chitosan especially in biomedical field.

This year, some of the board members have completed their term. I want to thank to them for their valuable contribution to our Society. I also want to welcome the new board members who have been elected for a 4-year term. We have already started working intensively with the new board with the goal of achieving more successful and fruitful period.

I wish you all the best in good health and peace!

Sevda Senel

General Assembly of the European Chitin Society

A General Assembly of the members of European Chitin Society was held on 22nd May 2011 at the Hotel Azimut (St. Petersburg, Russia). Twenty one members were present.

President's Report:
Prof. Sevda Senel, the President of European Chitin Society, presented information on the Society and mentioned that at the present moment there were 176 active members.

The jury, with Professor Senel as Chairperson and Prof. George A.F. Roberts, Prof. Vincent G.H. Eijsink, Dr Verena Seidl, Prof. Bruno Moerschbacher as members, had selected the winner of the Braconnot Prize who was awarded during the closing ceremony.
Prof. Senel also announced that there will be also three Poster Awards. The jury (Dr C. Caramella, Dr M. Jaworska and Dr F. Goycoolea) after careful selection chose the winners who were announced during the closing ceremony.

The series of "Advances in Chitin Science" was continued and the following volume (Volume XI, ISBN 978-5-4253-0133-8) was available during the conference. The Assembly expressed its appreciation to the editors (Prof. V.Varlamov, Dr. S.Bratskaya, Ms I. Yakovleva, Prof. S. Senel) for such rapid publication of the book.

Treasurer's Report
The treasurer, Dr Martin Greave, was not able to attend the General Assembly, but he sent the financial report which was presented to the General Assembly.
The Financial report was accepted by the General Assembly with no abstentions or abjections.

Election of the new Board of Directions
According to the statute of the European Chitin Society, Section III, part of the members of the Board of Directions must be elected every four-year period. This year the following members stood down from service: Dr Vincent Eijsink, Dr Martin Graeve, Dr Eric Guibal, Prof. George Roberts, Prof. Kjell Varum,
On their place the new members were proposed: Dr Suzana Vilchez (Spain), Prof. Jacques Desbrieres (France), Prof. Francisco Goycoolea (Germany), Dr Svetlana Bratskaya (Russia), Dr Kurt Draget (Norway), Dr Ruth Harris (UK), Prof. Carla Caramella (Italy).
The General Assembly chose new members to sit on the Board of Directors.
The new Board held its first meeting after the General Assembly.

EUCHIS'13 Conferences:
The President informed the meeting that she had not received any official proposal for organising the next EUCHIS conference, so this subject will be further discussed during the meeting of the Board of Directors.

Meeting of the Board of Directors of European Chitin Society

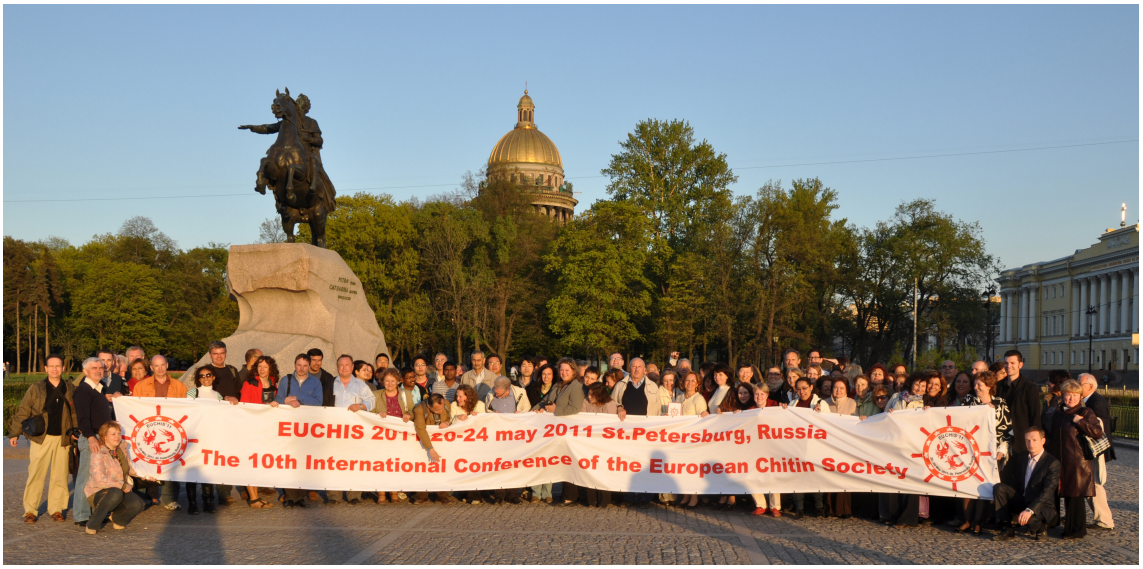
The meeting of the Board of Directors was held after the general Assembly. In the first step the Committee of the Board was elected:

President:	Sevda Senel
Vice-President:	Angela Heras
Vice-President	Bruno Moerschbacher
Secretary:	Malgorzata Jaworska
Assistant Secretary:	Jacques Desbrieres
Treasurer:	Francisco Goycoolea
Assistant Treasurer:	Laurent David
Members	Svetlana Bratskaya
	Carla Caramella
	Massimiliano Fenice
	Katia Heppe-Richter
	Henryk Pospieszny
	Verena Seidl-Seiboth
	Valery Varlamov
	Suzana Vilchez
Ad-hoc member	Martin Graeve

The Committee would like to thank Prof. George AF Roberts for his services as Secretary and, as a token of his contribution in preparation of the Chitin Newsletter, to confer on him the title of Honorary Secretary of the European Chitin Society.

The Committee discussed also the problem of the next EUCHIS conference. Unofficially two locations were suggested: Barcelona (Spain) and Potsdam (Germany). This will be the subject of further discussions. The Committee agreed that the organizers of EUCHIS'13 should be announced by the end of the year.

10th International Conference of the European Chitin Society
EUCHIS'11, St. Petersburg (Russia)



The 10th International Conference of the European Chitin Society was held on 20-24 May 2011 in St. Petersburg (Russia), in the Hotel Azimut (http://ecs-11.chitin.ru/). The conference started with the Welcome Party when Prof. Valery Varlamov wished a good time to all participants. After the official part of the evening, the banquet started. We were able to try excellent Russian food as well as taste Russian alcohols including vodka. This party gave us an opportunity to meet friends and discuss not only chitin/chitosan matters.

The scientific program of the Conference was divided into 5 parts:

A. Production of chitin, chitosan and its derivatives (16 oral presentations)
B. Chitin and Chitosan: characterization and analysis (4 oral presentations)
C. Nanochitosan – current status and future prospects (12 oral presentations)
D. Biomedical, pharmaceutical, agricultural and other applications (12 oral presentations)
E. Chitin and chitosan: biotechnological and enzymatic aspects (7 oral presentations).

Besides the oral presentation, there were nearly 80 posters focused on different aspects of chitin and chitosan science.

We were able to listen to 3 invited plenary lectures given by:

1. Prof. Angela Heras (Spain): "Chitin and chitosan: looking back, around and forward"
2. Mr Torsten Richter (Germany): "Standardisation of chitosan – first results of the interlab study"
3. Prof. Bruno M. Moerschbacher (Germany): "Enzymatic/ mass spectrometric fingerprinting of partially acetylated chitosans"

All of them were very interesting, showing successes and problems met in chitin/chitosan investigation and applications.

Except the scientific program, the organizers offered us unforgettable excursion to Peterhof and Conference dinner on a boat. Peterhof is an amazing combination of a park with thousands of fountains and small palaces build by Peter, the Great, as a summer resident. The beauty of this place will remain in our minds.

After the toil of visiting Peterhof, we were pleased to rest on the boat on the trip back to St.Petersburg, enjoying excellent food and listening to a gypsy band.

To end, I would like to thank the organisors for choosing this period of the year when we could enjoy the “white nights”. This let us visit St. Petersburg not only by daytime but also by night. By the way, next time we need to remember that they open the bridges at night and we need to be on the right side of Neva or we will enjoy St.Petersburg till sunrise.

Braconnot Prize Winner 2011

We are pleased to announce that the winner of the Braconnot Prize 2011 is

Dr Ander Abarrategi Lopez

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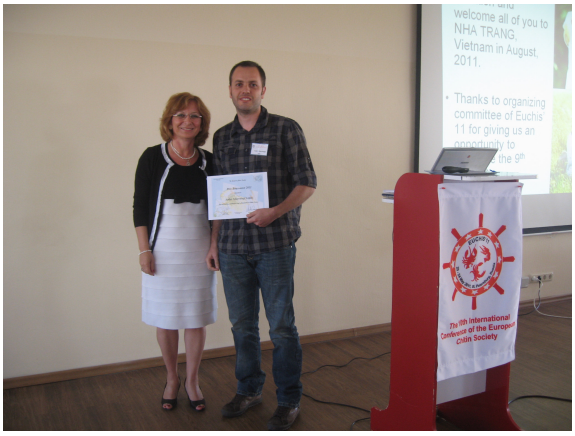

EDUCATION

PhD Degree : October 2008, Complutense University, Mardid (Spain)
Thesis title: "Chitosan as BMP-2 carrier: material development, characterization and applicability in bone tissue engineering"
Thesis available online: http://eprints.ucm.es/8615/1/T30747.pdf

Bachelor's Degree: 2002, Biology, Complutense University, Mardid (Spain)

PUBLICATIONS

1 - Y. Lopiz, **A. Abarrategi**, et al. In vivo comparison of the effects of rhBMP-2 and rhBMP-4 for Osteochondral Tissue Regeneration. European Cells and Materials, 20, 367-378, 2010.
2 - **A. Abarrategi**, Y. Lopiz, et al. Chitosan Scaffolds for Osteochondral Tissue Regeneration. Journal of Biomedical Materials Research A, 95A, 1132–1141, 2010.
3 - **A. Abarrategi**, J. Garcia-Cantalejo, et al. Gene Expression Profile on Chitosan/rhBMP-2 Films, a Novel osteoinductive Coating for Implantable Materials. Acta Biomaterialia, 5 (7), 2633-2646, 2009.
4 - M.J. Hortiguela, M.C. Gutierrez, et al. Urea assisted hydroxyapatite mineralization on MWCNT/CHI scaffolds. Journal of Materials Chemistry, 18, 5933-5940, 2008.
5 - **A. Abarrategi**, C. Moreno-Vicente, et al. Improvement of porous β-TCP scaffolds with rhBMP-2 chitosan carrier film for bone tissue application. Tissue Engineering, 14 (8), 1305-1319, 2008
6 - **A. Abarrategi**, A. Civantos, et al. Chitosan film as rhBMP-2 carrier: Delivery properties for bone tissue application. Biomacromolecules, 9(2), 711-718, 2008.
7 - **A. Abarrategi**, M.C. Gutierrez, et al. Multiwall carbon nanotube scaffolds for tissue engineering purposes. Biomaterials, 29, 94-102, 2008.
8 - **A. Abarrategi**, C. Moreno-Vicente, et al. rhBMP-2/chitosan film as titanium implant coating. Regenerative Medicine, 2(5), 587, 2007.
9 - J. L. Lopez-Lacomba, J. M. Garcia-Cantalejo, et al. Use of rhBMP-2 Activated Chitosan Films To Improve Osseointegration. Biomacromolecules, 7 (3), 792 -798, 2006.

10 - V.M. Ramos, N.M. Rodriguez, et al. Poly(ethylene glycol)-crosslinked Nmethylene phosphonic chitosan. Preparation and characterization. Carbohydrate Polymers, 64, 328-336, 2006
11 - **A Abarrategi**, C Moreno-Vicente, et al. Bone growth induction on rhBMP-2 coated implants. Cytotherapy, 8(S2), 61, 2006.
12 - R. Martinez-Corria, **A. Abarrategi**, et al. In vivo testing of chitosan coating as BMP-2 carrier: bone substitutes and titanium implants. In "Proceedings of First Branemark Scientific Symposium, Gothenburg 2009". Edited by R. Gottlander, D. van Steenberghe. Quintessence Publishing Company Berlin, p. 105-116, 2009.
13 - **A. Abarrategi**, Y. Lopiz, et al. In vivo study of chitosan scaffolds for osteochondral tissue regeneration. In "Advances in Chitin Science Volume XI. Proceedings of the 9th International Conference of the European Chitin Society". Edited by F. Rustichelli, C. Caramella, S. Senel, K.M. Varum. Venice, 2009.

TECHNOLOGY TRANSFER EXPERIENCE

- Participation Noricum S.L, which is a spin-off company based on technological innovations. This company has generated an international patent related to the thesis main topic.
- Person in charge of the starting-up of the Noricum's new R&D laboratory (65 m2).

Author: Ander Abarrategi
Thesis Title:
"Chitosan as BMP-2 Carrier: Material Development, Characterization and applicability in bone tissue engineering".

Advisor: Dr. Jose Luís López-Lacomba **Co- Advisor:** Dr. Viviana Ramos
Instituto de Estudios Biofuncionales. Universidad Complutense Madrid, Spain.

Abstract:

Tissue engineering (TE) is an emerging and multidisciplinary science, basically focused on the recovery of tissue functions via new functional tissue generation. In this sense, TE needs: biomaterials which provide an appropriate environment; cells that will form the new tissue and; growth factors that will induce this tissue formation. In this context, the aim of this work is to provide osteoinductive properties to clinically available implantable materials using chitosan films, rhBMP-2 and stem cells.

rhBMP-2 is one of the best known Bone Morphogenetic Factors. This rhBMP-2 was synthesized and its activity was exhaustively evaluated *in vitro* on cultured cells. The performed DNA microarray assays show the transcriptional changes induced by the rhBMP-2 and its osteoinductive effect on cultured cells.

On the other hand, chitosan films were evaluated as biocompatible material. For this purpose, C2C12 and mesenchymal Stem Cells were seeded onto these films and they adhere, grow and proliferate properly. The performed transcriptional studies show that cells slightly recognize this growing surface as a foreign chemical structure.

Chitosan films were also evaluated as rhBMP-2 carrier material. *In vitro* assays were performed in order to simulate the *in vivo* rhBMP-2 delivery: by partial film dissolution in the initial wound healing process; by low diffusion from the film and; by film enzymatic biodegradation. Performed assays show that rhBMP-2 is active on the film and also after release, both *in vitro* and *in vivo*.

Finally, chitosan/rhBMP-2 film was studied as coating of titanium implants and porous β-TCP ceramics. The performed in vivo assays show that the coating induces the bone formation around the implants and improved the properties of the porous β-TCP scaffolds.

All these results show both *in vitro* and *in vivo* that chitosan/rhBMP-2 films are a biocompatible and osteoinductive material applicable as coating for titanium implants and porous β-TCP ceramics.

Poster Awards EUCHIS'2011

Three Poster Awards were given during the Conference of EUCHIS'11. The winners are:

1st Prize: E.V.Korchagina, O.E. Philippova "Aggregation of chitosan and its hydrophobic derivatives in dilute aqueous solutions"

2nd Prize: E.Turano, S. Marconi, S. Sotgiu, B. Bonetti "Role of chitosan in Alzheimer: a new cytotoxic pathway in an old disease"

3rd Prize: I.Koppowa, J. Simunek "Modulation of intestinal bacterial population of healthy rats during administration of chitosan and chitooligosaccharides *in vivo*"

AGGREGATİON OF CHİTOSAN AND ITS HYDROPHOBİC DERİVATİVES İN DİLUTE AQUEOUS SOLUTİONS

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The present work is devoted to the study of the behavior of chitosan in dilute aqueous solutions. Chitosan, a (1→4)-linked linear copolymer of 2-amino-2-deoxy-β-D-glucan (GlcN) and 2-acetamido-2-deoxy-β-D-glucan (GlcNAc), is produced commercially by alkaline N-deacetylation of chitin, a second most abundant polysaccharide in nature after cellulose. Being nontoxic, biocompatible and biodegradable polymer chitosan is very promising for various applications in pharmacy, biotechnology, cosmetics etc.

In low acidic media chitosan is a polyelectrolyte having tendency to association. Similar to any associating polyelectrolyte, chitosan being dissolved in water forms spontaneously clusters of some optimum size determined by the competition of hydrophobic association inducing growth of aggregates and electrostatic repulsion limiting their growth. By playing with these counteracting effects one may easily obtain clusters of the desired size. Multichain clusters of chitosan and its hydrophobic derivatives can be used as prospective carriers for drug delivery.

Chitosan samples under study have molecular weights of 55000, 70000 and 125000 g/mol and the degree of acetylation 5 %. Hydrophobically modified (HM) chitosan contains 2 % or 4 % side n-dodecyl groups. Polymer solutions were prepared in 0.3M CH_3COOH containing different amount of CH_3COONa and NaCl.

By dynamic light scattering it was shown that all chitosan samples have bimodal particle size distribution. The smaller particles correspond to individual macromolecules in coil conformation and the bigger ones correspond to clusters consisting of several polymer chains.

The hydrodynamic radius of clusters ranges from 130 to 200 nm. It was shown that in solvent containing 0.05 CH_3COONa the size of clusters of HM chitosan is much larger than for its unmodified precursor and clusters of chitosan bearing 4% of side n-dodecyl groups are larger than those of chitosan with 2% of side n-dodecyl groups. The presence of chitosan clusters was confirmed by transmission electron microscopy. Chitosan clusters of polymer with 4% of side n-dodecyl groups have higher aggregation number than those of polymer with smaller (2%) content of side n-dodecyl groups in CH_3COONa. In solvent containing NaCl the size of aggregates does not depend on content of hydrophobes and with increasing amount of added low molecular weight salt the clusters of chitosan become smaller and change their structure. At the same time, the weight fraction of clusters increases at the addition of salt.

ACKNOWLEDGEMENTS

The financial support of Russian Federal Education Agency in the framework of the program "Scientific and educational staff of innovative Russia" in 2009-2013 is gratefully acknowledged.

Keywords: self-aggregation, chitosan, associating polyelectrolytes, nanogels.

ROLE OF CHITIN IN ALZHEIMER: A NEW CYTOTOXIC PATHWAY IN AN OLD DISEASE

Ermanna Turano[1], Silvia Marconi[1], Stefano Sotgiu[2], Bruno Bonetti[1].

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Alzheimer disease (AD) is a progressive neurodegenerative disease characterized by the presence of extracellular amyloid plaques and neuronal neurofibrillar tangles in the brain, identified by Alois Alzheimer more than a century ago. The main constituent of AD plaques are fibrillary aggregates of the β-amyloid protein, which is believed to play a major role in the pathogenesis of AD. The increased production and subsequent accumulation of β-amyloid protein results in direct neuronal toxicity and in microglial activation which, through the production of inflammatory mediators, contributes to neuronal damage.

However, in recent years the scientific community has raised some questions and doubts regarding the exclusive pathogenetic role of amyloid; indeed amyloid plaques contain a wide range of molecules, whose significance is not yet clear investigated. Among these, we have confirmed the presence by Calcofluor and Chitin-binding Probe of chitin in AD autoptic brains, suggesting a strict relationship between chitin and β-amyloid in pathological conditions. The aim of our study is to investigate whether chitin participates in the pathogenesis of AD by assessing its biological effects on neurons and microglia.

The exposure of microglial cultures to exogenous chitin showed that the cells were able to phagocyte small chitin particles, and the process was significantly inhibited by β-amyloid.

Similarly to what described with β-amyloid, phagocyted chitin deposits induced a marked proliferation and activation of microglial cells. In addition, experiments with neuronal cultures, clearly showed that chitin is cytotoxic for neurons. On the contrary, deacetylated chitin, chitosan, didn't show any effect on cell cultures.

A central point of this research concerns the production of chitin by mammalian cells, which however lack chitin synthase. We demonstrated that in the presence of activated N-acetyl-glucosamine, murine microglial cells undergo activation and are able to produce Calcofluor-positive chitin-like deposits, which are then released in the extracellular medium. In addition, activated N-acetyl-glucosamine induced marked cytotoxicity on neuronal cells, mimicking the effect of exogenous chitin. In both microglial and neuronal cultures incubated with activated N-acetyl-glucosamine, HPLC-MS analysis showed the presence of "new-formed" chitin-like compounds.

Our results indicate that in particular conditions of altered glucose metabolism both microglia and neurons produce chitin-like polymers which induce direct neuronal cytotoxicity and on the other hand activate microglia with consequent release in the extracellular space of chitin-like deposits contributing to neuronal damage.

These results might suggest a cytotoxic role of chitin-like molecules and offer new insights in the pathogenesis of AD.

Keywords: Chitin, Alzheimer, β-amyloid, N-acetylglucosamine, neurotoxicity.

MODULATİON OF INTESTİNAL BACTERİAL POPULATİON OF HEALTHY RATS DURİNG ADMİNİSTRATİON OF CHİTOSAN AND CHİTOOLİGOSACCHARİDES *IN VİVO*

Ingrid Koppová, Jiří Šimůnek

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Chitosan (CS) is a functional and linear polysaccharide, which is prepared by partial *N*-deacetylation of chitin. The fragmented chitosan with molecular weight 0.5-3 kDa (COS), that is in the focus of our scientific interest, exhibits various types of the potent biological activities useful for medical applications and health care. Only little information is known about the effect of chitosan or chitooligosaccharides on bacteria of gastrointestinal tract. The changes in the bacterial population, followed by the shifts in the bacterial metabolites production can significantly alter condition in gastrointestinal tract and health status of the host. In our study we performed to modulate the changes of fecal bacterial population in healthy rats during administration of chitosan and chitooligosaccharides. Rats were fed commercial diet during two weeks, after that were divided in four groups (n=6) and diet were supplemented with 0.2% chitosan (group A, B) or 0.2% chitooligosaccharides (group C,D). CS/COS was added to the drinking water (A, C) or to the diet (B, D). This period was followed by no CS/COS 2-weeks period. PCR-DGGE analysis was used to detect possible changes in bacterial population. The DGGE band fragments that intensified, newly appeared or by contrast disappeared after CS/COS ingestion were further characterized by sequencing analysis. For the quantification of total and selected bacterial DNA (subpopulation of bifidobacteria, bacteroides, lactobacilli and butyrate producing bacteria) was used real-time PCR analysis. DGGE profiles revealed high complexity and individuality for each subject. CS/COS changed the composition of microbial ecosystem of gastrointestinal tract of healthy rats. Marked changes were observed when chitosan was added. Nevertheless, the responses of fecal bacterial population to chitosan feeding were subject-specific.

ACKNOWLEDGEMENTS

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