EUROPEAN CHITIN SOCIETY

NEWSLETTER

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- 2007 Board Meeting
- 2007 Conference Reports
- Awards
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January, 2008
No. 24

Minutes from the EUCHIS board meeting (Antalya, September 9th, 2007)
Present:
Kjell M. Vårum (President and Chair)
Vincent Eijsink
M. Fenice
B. Kochanska
Bruno Moerschbacher
Henryk Pospieszny
George A.F. Roberts
Sevda Senel
Also present:
Prof. Alain Domard (former president of EUCHIS, observer)
Prof. Martin Peter (former president of EUCHIS, observer)

1. Time and place for the EUCHIS '09 meeting.
Professor Rustichelli presented the plans for the next EUCHIS meeting to the Board. Based on the presentation, the board decided that the next EUCHIS meeting was to be arranged in Venice, Italy, provided the date could be moved from September to earlier in the year, so avoiding a clash with the 11th ICCC which is to be held in Taiwan in September 2009. Subsequent to the Board meeting Professor Rustichelli was able to confirm that the date had been changed to May 23-27, 2009.
The conference venue will be the San Servolo island, which is in the lagoon about 5 minutes by water-taxi from St Mark's Square.

Conference Chairman:
Prof. Franco Rustichelli, University Politecnica delle Marche, Italy

Conference Co-Chairpersons:
Prof. Carla Caramella, University of Pavia, Italy
Prof. Sevda Senel, Hacettepe University, Turkey
Prof. Kjell M.Vårum, Norwegian University of Science and Technology, Norway

For further information see http://www.sanservolo.provincia.venezia.it) and http://alisf1.univpm.it/euchis2009.

2. Finances
An update on the economy of EUCHIS was presented by Peter Graeve on behalf of the treasurer, Martin Graeve.
The current economy of EUCHIS is in good (see also the Financial Reports on the following page for details)
In view of this it was decided to use more money related to travel awards.

3. Other issues
Discussions concerning the membership categories.
EUCHIS has so far offered reduced membership fees for the former active EAST members. It is the opinion of the board that this should be changed, and this should be included on the agenda of the next General Assembly (at EUCHIS'09).

EUCHIS ‘07

EUCHIS ’07 was held on 8th-11th September in Antalya, a resort town on the southern coast of Turkey, the actual venue being the Conference Centre of the Divan Hotel. This was convenient for the majority of delegates who were accommodated in the Divan Hotel itself. It was a good choice of venue by the Organising Committee and I do not think that anyone who experienced them will ever forget the beauty and tranquillity of breakfast on the terrace of the hotel.

However a conference should not be judged solely on the comfort of the hotel and the beauty of the surroundings and the content and level of the lecture programme are obviously the most important factors by far. In these aspects EUCHIS ’07 was definitely not a disappointment, having a wide programme covering most technical areas relevant to both producers and users of chitin and chitosan as well as academic researchers, and with most of the lectures being to a high technical level. A quick look through the titles confirms that the shift in interest towards the biomedical field is continuing in both pure and research on chitin and chitosan.

There were 213 delegates, which is about average for EUCHIS meetings, representing 35 countries including those of Europe, North and South America, Africa and Asia. The host nation, Turkey, has the largest group while the Korean delegation was the second largest. EUCHIS meetings benefit from this open-door policy, both in terms of the resultant presentations and of the world-wide contacts that can be made at what is nominally a European event. Long may this aspect continue.

As is usual at EUCHIS meeting there is the presentation of the biennial Braconnot Prize which was awarded to Dr Sebastien Ladet (France). Also there is the presentation of awards for the best posters, an idea that began at the 10th ICCC/7th EUCHIS meeting in 2006. The winners were:
1st: M Mitsutomi, M Watanabe, K Hirano and K Seki (Japan)
2nd: M K Yoo, I Y Kim, S S Park, H S Na, H C Lee, S K Kim and C S Cho (Korea)
3rd: A Chenite, C Jarry, M Garan and E A DesRosiers (Canada)
A profile of Dr Ladet and summaries of the winning posters together with profiles of the senior authors can be found elsewhere in the *Newsletter*.

One item of particular interest was the final technical session which was a panel/audience discussion on regulatory issues of concern in applications of chitin and chitosan. There was quite a few opinions expressed from the audience and one suggestion was to get a spokesperson from the FDA or the EU Commission to address EUCHIS ’09. However it was pointed out that regulatory issues have been discussed at previous conferences and it would be better to act sooner rather than delay. It is hoped to have an account of this discussion published either in the next *Newsletter* or else on our website. It would also be of considerable help and interest if any members with a contribution to make on this issue were to send in their thoughts for publication in the *Newsletter*.

Finally, EUCHIS ’07 had one unique feature that made it different from any other conference I have ever attended – delegates received their copy of the *Proceedings*

before the conference presentations started. This was achieved by having a deadline for submission of manuscripts about 1 month ahead of the conference and a very large amount of last minute work by the organisers and the printers. Unfortunately some authors missed the deadline but I understand that a Supplement containing those papers and posters will be published soon.

POLISH CHITIN SOCIETY WORKSHOP

A workshop on "New Aspects of Chemistry and Application of Chitin and its Derivatives" was held in Wroclaw, Poland on 17th-19th September 2007. This was the 13th annual workshop to be held following their inception by the late Professor Henryk Struszczyk. They are relatively small meetings with normally 40-45 delegates, this makes for a very relaxed and informal atmosphere. Although the majority of delegates are from Poland, the meetings are open to other nations and this year there were scientists from Russia and from the United Kingdom. To cater for other nationalities the abstracts, which are available prior to the start of the presentations, are in English as well as Polish, and the Proceedings are also published in English.

Most of the presentations this year, both oral and poster, dealt with applications or potential applications of chitin and chitosan, particularly in the fields of biomedicine and of agriculture, two areas where Polish researchers appear to be concentrating their efforts. However other topics were covered, including enzymatic deacetylation of chitosan, thermosensitive chitosan hydrogels, and sonication of chitin solutions.

The programme also included a guided tour round Wroclaw, the General Assembly of the Polish Chitin Society, and a Ceremonial Dinner.

George Roberts

EUCHIS Financial Report 2006

31. December 2006 Account at Deutsche Bank, Bonn

POSITIVA				
Balance per 31.12.2005			EUR	9.720,18
Members fee				
- collective members	EUR	1.080,00		
- active members	EUR	2.158,80		
- associate members	EUR	92,00		
- student members	EUR	384,00		
	EUR	3.714,80	EUR	9.720,18
total			EUR	13.434,98

NEGATIVA				
Bank charges	EUR	-384,73		
10th ICCC (Graeve, M.)	EUR	-600,00		
Reimbursement (Cartier)	EUR	-35,00		
Office expenses	EUR	-260,00		
Internet charges	EUR	-215,76		
Euchis travel award 2006	EUR	-2.544,05		
Euchis Poster Price 2006	EUR	-1.000,00		
Prix Bracconnot 2006	EUR	-1.000,00		
total			EUR	-6.039,54
Balance per December 31 2006			EUR	7.395,44

Bremen, 31.12.2006

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EUCHIS Financial Report 2007

31. December 2007 Account at Deutsche Bank, Bonn

POSITIVA				
Balance per 31.12.2006			EUR	7.395,44
Reimbursement Montpellier	EUR	4.500,00		
Members fee				
- collective members	EUR	900,00		
- active members	EUR	1.593,00		
- associate members	EUR	420,00		
- student members	EUR	464,00		
			EUR	7.877,00
total			EUR	15.272,44

NEGATIVA				
Bank charges	EUR	-356,98		
Office expenses	EUR	-260,00		
Internet charges	EUR	-221,19		
Euchis travel awards 2007	EUR	-1.529,05		
Euchis Poster Price 2007 (to be paid in 2008)	EUR	-0.000,00		
Prix Bracconnot 2007	EUR	-1.000,00		
total			EUR	-3.367,22
Balance per December 31, 2007			EUR	11.905,22

Bremen, 31.12.07

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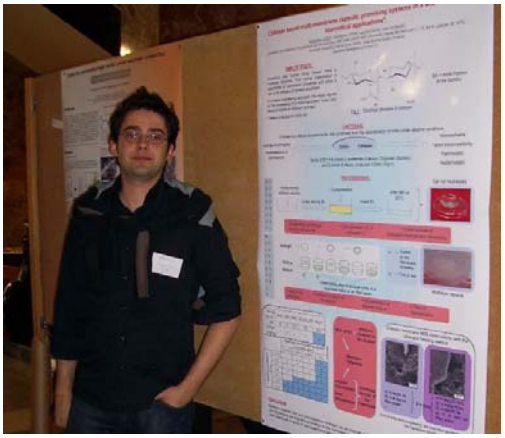
BRACONNOT PRIZE 2007

There were three candidates for the Braconnot Prize: Dirk Peikow from Institute of Chemistry, University of Potsdam, Germany; Aslak Einbu from NOBIPOL, Department of Biotechnology, NTNU, Trondheim, Norway; and Sebastien Ladet from Laboratoire des Matériaux Polymères et des biomatériaux, CNRS, Villeurbanne, France.

The Braconnot jury has decided that Dr. Sebastien Ladet should receive the Braconnot Prize for 2007. One of the major achievements in the thesis is the basis of a paper very recently accepted for publication in *Nature*. More about this in the next EUCHIS Newsletter.

Dr. Sebastien Ladet receives the Braconnot Prize from the jury leader, EUCHIS secretary George A.F. Roberts on the banquet dinner at EUCHIS'07



Profile : Sébastien Ladet

Date of birth: May 29th, 1979 in Rochefort (France)

Address: Covidien, *116 avenue du Formans, ZAC de Forquevaux, 01600 Trévoux – France*
Tel: (33) 04 74 08 90 00
Email: sebastien.ladet@covidien.com

Education :

March 2007 R&D Project Manager in medical device development, Sofradim production-Covidien (Trévoux, France).

2003-2006 PhD., Thesis title: Elaboration and study of the physical and biological properties of a multi-membrane bioreactor.
Laboratoire des Matériaux Polymères et des biomatériaux (CNRS 5627, Villeurbanne). Under Pr. A. Domard and Pr. L. David supervision.

2001-2003 Master in biomolecules chemistry, University of Montpellier II.

-Synthesis of mesoporous materials by sol/gel process. Drugs encapsulation study. Laboratoire des matériaux catalytiques et catalyse en chimie organique (CNRS 5618, Montpellier)

-Asymmetric synthesis of silanated modified amino acid in order to optimise peptide biodisponibility. Laboratoire des aminoacides peptides et protéines (CNRS 5810, Montpellier)

2000-2001 Licence ès sciences, University of Perpignan.
Characterisation of biomolecules extracted from marines sponges. (Centre de Phytopharmacie, UMR CNRS 5054, Perpignan).

Patent and publications :
"Capsules plurimembranaires de polysaccharides et en particulier de chitosane et leur procédées de préparation " Ladet, S.; Viton, C.; Domard, A. (ref.: WOFR06050191)

"Multi-membrane hydrogel" Ladet, S.; David, L.; Domard, A. (To be published in Nature)

Main Communications:
European Conference on Biomaterials, Nantes, France, Sept. 2006. "New generation of biomaterials: multimembrane onion-like architecture, a template for tissue regeneration" Ladet, S.; Tahiri, K; David, L; Corvol, M-T.; Domard, A.

International Congress of Chitin and Chitosan, Montpellier, France, Sept. 2006. "A new design architecture for tissue engineering" Ladet, S.; David, L, C.; Domard, A.

1st Poster Prize

Classification of chitosanases by hydrolytic specificity against N, N^4-diacetylchitohexaose

Masaru Mitsutomi*, Masamichi Watanabe, Katsuaki Hirano, and Kiyohiko Seki

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Chitosanases (EC 3.2.1.132) are glycoside hydrolases that catalyze the degradation of chitosan, which is a copolymer of D-glucosamine (GlcN) and *N*-acetyl-D-glucosamine (GlcNAc). Chitosanases can be divided into three subclasses according to their specificity for the hydrolysis of the β-glycosidic linkages in partially *N*-acetylated chitosan molecules: subclass I chitosanases split the GlcNAc-GlcN and the GlcN-GlcN bonds, subclass II chitosnases split only the GlcN-GlcN bonds, subclass III chitosanases splits the GlcN-GlcNAc and the GlcN-GlcN bonds. The hydrolytic specificities were determined by sequence analysis of the chito-oligosaccharide products obtained from the enzymatic degradation of partially *N*-acetylated chitosan. In this study, we examined the cleavage specificities of chitosanases against *N*, *N* ^{4}-diacetylchitohexaose [$(GlcN)_2$-GlcNAc-$(GlcN)_2$-GlcNAc].

N, *N* ^{4}-diacetylchitohexaose was prepared by enzymatic hydolysis of partially *N*-acetyated chitosan. Subclass I chitosnase from *Aspergillus fumigatus* hydrolyzed $(GlcN)_2$-GlcNAc-$(GlcN)_2$-GlcNAc to produce $(GlcN)_2$-GlcNAc. *Bacillus* sp. No. 7-M chitosanase (subclass II) could not hydrolyze the chito-oligosaccharide. Subclass III chitosanases from *Bacillus circulans* WL-12 and *Bacillus circulans* MH-K1 hydrolyzed the chito-oligosaccharide into $(GlcN)_2$ and GlcNAc-$(GlcN)_2$-GlcNAc. On the other hand, $(GlcN)_2$, $(GlcN)_2$-GlcNAc, and GlcNAc-$(GlcN)_2$-GlcNAc were detected in the hydrolysis of the *N*, *N* ^{4}-diacetylchitohexaose by chitosanses from *Pseudomonas* sp. A-01 and *Amycolatopsis* sp. CsO-2. The results indicated that the enzymes split the GlcN-GlcNAc bonds and the GlcNAc-GlcN bonds in addition to the GlcN-GlcN bonds.

These results suggested that chitosanases are classified into four subclasses according to their specificity for the hydrolysis of the β-glycosidic linkages in *N*, *N* ^{4}-diacetylchitohexaose, which is a useful substrate to determine the cleavage specificity of chitosanase.

Masaru Mitsutomi graduated from the Faculty of Agriculture, Saga University in 1976 and received his master degree from the same University in 1978. He received his Ph.D. in Agricultural Sciences from Kyushu University in 1992. After working at Hayashibara Biochemical Laboratories Inc. as a researcher from 1978 to 1980, he joined the Department of Agricultural Chemistry at Saga University as a Research Associate (supervised by Professor Akira Ohtakara) from 1980 to 1992. He was an Associate Professor at Laboratory of Food Chemistry from 1992 to 2000. He has been Professor at the same laboratory since 2000. His research interests include structures and biological functions of chitin-related enzymes and enzymatic synthesis of functional oligosaccharides.

2nd Poster Prize

Evaluation of Semi-Interpenetrating Polymer Networks Composed of Chitosan and Poloxamer for Wound Dressing Application

Mi-Kyong Yoo[1], In-Yong Kim[1], Sung-Sik Park[2], Hee-Sam Na[3], Hyun-Chul Lee[3], Se-Kwon Kim[4], Chong-Su Cho[1*]

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[2] Department of Anatomy, Chonnam National University Medical School, Kwangju 501-190, Korea
[3] Department of Microbiology, Chonnam National University Medical School, Kwangju 501-190, Korea
[4] Department of Chemistry, Pukyong National University, Busan 608-737, Korea
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INTRODUCTION : Wound dressing is to protect a skin defect from infections and dehydration in the intervening period between hospitalization and grafting. Various natural and synthetic polymers with good biocompatibility have been used in order to develop wound dressing materials. Chitosan (CS) is an N-deacetylated product of chitin and one of the most abundant polysaccharides in nature. CS has many advantages for wound dressing, namely biocompatibility, biodegradability, hemostatic activity, anti-infection and wound healing acceleration properties. However, CS alone has a poor mechanical property due to their brittleness, whereas its application to wound dressing requires sufficient mechanical strengths. Therefore, addition of other synthetic polymers is necessary to achieve films or sponge with improved strength and elasticity. In our system, poloxamer-407 macromer, having an acrylated-terminated PEO derivative, was chosen as blending material and crosslinked in the presence of CS to form the semi-interpenetrating polymer networks (SIPNs). The advantage of this system is the formation of mechanically stronger hydrogel, increasing the compatibility of the polymer blends, which exhibit favorable properties of phase-separated materials. Therefore, CS/poloxamer SIPNs are expected to have greater enhanced mechanical strength and the ability of wound healing than those of CS alone.

OBJECTIVE : To develop the chitosan (CS)-based matrix as a skin substitute in wound management through the introduction of SIPNs system using a poloxamer macromer.

EXPERIMENTAL : Porous SPINs hydrogel composed of CS and poloxamer macromer terminated with acrylate groups were prepared by freeze-drying after UV irradiation. The properties required for ideal wound dressing, such as equlibrium water content, water absoption, water vapor transmission rate (WVTR), and evaporative water loss were examined. *In vitro* degradation and cytotoxicity of the CS/poloxamer SIPNs were also assessed. The wound healing efficiency of the SIPNs hydrogel was evaluated on experimental full thickness wounds in a mouse model.

RESULT : The CS/poloxamer SIPNs was found to have a water content of 90% of its weight which could prevent the wound bed from accumulation of exudates and also have excellent water adsorption. The WVTR of CS/poloxamer SIPNs was found to be 2508.2 ± 65.7 gm-2day-1, indicating that the SIPNs can maintain a moist environment over wound bed in moderate to heavily exuding wound which enhances epithelial cell migration during the healing process. Also, the CS/poloxamer SIPNs in vitro assessment showed proper biodegradation and low cytotoxicity for wound dressing application. The wounds covered with the SIPNs were completely filled with new epithelium without any significant adverse reactions after 3 weeks.

CONCLUSION : *In vitro* and *vivo* data support that the CS/poloxamer SIPNs can be safely used as good wound dressing systems.

Mi Kyong Yoo received her Ph.D. degree in 1998 from the Department of Chemistry, Dongguk University, Korea. She carried out her Ph.D. studies on “Effect of polyelectrolyte on the cloud point of temperature and pH sensitive polymers” Following a research intern program at Dongguk University, supported by Korea Research Foundation, as a post-doctoral fellow, from 1998 to 2001. In 2002, she joined the Department of Agricultural Biotechnology, Seoul National University as a post-doctoral fellow, supported by Brain Korea 21 (BK 21) grant. Her research focuses on chitosan scaffold for tissue engineering and contrast agent design for targeted MR imaging.

3rd Poster Prize

Reinforcement of Chitosan-Glycerophosphate Hydrogels with Glyoxal Cross-Linker

A. Chenite, C. Jarry, M. Garon and E. A. DesRosiers

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Summary

Chitosan-based hydrogels are a class of biomaterials that have shown great promise as a scaffold for tissue engineering, they benefit from the well-documented advantageous biological properties of chitosan. The thermo-sensitive chitosan-glycerophosphate hydrogels have been proposed as implantable delivery systems which may form *in situ* at the site of injection and can encapsulate, carry and deliver therapeutic cells or drugs. However, despite the overall performance of chitosan-glycerophosphate hydrogels, their use is restricted by limited mechanical robustness, which could be insufficient for some applications. A solution is to reinforce the hydrogels by incorporating glyoxal, which is recognized as a potent chemical cross-linker for water-soluble polymers.

Very low concentration of glyoxal (between 0.125 and 0.25 mM) was shown to be sufficient to impart to the hydrogel enough strength and make it more resistant to eventual in vivo shear forces, so that it can ensure a better protection of encapsulated cells and nascent tissue. This was rendered possible by the improvement of the reactivity of chitosan amino groups toward glyoxal due to the quasi-neutral pH of chitosan-glycerophosphate solution. In a previous study, we reported that this low glyoxal concentration was largely below the toxicity level and did not affect the cytocompatibility of the hydrogel.

The present work is intended to investigate the properties of chitosan-glycerophosphate hydrogels reinforced by chemical cross-linking with glyoxal. We demonstrated that the cross-link density can be controlled by the pH, the glyoxal concentration and the degree of deacetylation (DDA) of chitosan, as this later parameter expresses the NH_2 concentration. The biomechanical properties were investigated in indentation on hydrogel samples enclose in petri dish using a cylindrical indenter mounted on a micromechanical system (Mach-1™, Bio Syntech Canada Inc). The equilibrium stress, when the hydrogel is indented by 50 % of its thickness, is between 0.4 kPa and 2.2 kPa for hydrogels composed of 2% in chitosan and about 93 to 95% of water. These results also indicate a direct relationship between the hydrogel equilibrium stress and the cross-linking density. Another indentation procedure showed the ability of the hydrogels to recover to their original mechanical strength after four indentation cycles.

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Dr. Chenite joined BioSyntech in 1995, and has the distinction of being the inventor of the BST-Gel® platform technology. This technology provides a family of thermosensitive chitosan hydrogels formulated at physiological pH, which allow the conception and the design of injectable devices to carry therapeutic materials for minimally invasive implantation *in vivo*. His 22 years of materials research has contributed to BioSyntech's extensive chitosan knowledge base. He currently researches novel material modifications for future BioSyntech medical devices and therapeutic delivery systems. Dr. Chenite obtained a Doctoral degree in Material Science, in 1983, from the University of Nancy, France. After that he was offered an Assistant Professor position at the *École Normale Supérieure* in Marrakech, Morocco, where he worked during 6 years. Then, he joined the University of Montréal, Canada, from where he obtained a Ph.D. in Polymer Chemistry, in 1992. Before joining BioSyntech, he worked during 3 years as a Research Associate at the National Research Council, in Ottawa, Canada. He has published more than 30 peer-reviewed scientific articles and authored patent applications for 8 distinct inventions.